AN INVESTIGATION INTO THE PHARMACOLOGICAL PROPERTIES OF PROPOLIS

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7. Hu FL, Zhu W, Li YH, Chen ML, Ying HZ (2004) Determination of the anti-tumor and anti-inflammation effects of the flavonoid component of different propolis extracts. *Apiculture of China* **55**(3): 4-6. (in Chinese)

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讀萬卷書，行萬里路

ABSTRACT

Propolis is a traditional value-added product from beekeeping, and has been widely used in medicine and the chemical industry because of its extensive biological activities. In this dissertation some pharmacological properties of propolis extracted by ethanol (EEP) and water (WSP) were studied by means of modern pharmacological methods. The results of the experiments show the following:

1. Both EEP and WSD led to decreased levels of fasting blood glucose (FBG), glycosylated haemoglobin (HBAlc), Fructosamine (FRU), blood urea nitrogen (BUN), uric acid (UA), malonaldehyde (MDA), nitric oxide (NO), nitric oxide synthetase (NOS), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and very-low density lipoprotein cholesterol (VLDL-C) in the serum of fasting rats; and, to increased serum levels of high-density lipoprotein cholesterol (HDL-C) and superoxide dismutase (SOD) in rats with diabetes mellitus. This suggests that propolis can control blood glucose and modulate the metabolism of glucose, blood lipid and protein, leading to decreased outputs of lipid peroxidation and scavenging free radicals in rats with diabetes mellitus.

2. Both EEP and WSD showed inhibitory effects on swelling induced by Freund's complete adjuvant and decreased the degree of local inflammatory responses; significantly inhibited the increase of interleukin-6 (IL-6) in inflamed tissues, but had no significant effect on levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ). The results are consistent with the interpretation that EEP and WSD may exert these effects by inhibiting the activation and differentiation of mononuclear macrophages.

3. Both EEP and WSD exhibited significant anti-inflammatory effects in animal models with respect to thoracic capillary vessel leakage in mice, Carrageenan-induced oedema, Carrageenan-induced pleurisy and acute lung damage in rats. The mechanisms for the anti-inflammatory effects probably involve decreasing prostaglandin and NO levels.

4. Both EEP and WSD had an inhibitory effect on the level of TG, TC, LDL-C, glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) in serum, and TC, TG and MDA in liver of hyperlipidemic SD rats; but were without effects on HDL-C, MDA, SOD and NO in serum. EEP also reduced body weight, liver weight and liver index, but WSD did not reduce those indexes. The results showed that the two extracts contribute to the improvement of lipid metabolism in hyperlipidemic rats and provide them with the required anti-oxidative activity.

5. The 80% ethanol extracts of propolis had the highest flavonoid content. The flavone content of water extracts of propolis were obtained with a natural surface-active agent, under ultrasonic perturbation at 80°C, for 12 h and was 6.7 times greater than that of propolis extracted with water at room temperature. Compared to EEP, WSD has the same or a greater anti-tumor or anti-inflammation effect at the same dosage, and shows considerable pharmacological potential especially because of its low side effects and ease of preparation.

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CHAPTER 1

1. INTRODUCTION

1.1 The chemistry and plant origins of propolis

Propolis is a resinous material that honeybees (*Apis mellifera* L.) collect from various plants, and mix with wax and other secretions. Propolis has been widely used in medicine and the chemical industry because of its extensive biological activities, including anti-bacterial, anti-fungal, anti-viral, anti-protozoan, anti-inflammatory, anti-ulcer, anti-tumor, hepatoprotective, local anaesthetic, immunomodulation and free radical scavenging properties (Ghisalberti, 1978; Marcucci, 1995; Marcucci *et al.*, 2001; Basnet *et al.*, 2002). Unlike traditional Chinese herbal medicines, the components of propolis significantly differ from source to source and this is reflected in their biological activities. Thus it is necessary to study the chemical composition of propolis in relation to plant sources to provide a foundation for the standardization of propolis.

1.1.1 Plant sources of propolis

The issue about plant sources and the formation of propolis has long been a matter of scientific debate. Meyer (1956) recorded the whole process of bee collecting and processing propolis in detail by camera, which provided proof for some of the sources and formation of propolis.

Botanical sources of propolis in temperate areas such as Europe, North America and North Africa are mainly *Populus* species and their hybrids; in New Zealand, introduced poplar species are the main source; in Australia however the main botanical source is *Xanthorrhoea spp.*, in South America *Clusia major* and *Clusia minor,* and in Asia the

botanical sources of propolis are poplar (*Populus*), birch (*Betula*), elm (*Ulmus*), alder (*Alnus*), beech (*Fagus*), conifers and the horse-chestnut (*Aesculus*). Botanical sources of propolis in the world are shown in table 1.1 (Burdock, 1998).

Table 1.1 Botanical sources of propolis in the world

Genus and species	Geographic location
Populus nigra, P. italica, P. tremula	Belgium
Populus nigra	Albania
Populus suaveolens	Mongolia
Populus euramericana, P. cuminata, P. acutifolia	America (mainland, Hawaiian islands)
Populus euramericana	England
Betula, Populus, Pinus, Prunus and Acacia spp. Aesculus hypocastane	Hungary
Betula, Alnus spp.	Poland
Delchampia spp., Clusia major	Tropical zone areas
Clusia spp.	Venezuela
Xanthorrhoea	Australia
Populus, Betula, Ulmus, Alnus, Fagus, Aesculus, conifers	North temperate zone

1.1.2 Studies on the history of the chemical composition of propolis

Studies on the composition of propolis began in Germany, when Kustenmacher (1911) identified cinnamic acid and cinnamon. Limited by the then available methods of analysis, few components of propolis were identified for a long time. After the 1950s', with the development of analytical methods such as TLC, HPLC and GC, more and more

components of propolis were identifed. Ghisalberti (1978) published the first review on propolis, which included more than 200 components of propolis, and more compounds are reported every year. The new components reported in recent years are shown in table 1.2.

Table 1.2 Recently identified components of propolis

Source of propolis	Composition of propolis	References
Brazil	3-caffeoylquinic acid; 4-caffeoylquinic acid; 5-caffeoylquinic acid; 3,5-dicaffeoylquinic acid; 3,4-dicaffeoylquinic acid; 4,5-dicaffeoylquinic acid methyl ester; 3,4-dicaffeoylquinic acid methyl ester; 3-[4-hydroxy-3-(3-oxobut-1-enyl)-phenyl]acrylic acid; (+)-treo-1-C-quayacylglycerol	Tatefuji *et al.*, 1996
	Flavonoid: 5,7,4'-trihydroxy-6,8-dimethoxyflavone; 5,6,7 –trihydroxy-3,4'–dihymethoxyflavone; aromadendrine-4'-methyl ether; 3,5,7-trihydroxy-6,4'-dimethoxyflavone **Acetophenonederivatives**: 2-[1-methyl]-vinyl-5-acetylcumarane; 2-[1-hydroxymethyl]-vinyl-6-acetyl-5-hydroxycumarane; 2-[1-acetoxymethly]-vinyl-6-acetyl-5-hydroxycumarane **Diterpeneic acid**: 8(17),13E-labdadien-15,19-dioic acid; 8(17),13E–labdadien-15,19-dioci acid-15-methyl ester; 19-oxo-8(17), 13E-labdadien-15-oic acid; 13-hydroxy-8(17),14-labdadien-19-oic acid	Bankova *et al.*, 1998

Brazil	**Prenylated p-coumaric acid:** 3,5-diprenyl-4-hydroxycinnamic acid; 3-prenyl-4-dihydro-cinnamoyloxycinnamic acid; 2,2-dimethyl -6–carboxyethenyl-2-2H-1-benzopyra; 9-E-2-dimethyl-6-carboxyethenyl-8-prenyl-2H-1-benzopyran; 3-prenyl-4-(2-methoxypropionyl)-cinnamic acid; (E)-3-[2,3-2H-2-(1-hydroxy-1-methylethyl)-prenyl-benzofuran-5-yl]-2-propenoic acid	Boudourova-Krasteva *et al.*, 1997
Sonoran Desert	**Flavonoid**: 5,7,4'-trihydroxy-6,8-dimethoxy flavone; sideritiflavone	Wollenweber & Buchmann, 1997
Tunisia	Myricetin 3,7,4',5'-tetramethyl ether; quercetin3,7,3'-trimethyl ether	
Chile	1-(4-hydroxy-3-methoxyphenyl)1,2-bis[4-[(E)-3-acetoxypropen-1-yl]-2 -methoxyphenoxy-]-propan-3-ol; 1-(4-hydroxy-3-methoxyphenyl)-2-[4-[(E)-3-acetoxypropen-1-yl]-2- methoxyphenoxy] propan-1,3-diol-3-acetate; 3-acetoxymethyl-5-[(E)-2-formylethen-1-yl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran	Valcic *et al.*, 1998
Canary Islands	Ent-17-hydroxy-3,13Z-clerodadien-15-oic acid; communic acid; 15-oxo-3,13Z-kolavadiene-17-oic acid and its E-isomer; imbricatoloic acid; isocupressic acid; acetylisocupressic acid	Bankova *et al.*, 1996, 1998
	Furofuran lignas: sesamin; aschantin; sesartenin; yangambin **Sesquiterpenes:** ledol; spatulenol; germacrene **Sugars and sugar alcohols:** melibiose; maltose; galactose; gluuronic acid; lactose; xylitol; mannose; erytritol; xylose; inositol	

1.1.3 Local differences in the chemical composition of propolis

The chemical composition of propolis is very complex and depends on the flora in the areas where it is collected. The composition of propolis in Europe, North America, South America, and Asia is different, and these are related to the local climate and botanical sources. The precise composition of raw propolis varies with the source. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances including organic debris. Propolis has been widely used in folk medicine, the food and chemical industries of Asia because of its extensive biological proper ties.

In the temperate zone, including Europe, Asia and North America, the composition of propolis samples originating from these regions are characterized by similar chemical composition, the main constituents being phenolics: flavonoid aglycones, aromatic acids and their esters. Samples originating from tropical areas are quite different.

Markham *et al.* (1996) identified in samples from New Zealand the unusual "poplar" phenolics, together with two new compounds: 5-phenyl-trans, trans-2, 4-penadienoic acid and 5-phenyl–trans-pentenoic acid. In a sample from Egypt, along with poplar bud constituents, esters of caffeic acid with long-chain fatty alcohols (dodecanol, tetradecanol, tetradecenol and hexadecanol) were identified (Christov *et al.,* 1998). Marcucci *et al.* (1998) identified triterpenes, β -amyrin and cycloartenol in samples from Egypt.

In South America, the chemical composition of propolis is significantly different from other continents. Propolis samples with new chemical components having important biological properties have been found in recent years especially in Brazil. The new chemical components that have been recently identified are shown in table 1.2.

1.2 The biological properties and toxic allergic reactions

1.2.1 Biological activity of propolis

Propolis has extensive biological activity and has anti-oxidative, anti-viral and anti-bacterial properties. It can also be used to scavenge free radicals, improve immunity, and ameliorate afflictions such as colds and sore throats, skin problems, stomach ulcers, burns, hemorrhoids, gum diseases, wounds, tumors and diabetes mellitus (Burdock, 1998).

1.2.1.1 Anti-tumor activity of propolis

There are some components of propolis, which have cytotoxic and chemopreventive effects including artepillin C, clerodane diterpenoids, propolis benzofuranes, and other yet to be defined methanol-, ethanol- and water extracts (Banskota *et al.*, 2000a). Specifically, caffeic acid phenethyl ester (CAPE) may be involved in restoring intercellular gap junction communication, induction of natural cell death (apoptosis), growth inhibition, and exerting a cytotoxic effect on malign tissues. CAPE can ameliorate inflammation caused by the tumour-promoter (12-o-tetradecanoylphorbol-13-aacetate), indicating that CAPE may be acting by interfering with the oxidative capacities of the cells rather than by being anti-oxidants (Jeng *et al.,* 2000).

Animal studies showed that CAPE, artepillin C, and the clerodane diterpenoid prevented cancers of the breast, skin, kidney, and the colon (Mitamura *et al.,* 1996; Kimoto *et al.,* 1998, 2000). In addition, studies have shown that a combination of propolis and a chemotherapeutic drug (blemoycin) showed no synergistic effects. On the contrary, propolis reduced the activity of bleomycin (Scheller *et al.*, 1989).

Ghazaly and Khayyal (1995) found that aqueous and ethanol extracts of propolis prevent radiation-induced damage during treatment by preventing inflammatory responses

such as edema. Velikov and Zanev (1989) showed that the application of ethanol-extracts of propolis minimized the probability of treatment delays due to side effects of radiation of the pharynx, and is thus believed to reduce the probability of metastasis and relapse (Velikov & Zanev, 1989). The origins and methods of preparation of propolis are very important. Kawabe *et al.* (2000) reported that propolis from Uruguay was found to be an inhibitor of mammary gland carcinogenesis in comparison to water-extracted propolis and water- and ethanol-extracted propolis from Brazil (Kawabe *et al.*, 2000).

The activation of all chemical carcinogens relies on cytochrome 450 in the body. Studies have shown that quercetin (one of the flavonoids) can inhibit the activity of cytochrome 450. Besides these, some flavonoids can inhibit the growth of tumor cells, antagonize carcinogens or modulate the enzyme system to inhibit chemical toxicity and carcinogenicity. The enzymes of flavonoids include hydrolases and alkaline phosphatase. Propolis has exhibited similar effects inhibiting glycosyltransferases of cariogenic *Streptococci*, myeloperoxidase activity of inflammation, ornithine decarboxylase, lipooxygenase, tyrosine protein kinase and arachidonic acid metabolism (Havsteen, 1983).

1.2.1.2 Anti-oxidative activity of propolis

Propolis has little effect on benign tissue and has a protective effect because of its anti-oxidative properties (Matsuno *et al.*, 1997). Some metabolic oxygen is converted to peroxides, superoxide anions and hydroxide free radicals during oxidation reactions with age and growth. The accumulation of the products of oxidation such as active oxygen and free radicals are positively associated with ageing. Besides these, the reaction of active oxygen and unsaturated fatty acids produce over-oxidation lipidemia and protein, and over-oxidation lipidemia is the main material that causes the atherosclerotic condition.

Lin *et al.* (1999) studied the protective function of water- and ethanol-extracts of propolis, and the experiments showed that liver cells (hepatocytes) were protected against

several toxic substances like ethanol, carbon tetrachloride, galactosamine and allyl alcohol in the presence of propolis extracts. Dicaffeoyl quinic acid derivatives from water-extracted propolis were found to be especially protective. Gonzalez *et al.* (1995) found propolis had the ability of protecting the liver because of the anti-oxidative effects during his study on the toxicity of paracetamol. However, the protective effects of propolis form different sources and by different extraction methods vary (Banskota *et al.,* 2000b). These effects include protection of pancreatic beta-cells against streptozotocin and the heart muscle against doxorubicin, which are very important in preventing diabetes mellitus and myocarditis caused by chemical treatments (Chopra *et al.,* 1995).

The cause of anti-oxidative activity of some components in propolis is due to the reaction between flavonoids and superoxide anions that inhibit the initiation of free radicals, binding heavy metal ions, or reacting with lipid peroxide to prevent the process of lipid peroxidation. Furthermore, propolis absorbed in the body obviates the need for Vitamin C and other hydrophilic oxidants (Fang *et al.,* 2000).

1.2.1.3 Anti-viral properties of propolis

There has been a long history of research on the anti-viral properties of propolis. Besides herpes virus, which can be inhibited by CAPE, other viruses, adenoviruses and influenza, are also inhibited by components of propolis (Kujumgiev *et al.,* 1999). Amoros *et al.* studied the effects of propolis on the viruses including HSV-1, HSV-2, adenoviruses-2, VSV and poliomyelitis-2, and found that propolis could inhibit the activity of enzymes such as H^+-ATP and phosphodiesterase and so influence phosphorylation of virus transfer genes, and inhibit synthesis of DNA and RNA (Luo, 1997). Propolis could also inhibit the replication of influenza A and influenza B, vaccinia virus, newcastle virus; furthermore, propolis could eliminate the enveloped viruses such as HSV and VSV. Some recent studies also indicated activity against the human immunodeficiency virus (HIV) (Harish *et al.*, 1997).

Although the components of propolis, flavonoids and CAPE, were from various sources, the anti-viral activity was common to all of them. Vynograd *et al.* (2000) studied the efficacy of ointments prepared from Canadian propolis versus acyclovir and a placebo in patients with confirmed genital herpes infection. The study reported a significantly faster and a higher percentage of healing in the propolis group within 10 days after the beginning of therapy (propolis 80%; 24/30 versus acyclovir 47%; 14/30 versus placebo 40; 12/30; $P = 0.0015$). In the propolis group, bacterial super-infections in the vagnia were eliminated in 55% of cases, while patients in the group of acyclovir and placebo showed no improvement effect.

1.2.1.4 Anti-bacterial activity of propolis

The anti-bacterial and anti-inflammatory properties of propolis and its positive influences on wound healing and in the treatment of infections contribute to the favorable properties of propolis, which have been reported in China since ancient times. Experiments showed that propolis not only had direct bacteriostatic and mycostatic effects on a variety of bacteria (especially to G^+) and fungi (*Candida albicans*), but also inhibited the adhesion of bacteria to the cell. Metesta tested the sensitivity of 75 bacterial strains, 69 strains belonged to S*taphylococcus* and S*treptococcus*. All the strains were sensitive to propolis extracts. The minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of propolis to *Staphylococcus aureus* respectively were 10 and 120 mg/kg (Liang & Gao, 1997). In contrast to its anti-viral properties, the activity of propolis against certain types of bacteria varies with its origin; likewise, propolis and anti-biotic (tuberculostatic) drugs are synergistic (Scheller *et al.*, 1999).

Ear infections of dogs (canine otitis externa) were successfully treated with a 70% propolis-castor oil or a propolis-glycerol mixture (Heinze *et al.*, 1996). Recent reports from Turkey investigated the effects of propolis extracts on wounds and infections of the eye in

rodents. These studies demonstrated that water extracts of propolis facilitate corneal epithelial wound healing and inhibit unfavorable neovascularization (Hepsen *et al.*, 1999). Ozturk *et al.* (1999) found that propolis had the same efficacy as corticosteroids (methylprednisolone) in endotoxin induced uveitis and arthritis.

The active anti-bacterial components of propolis are mainly flavonoids and aromatic acids and esters, and among these, the anti-bacterial activity of galangin, pinocebrin and pinobanksin are strongest. Besides these, benzyl ferulate and caffeic acid are also anti-bacterial components of propolis and flavonoids can inhibit the formation of free radicals (Burdock, 1998).

1.2.1.5 Other properties of propolis

Animal experiments showed that bee pollen and propolis as additive reagents can improve body weight, the utilization of iron ions and the regeneration of hemoglobin in animals (Haro, 2000). CAPE can prevent local ischemia caused by higher NO in injuries of the testis (Koltuksuz *et al.*, 2000); furthermore, propolis also has the activity of protecting spinal cord damage (Ilhan *et al.*, 1999). Przybylski and Scheller found that water-soluble propolis and ethanol extracts of propolis can improve femur gangrene by arthrotic injection (Przybylski & Scheller, 1985).

Mahmoud *et al.* found that propolis had significant effects on teeth allergy in a study on 26 Sudanese women, and propolis had significant anti-hyperalgesic effects, and could improve dental rigidity (Mahmoud *et al.*, 1999). In addition, ethanol extracts of propolis could act independently to release or activate the opioid system (De Campos *et al.*, 1998).

Propolis has no activity against eukaryotic parasites like *Entamoeba histolytica, Toxoplasma gonii*, *Trichomonas vaginalis* or *Trypanosoma cruzi in situ*, but in healthy volunteers it led to an enhancement of immune reactivity (Burdock, 1998).

1.2.2 Toxicity and allergic reactions to propolis

1.2.2.1 Toxicity of propolis

Because extraction methods for propolis have not been standardized, various published results on propolis toxicity are significantly different. Arvouet-Grand (1993) reported the oral LD_{50} of propolis extract in the mouse to be greater than 7340 mg/kg (Arvouet-Grand, 1993), while Hrytsenko (1997) reported the oral LD_{50} of 2050 mg/kg and an LD_{100} of 2750 mg/kg. Ghisalberti (1978) reported that cats tolerated subcutaneous administration of 100 mg/kg of an ether extract of propolis. Dobrowolski (1991) administered approximately 700 mg/kg orally to groups of 10 mice (five males and five females) and monitored them 48 h post-dose. They reported that the propolis preparations were well tolerated and no death occurred during the 48 h observation period. While the food analysis center of Japan reported that the oral LD_{50} of propolis from China and Brazil was 3600 mg/kg in acute toxicity experiments (Chen & Yan, 1999), Burdock (1998) found that none of his experimental animals died after administration of 200-5000 mg/kg/day. Despite the disparity in the reported toxicities, there is nevertheless, a rather low innate toxicity for propolis extracts. However, their use in humans should include a safety factor of 1000 considering the lack of chronic toxicity studies. A safe dose in humans would be 1.4 mg/kg body weight/day or approximately 70 mg/day (Karsten, 2001). Although reports of the toxicity of propolis are in disagreement, all the investigators consider propolis as a material on low toxicity, and a safe additional reagent in the food industry.

1.2.2.2 Allergic reactions to propolis

An allergic reaction to propolis may be caused by direct contact, but propolis taken orally would not cause an allergic reaction. Studies show that the allergic reaction of propolis is usually indirect, the main cause of allergic reactions for people are those with a

high sensitivity to the secretions of poplar (Burdock, 1998).

Use of propolis in cosmetics, tooth pastes and self-treatment for various diseases led to a steady increase in the cases of propolis allergy which, formerly, was more common among beekeepers. The main allergens in propolis include a mixture of 3-methyl-2-butenyl caffeate (54%), 3-methyl-3-butenyl caffeate (28%), 2-methyl-2-butenyl caffeate (4%), phenylethyl caffeate (8%), and caffeic acid (1%), benzyl caffeate (1%). Apart from allergic reactions, no side effects of propolis treatment have been reported. Miyataka has reported that propolis also contains an unknown, water soluble non-flavonoid with anti-allergic action (Karsten, 2001).

1.3 Anti-tumor mechanisms of propolis

In this chapter, advances on the analysis of the functional components and anti-tumor mechanisms of propolis are investigated to further develop base-line data on the pharmacological usefulness of propolis.

1.3.1 Functional components of propolis

Propolis has a wide spectrum of pharmacological activities, which largely derive from the functional components: flavonoids and other volatiles such as flavonoids, flavonols, flavanones and flavanonol. More than 60 flavonoid components have been identified to date. The common components in propolis are shown in table 1.3.

Table 1.3 The common chemical components of propolis frequently encountered

flavonoids	Cicheriin; 5-hydroxy-4',7-dihmethoxyflavone; tectochrysin
flavonols	galangin; galangin-3-methylether; galangin-6-methylether; kaempferitrin; kaempferol; kaempferol-4,7-dimethylethe; kaempferol-3,4-dimethyether; quercetin; quercetin-3,3-dimethyether; fagopyrol; apigenin; mulberrin; rhammetin; isorhammetin
flavanones	5-hydroxy-4',7-dimethoxyflavanone; 2,5-dihydroxy-7-methoxyflavanone
phenolic acid	benzoic aicd; hexbenzoic acid; vanillin; isovanillin; 3,5-dideoxy-phenylmethanol; cis-methylsulfone methanol; P-coumaric acid; caffeic acid; phenolic acid; isoferulic acid; 3,4-dimethylcinnamic acid; cinnamic acid; phenyl caffeic acid
coumarin	6,7-dihydroxycoumarine; 7-hydroxymethoxycoumarine
others	sorboside, eugenol; phenylethyl methanol; divinyl ether; nutmeg ester acid

1.3.2 Anti-tumor mechanisms of propolis

1.3.2.1 Prevention and inhibition of the production of carcinogens and potential carcinogens

As we know, nitrosamines and aflatoxins are highly carcinogenic (Tricker & Preussmann, 1991; Groopman & Kensler, 1993). The process of curing foods results in the production of nitrites, which yields nitrosamines, strong carcinogens, under the combined action of natural acids and bacteria in the stomach. However, substances like phenolic acid, Se, Vitamin C and β-carotene can interrupt the synthesis of nitrosamine. Furthermore, the flavonoids of propolis can induce benzpyrene hydroxylase to reduce the toxicity of aflatoxin. Pepeljnjak (1982) found that propolis extractions had a significant inhibitory

effect on ochratoxin A. Quercetin methyl ether and fagopyrol are the inhibitors of benzpyrene, a carcinogen associated with lung cancer. Siskin isoflavone, kaempferol and quercetin can denature DNA to po-isomerase.

Mahran (1996) studied the effects of propolis on liver damage caused by carbon tetrachloride (CTC), and found that water-soluble derivatives of propolis had a repair function: the main mechanism prevents lipid oxidation and modulates glutathione reductase. Basnet (1996) isolated two derivatives of quinate-2-cafferol diester: methyl 3,4-di-o-caffeoyl quinate and 3,4-di-o-caffeoyl quinic acid by chromatographic analysis, and experimentally demonstrated that that these two components had better effects in recovering from liver damage than liquorice. Sharma and Pillai (1998) found that propolis could prevent liver necrosis caused by paracetamol by adding 50 mg/kg to decrease the incidence of liver carcinoma.

1.3.2.2 Inhibition of cancer cells by propolis

Some flavonoids in propolis can inhibit the growth of tumor cells. In this context, the inhibitory activities of acacetin, mulberrin, and catecholamine were greater than other compounds. Kaempferol has a marked ability to inhibit cell proliferation; while nobiletin inhibits the multiplication of human pinacocyte tumor cells at the dose of 2-8 μg/ml. Caffetannic acid phenyl-2-ester and terpenes can also inhibit the multiplication of tumor cells by inhibiting DNA synthesis. Quercetin can lead to the death of cancer cells. Flavonoids can induce undifferentiated sarcomata to change to mature cells. CAPE can kill or inhibit DNA growth and synthesis of amoebocytes. In addition, the combined application of CAPE and MPA had a stronger inhibitory effect on cell proliferation than either substance used alone (Matsuno *et al.*, 1997). Mitamura *et al.* (1996) found during the course of experiments with mice that clerodane diterpenoids in methanol extractions of propolis could inhibit skin cancer induced by chemical materials by two mechanisms: one was to inhibit DNA synthesis at the beginning, the other was reduce DNA synthesis by a

remediation metabolism path, both of which inhibit the growth of tumor cells (Mitamura *et al.*, 1996). Kimoto *et al.* (1998) found that Artepillin C was an effective anti-bacterial, cytotoxic to malignant neoplasm, and significantly inhibited the growth of tumor cells through the induction of cell apoptosis. Artepillin C had inhibitory effects on solid tumors and leucocythemia in monocyte cytotoxicity experiments. Moreover, in experiments with mice it has clearly been demonstrated that Artepillin C terminates mitosis and induces transplantation tumors to atrophy. The mechanism of Artepillin C inhibiting tumor cells is associated with the prevention of a proliferation of tumor blood vessels cutting off the nutrition of tumor cells (Kimoto *et al.*, 1998). For growth and transfer, solid tumors rely on the formation of new blood vessels, which could deliver the necessary nutrients and oxygen supplied by new blood vessels; if the formation of new blood vessels were inhibited, the tumor cells would degenerate through lack of adequate nutrition.

1.3.2.3 Anti-mutational effects of propolis

Oxygen-derived free radicals (OFR) and lipid peroxidation (LPO) can lead to DNA damage and cross-linking thus initiating mutations in carcinoma. Superoxide dismutase (SOD) and flavonoids in propolis are the main effective components that scavenge free radicals and LPO in the body. Scheller and Wilczok (1990) studied the effects of ethanol extracts of propolis on free radicals by electron paramagnetic resonance and found that the flavonoids in propolis had strong inhibitory effects on the scavenging of free radicals such as 2,2-diphenyl-1-hydrazine. The component 3,4-dihydroxy-5-isopenene cinnamic acid in propolis was more effective in preventing oxidation of linoleic acid than butylhydroxytoluene. Kimoto and Kurimoto (1999) found that Artepillin C in propolis extracts could decrease renal damage caused by triacetic acid, nitrile iron, and prevent carcinomatous developments of the kidney and lung. Vachalkova (1995) found that quercetin, rhamneti and kaempferol etc. had significant inhibitory activity on mutants by polar spectrum analysis. Lin *et al.* (1999) studied the influence of ethanol extracts of propolis on glutathinone reductase and microsomal enzymes caused by chronic alcohol

toxicosis, and found that propolis, at the dose of 30mg/kg, could significantly inhibit the activity of cytochrome C reductase which directs the metabolism of cytochrome P-450, NADH II, so decreasing the production of glutathinone and hydroxy aniline to prevent lipid oxidation.

1.3.2.4 Anti-bacterial and anti-viral activity of propolis

Krochmal (1991) studied the anti-bacterial, anti-fungal, and anti-inflammatory effects of propolis and found that it had good inhibitory activity against bacteria and fungi located in the epidermis and mucosa. Serkedjieva (1992) found that prenyl ferulate strongly inhibited an influenza virus such as H_3N_2. Scheller *et al.* (1999) studied the combined effects of propolis and streptomycin, isoniazid, ethambutol hydrochloride and rifamycins to tubercle bacilli and found that propolis decreased the activity of tuberculostatics. Sato and Miyataka (1999) studied the effects of propolis on *Helicobacter pylori* which lead to gastric cancer and found that propolis could kill *Helicobacter pylori* and inhibit the activity of urease to accelerate the secretion of gastric fluids to reduce the emergence of stomach disease. Hegazi (2000) studied the components and anti-bacterial activity of propolis from Europe by GC and found that the main components of propolis were essentially the same, with trans-p-coumaric acid as the main component. The main chemical components of propolis from Germany were phenylethyl-trans-caffeate, benzyl ferulate and galangin. The main components of propolis from Austria were pinocembrin and coumaric acid, which effectively inhibited oidimycosis. The main components of propolis from France were mainly benzyl caffeate and pinocembrin, which have inhibitory activity against various bacteria, but the effect was no better than that of propolis from Germany and Austria.

1.3.2.5 The immunity modulation activity of propolis and changes in environmental factors

Although propolis has no anti-genic activity, it can be used as an immunity adjuvant to improve the production of anti-bodies, so as to increase the gamma globulin content of

total serum protein. In addition, propolis can also enhance the phagocytosis ability of white blood cells and macrophagocytes and improve the immune ability for specificity and non-specificity in the body. Ivanovska *et al.* (1995 a, b) carried out a series of studies on the immune effects of propolis *in vivo*, and found that cinnamic acid and caffeic acid effectively inhibited erythrocytorrhexis, so improving the immune system. In addition, the immune modulation mechanism on infections caused by Gram-negative bacteria was mainly via enhancing the activity of macrophagocytes. Water-soluble derivatives of propolis were better at improving immunity than other solutions of propolis. The immunity enhancement ability of propolis may well prevent leucocythemia and AIDS.

1.3.3 Exploitation prospects of propolis in health foods

With the developing ideas about life and health care, people now readily accept healthy foods with anti-carcinogenic properties. Propolis has been used since ancient times in China, and it is a natural product full of microelements, enzymes and flavonoids. In addition, it also can influence the synthesis of DNA and improve immunity. Because of its wide biological activity, propolis can be easily accepted by people. In addition, China is the largest of the beekeeping countries and propolis is very abundant so that the exploitation prospects of propolis are in an expanding phase.

1.4 The effects and mechanisms of propolis as an anti-diabetic preparation

Diabetes mellitus has long been known to man and modern medicine finds that it is an endocrine disease caused by the absolute or comparative absence of insulin in the body resulting in elevated blood glucose levels. The main symptoms are excessive drinking, production of urea, and intake of food, decreasing body weight, dizziness and fatigue, all of which further lead to a wide spectrum of other diseases. After cardiovascular diseases and tumors, diabetes mellitus is the third cause of disease-induced human mortality. It is reported that there are more than 120 million diabetes mellitus patients in the world, and there are

more than 30 million patients in China (Jiang, 1992). According to the forecast of WHO, there will have been a four-fold increase in the incidence of this disease by 2010, and the total number of of diabetes mellitus patients may reach about 240 million (King & Roglic, 1999).

1.4.1 Categories and pathogenesis of diabetes mellitus

(1) Type-1 diabetes mellitus: also called insulin-dependent diabetes (IDD), occurs in about 10% diabetes mellitus patients, most being children but it also occurs in patients more than 80-90 years old.

(2) Type-2 diabetes mellitus: also called non-insulin-dependent diabetes (NIDD), occurs in about 90% of diabetes mellitus patients, most of whom are more than 35 years old. There are evident family genetic tendencies to express the disease, and it has no association with the human leukocyte antigen (HLA), and there are neither insulin antibody cells nor insulin autoantibody in the serum.

(3) Pregnancy diabetes mellitus: this type occurs during pregnancy, and the main patients are elder or fat puerpera. The incidence of this disease is low.

Until now, the pathology and pathogenesis were not well known. Scientists only found some referenced pathogenesis such as autoimmune deficiency and genetic factors, virus infection, adiposity, ages and feeding customs. As far as we know, the pathogenesis of type-1 and type-2 diabetes mellitus are quite different (Rabinovitch, 1994; Hgoty, 1995; Terauchi *et al.*, 1997; UKPDS Group, 1998; Bloomgarder, 1999).

1.4.2 Effects and mechanisms of propolis on diabetes mellitus

1.4.2.1 Effect of propolis as an anti-viral preparation

There are some flavonoids and terpenes, which can accelerate glucose to synthesize liver hepatin and modulate blood glucose (Wang & Jia, 1998). Propolis could activate cells

to accelerate tissue regeneration, and repair insulin cells and tissue. Liu and Xu (2000) reported that the efficiency of propolis used to cure diabetes was 94%, and found that propolis could modulate endocrine function, accelerate blood glucose metabolism, and stimulate the secretion of insulin so as to decrease the blood glucose level. 3-o-methquercine could inhibit poliomyelitis, and quercine could decrease poliomyelitis. In addition, the effect of thistle extracts to inhibit pancreas inflammation has been established (Gao & Liu, 2000).

1.4.2.2 Effect on immuno-modulation of propolis

Propolis can strengthen immune systems and enhance immune activity. Propolis can not only improve the phagocytosis of macrophages to improve the quantity of antibodies, but also can improve the function of cell immunity and humoral immunity. In addition, it also can improve the whole immune system including thymus, spleen and other immune organs, which can maintain the homeostasis of immune function at an optimal level, and improve disease resistance and self-healing (Zhu & Lü, 1997; Orsi *et al.*, 2000; Murad *et al.*, 2002).

1.4.2.3 Anti-oxidative effects of propolis

A relationship has recently been established between the increase of free radicals and the increase of blood glucose, and the change of free radicals, lipid peroxidation and low-density lipoproteins participate in the progress of diabetes mellitus. Oxygen radicals would damage cells and decrease cell activity (Chen, 1998). Propolis is a natural anti-oxidative reagent. Propolis exhibits good anti-oxidative ability in very low concentrations and improves the activity of SOD of body. As a natural anti-oxidative reagent, propolis can scavenge redundant free radicals and reduce the production of lipofuscin and liposomes and enhance cell energy to modulate tissue to delay aging (Huang *et al.*, 2000).

Soto (1998) found that thistle could scavenge free radicals, stabilize cell membranes to protect insulin cells, decrease the level of malonaldehyde and increase the level of glutathione, as well as inhibiting the increase of blood glucose caused by alloxan.

1.4.2.4 Effects on cleaning blood of propolis

Propolis can enhance heart constrictions to facilitate breathing, modulate blood pressure, and blood lipid. Studies and clinical experiments on propolis proved that propolis could intenerate and decrease brittleness of blood vasculature, so as to smooth the blood stream and modulate cycles to prevent hardening of the blood vasculature thus avoiding arteriosclerosis and modulating blood cycle (Shi, 1998). Because hyperlipidemia and high cholesterol are often associated with diabetes mellitus, the effect of propolis on cleaning blood would aid in the cure of diabetes mellitus.

1.4.2.5 Effects of propolis on enzyme systems

A. Propolis can inhibit the activity of aldose reductase

Aldose reductase (AR) serves as a coenzyme for the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) and is the key restrictive enzyme in the process of sequestering alcohol. AR catalyse deoxidization reacts with hexose, which make glucose and lactose deoxidize to sorbol and galactol, and sorbol is oxidized to fructose under the effects of sorbol dehydrogenase, the second restrictive enzyme in the process of metabolizing alcohol, but it cannot oxidize galactol, so facilitating the metabolism of galactol causing galactosemia (Zuo & Yao, 1999). When the level of blood glucose is normal, AR is not activated, but in high concentrations of blood glucose, glucose is catalyzed to sorbol, but the activity of sorbol dehydrogenase does not increase with sorbol, and sorbol does not pass through cell membranes because of its polarity,

leading to an accumulation of sorbol (Song *et al.*, 1991a). Numerous experiments showed that chronic syndromes of diabetes mellitus such as diabetic retinopathy, diabetic peripheral neuropathy and diabetic nephropathy were all associated with the accumulation of sorbol (Chen, 1998).

Studies on natural sources of AR inhibitory action have made considerable progress and such compounds are mainly flavonoids, coumarin, and so on. Varma (1977) tested the inhibitory activity of AR in a series of studies with flavonoids and found that flavonoids, including quercetin and coumarin, were inhibitory to AR (Song & Han, 1994). Mao (1993) proved that flavonoids had inhibitory effects on AR, and the effect of quercetin was that the IC_{50} was 3.44×10^{-7} mol/L, nearly equal to that of Sorbinil which was the strongest inhibitory substance for AR.

B. Effect of propolis to inhibit α-glucosidase

Starch in food is digested by saliva and amylopsin to yield oligosaccharides, disaccharides, and trisaccharides. In the small intestine, all these kinds of saccharides are reduced to monosaccharides which are then absorbed. Normally, α-glucosidase exists in all the parts of the small intestine. The absorption of glucose is restrained in the upper areas of the small intestine after taking inhibitory α-glucosidase, and the absorption of glucose is centralized in the middle and lower areas of the small intestine, thus reducing the absorbing area and delaying absorption time, which is beneficial in decreasing postprandial blood sugar. Taking α-glucosidase as an inhibitory substance for a long time also could decrease fasting blood sugar, which probably is related with increasing insulin sensitivity.

There have been many studies on α-glucosidase inhibitory action since the 1970s' with notable achievements in natural medicines. A traditional natural medicine of Brazil used in curing diabetes mellitus significantly inhibited the activity of α-glucosidase (Bai & Cai, 1999). Further studies showed that the main components of decreasing blood glucose

were flavonols such as myricetin, 4'-methylmyricetin-α-glucosidase, quercetin and two other new flavonols of citrin-I and citrin-II. All these components could significantly inhibit hyperglycemia caused by alloxan. Studies *in vitro* also found that these components also significantly inhibit the activity of α-glucosidase to delay the absorption of carbohydrate and thus decreasing hyperglycemia (Yu & Li, 2000).

The main mechanisms of propolis on decreasing hyperglycemia were that the anti-oxidative activity of propolis, while not disrupting the stability of cell membranes, protects pancrease and modulating enzymes related to glucose metabolism affecting the absorption of glucose in the small intestine to delay hyperglycemia. In addition, propolis might also have a similar effect on insulin to modulate glucose metabolism and protect pancrease and inhibit the activity of AR and α-glucosidase to inhibit diabetes mellitus and syndromes.

1.5 The functional factors of propolis in the modulation of hyperlipidemia

In recent years, studies have found that propolis has significant effects on modulating hyperlipidemia and has been termed "scavenger of blood", which is related to many of the functional factors of hyperlipidemic modulation by propolis. There are more than ten modulating hyperlipidemic factors in modern clinical use, and among them factors such as flavonoids, unsaturated fatty acids and terpenes are found in propolis (Fang, 1998 a, b; Stefano & Francesco, 2002).

1.5.1 Flavonoids

Flavonoids have the ability to decrease blood lipid, scavenge free radicals and, enlarge coronary arterial blood vessels. There are more than 3000 kinds of flavonoids, the main components being flavones, flavonols, dihydroflavones, and so on. The contents of

flavonoids in different sources of propolis vary. The flavonoid content of propolis may exceed 12%, and is the principal basis of biological activity of propolis. More than 70 flavonoids have been extracted from propolis in the world and they are mainly flavanones, flavonols, and dihydroflavones and so on (Bankova *et al.*, 2000).

Flavonols are the main components responsible for a decrease in blood lipid. In recent years, more than 20 kinds of flavonols have been identified in propolis (Peng, 1996). The principle flavonols in propolis are fagopyrol, quercetin, kaempferol, iso-rhammetin and so on. All of these components can increase the elasticity of capillary vessels to avert cerebral hemorrhage. Quercetin, which is the main component of many Chinese traditional medicines, is abundant and has wide biological properties, such as enlarging blood vessels, decreasing blood lipid, inhibiting the collecting of platelets and so on (Banskota *et al.*, 2000 a, b). Quercetin has the effect of inhibiting platelet collecting induced by arachidonic acid, and inhibits endotheliocytes from releasing endothelin to decrease the strain on blood vessels thus preventing the formation of thrombi. Fagopyrol and kaempferol are also abundant in propolis (Fang, 1998b). Zhao (2000) showed that the content of fagopyrol, kaempferol, and rhammetin in ethanol extracted propolis from the Beijing area were respectively 0.19g/100g, 0.17g/100g and 3.89g/100g.

The other two large fractions of flavonoids in propolis are flavanones and dihydroflavonols. They can decrease blood lipid and strengthen capillary vessels and anti-inflammatory reactions. Compounds such as poplus and tectochrysin are the main components of propolis in temperate zones, and they have significant effects in decreasing blood lipid. Chrysin, caffeate, 4,6-dihydroxyl-hihydroflavone, and 3,5,7-trihydroxyl flavanol are also found widely in propolis and affect hyperlipidemia (Wu, 2000).

In addition, the activity of superoxide dismutase (SOD) decrease, which causes an imbalance of prostacyclin and thrombus A2 and the collecting of platelets as well as releasing 5-hydroxytryptamine and increasing the activity of thrombin, which would cause

damage to the endothelium, hyperplasia of smooth muscle cells to form foam cells and finally to form arteriosclerosis (AS) (Feher, 1987). Scheler's (1990) studies showed that flavonoids in propolis are the effective components in scavenging free radicals, and also alkyloxyly negative ions and decrease the production of peroxide. 3,4-hydroxyl in the B-ring of flavonoids is the key structure to scavenge free radicals, others such as hydroxyls act synergistically (Yu, 1986).

1.5.2 Polyunsaturated fatty acids (PUFA)

Studies and clinical experiments show that PUFA has significant effects on decreasing triglycerides, total cholesterol, and increasing the level of high density lipid cholesterol, decreasing blood viscosity and platelet collecting to reduce thrombosis production (Xie, 1998). PUFA is subdivided into n-3 and n-6 series, such as linolenic acid (18 : 3n-3) and linoleic acid (18 : 2n-6) and arachidonic acid (20 : 4n-6). The effects of linoleic acid, linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in PUFA on modulating hyperlipidemica are most significant (Hansen, 1994).

The mechanisms of PUFA decreasing total cholesterol (TC) and triglycerides (TG) are mainly to inhibit the synthesis of endogenous cholesterol and triglycerides. PUFA can significantly increase the level of high-density lipoprotein (HDL) by adding the activity of lecithin-cholesterol acyltransferase (LCAT) and LPL and inhibiting the activity of HEL, CAT and LPL. This would accelerate the production and maturation of HDL, and HEL would inhibit its decomposition (Liu, 1991). In addition, PUFA also has the ability to inhibit the collecting of platelets, which are related to n-3 PUFA, arachidonic acid and catalysis reactions caused by lipid oxidase, cyclooxygenase (COX), and prostate synthease (Wang, 1994).

Propolis has a low amount of PUFA, the main components of which include linoleic acid, linolenic acid and arachidonic acid and so on. Mao (1998) studied 10 kinds of fatty

acids in ethanol extracted propolis, and found that the content of PUFA in propolis was more than 57.9%, and mono-unsaturated fatty acids (MUFA) were 34.8%, PUFA, with two double bonds, was 23.4%, and saturated fatty acids was 37.63%, the proportion of saturated fatty acids (SFAS): MUFA: PUFA was 1: 0.93: 0.61. The ratio of unsaturated fatty acids (UFA) to SFAS more than 1 is the signal in natural food which has the biological properties to protect cardiovascular disease and decrease hyperlipemia (Sinclair, 1990).

1.5.3 Terpenes

Propolis contains substantial quantities of terpenes, which can reduce the effects of hyperlipidemia. The terpenes in propolis mainly include triterpenes which decrease cholesterol in the serum of rats by inhibiting cholesterol absorption in the intestine (Enomoto, 1977), and diterpenic acids such as 8(17), 13E-labdadien-15, 19-dioic acid 15-methyl ester, 19-oxo-8(17), 13E-labdadien-15-oic acid, 13-dydroxy-8(17), 14-labdadien-19-oic acid, and sesquiterpenes such as ledol, germacrene, spatulenol, isopatulenol and so on, which mainly act by inhibiting lipid synthetase such as acetyl coenzyme A carboxylase, citric acid lyase, acetyl coenzyme synthetase, and hydroxyl methylglutaryl coenzyme A reductase (Hall, 1980).

1.5.4 Amino acids

Some amino acids have the ability to decrease the level of blood lipid, the main mechanisms are by accelerating the excretion of cholesterol in the intestines, and combining with cholesterol to block the absorption of cholesterol, which affects patients with high cholesterol but has no effect on normal people. For example, thioproline can shorten the length of thrombi, decrease the wet weight and dry weight of thrombi, and decrease the conglutination of platelets, thus significantly inhibiting platelet collecting induced by adenosine diphosphate (ADP) in rabbits or rats. Thioproline is weakly

antagonistic to calcium, whereas thrombosis and platelet conglutination and depend on participation of calcium. Thus the mechanism of thioproline to inhibit AS was related with that of calcium antagonists (Hao *et al.*, 1990). Studies showed that glycine ramification could decrease the level of triglycerides. There are more than 20 amino acids in propolis, which can to some degree affect the modulation of blood lipid (Guo & Zhou, 2000).

1.5.5 Steroids

The components of steroids in propolis are mainly lanosterol, cholesterol, fucosterol, stigmasterol and β-dihydrofucosterol. The skeleton of steroids has free hydroxyls in positions $C_5 - C_6$ and $C_{22} - C_{23}$, which are necessary to decrease cholesterol. Steroids mainly inhibit the absorbability of cholesterol in the intestine, the main mechanisms are that steroids and cholesterol would synthesis infusibility material to deduce the absorbability of cholesterol, the activity is related with alkyl in C_{24}, Δ^{22} of steroids. Secondly, steroids can inhibit the metabolism of cholesterol, for example β-sitosterine inhibits the activity of hydroxymethylglutaryl coenzyme A, which is the key enzyme in the synthesis of cholesterol (Wang, 1983).

In summary, there are several lipid-modulating factors in propolis, but they do not exert unique effects alone but operate synergistically, so the mechanism of propolis modulating hyperlipidemiac is somewhat unique. In addition, the components in propolis are complex, and chemical components from different sources of propolis are significantly different (Bankova *et al.*, 2000), so quality control must be taken when propolis is used to modulate hyperlipidemia.

1.6 Research objectives

In this dissertation the results of experiments performed to determine the effects (1) propolis on diabetes mellitus in rats, (2) compare the anti-inflammatory effects of ethanol

and water extracts of propolis, (3) effects of propolis in the treatment of hyperlipidemia, (4) the effectiveness of propolis in the treatment of induced tumors, and (5) ethanol and water extracts of propolis in general are reported.

CHAPTER 2

2. EFFECTS OF PROPOLIS ON DIABETES MELLITUS IN RATS

2.1 Summary

The effects of ethanol (EEP) and water (WSD) extracts of propolis on diabetes mellitus in rats were analyzed. Both WSD and EEP decreased levels of fasting blood glucose levels (FBG), glycosylated haemoglobin (HBAlc), fructosamine (FRU), triglyceride (TG), total cholesterol (TC), creatinine (CREA), blood urea nitrogen (BUN), uric acid (UA), malodialdehyde (MDA), total protein (TP) and albumin (ALB), and decreased the ratio of kidney to body weight in rats with diabetes mellitus. The results indicate that both EEP and WSD improved metabolism of glucose, fat and protein and afforded renal protection for rats with diabetes mellitus.

2.2 Introduction

Recently, a few publications on the efficacy of propolis in the treatment of diabetes mellitus have appeared (Gao & Liu, 2000), but the literature reflects limitations including the absence of uniform and standard methods for the extraction of propolis. Likewise, the relative effects of propolis extracts obtained by different extraction methods on diabetes mellitus remain unknown. In this chapter, the effects of ethanol and water (EEP and WSD) extracts of propolis on diabetes mellitus induced in rats are assessed.

2.3 Materials and methods

2.3.1 Drugs and reagents

A bulk sample of propolis was obtained from honeybee colonies, *Apis mellifera ligustica*, in North China in 2001 and the main plant origin was poplar (*Populus sp.*). A 30 g sample of propolis was pulverized and extracted in water at 80°C for 12 hours (WSD). A similar sample was extracted in 80% ethanol (EEP). Alloxan was obtained from Sigma Company. Reagents for blood glucose, total cholesterol, triglycerides, creatinine, blood urea nitrogen, uric acid, total protein and albumin were obtained from Shanghai Fudan-Zhangjiang Bio-pharmaceutical Company, China. Reagents for malonaldehyde and glycosylated haematoglobin protein were obtained from Nanjing Jiancheng Biology Engineering Company, China.

2.3.2 Laboratory animals

Male rats (strain SD) 300 ± 20 g were provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of the Laboratory of Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. The rats were allowed a standard pellet diet and free access to water and maintained at 25°C under a 12/12 hr light/dark cycle. The animals were maintained and the experiments performed according to the principles of the Helsinki accord. The experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, Hangzhou.

2.3.3 Induction and treatment of diabetes mellitus

Diabetes mellitus was induced in rats by intravenous injection of alloxan (40 mg/kg) dissolved in physiological saline through the tail vena. Control rats were given intravenous injections of physiological saline. 7 days later, blood was taken from all rats at the tip of the tail and the serum centrifuged to measure blood glucose levels. 60 rats with a blood glucose concentration about 20 mmol/L were then randomly divided into 6 groups: a diabetes mellitus (model) control group, WSD1, WSD2, EEP1, EEP2 treated groups and an additional 10 rats as normal, healthy controls.

2.3.4 Methods of drug administration

WSD groups: the concentration of dry propolis material in the WSD solution was 15mg/ml and the WSD1 solution was prepared by diluting the stock solution 5 times and the WSD2 solution diluted 10 times. Rats in the WSD1 and WSD2 groups were injected intragastrically with WSD1 and WSD2 solutions (1 ml/100 g) respectively and treated twice (09:00 and 15:00) daily continuously for 4 weeks.

EEP groups: the concentration of dry propolis material in the EEP solution was 100 mg/ml and the EEP1 solution was prepared by a 25-fold dilution of EEP and the EEP2 solution diluted 50 times. Rats in the EEP1 and EEP2 groups were injected intragastrically with EEP1 and WSD2 (1 ml/100 g) respectively and treated twice daily (09:00 and 13:00) continuously for 4 weeks.

Positive control group: acarbose was diluted in physiological saline (1 mg/ml). Rats in the positive control group were injected intragastrically with the acarbose solution (1 ml/100 g) and treated twice daily (09:00 and 15:00) continuously for 4 weeks.

Rats in the normal control group and diabetes mellitus model group were injected intragastrically with 0.9% physiological saline (1 ml/100 g) and treated twice daily (09:00 and 15:00) continuously for 4 weeks.

2.3.5 Methods of measurement

Blood was taken weekly from all rats at the cut tip of the tail, and the serum centrifuged at 3000 rpm/min to test blood glucose. At the end of the experiment, blood was taken from the eye for measurement of the other biochemical indexes.

2.3.6 Data analyses

Results are presented as means ± standard deviations. The statistical analyses were performed by using ANOVA procedures and Tukey and Fisher's LSD multiple comparison tests with $P < 0.05$ considered as significant.

2.4 Results

2.4.1 Effect of propolis on blood glucose in diabetes mellitus

The effects of propolis on blood glucose levels in rats with diabetes mellitus SD rats are shown in table 2.1. The experiment lasted 5 weeks. The first week involved the induction and treatment of diabetes mellitus and the subsequent 4 weeks involved drug administration. The results of table 2.1 show that blood glucose concentration in the diabetes mellitus model group increased significantly with time ($F_{1,48} = 45.86$, $P < 0.0001$), whereas the increases in blood glucose concentration in the experimental groups were slower than that of diabetes mellitus model group (WSD1: $F_{1,48} = 15.61$, $P < 0.001$; WSD2: $F_{1,48} = 20.64$, $P < 0.0001$; EEP1: $F_{1,48} = 27.70$, $P < 0.0001$; EEP2: $F_{1,48} = 32.58$, $P < 0.0001$; Positive group: $F_{1,48} = 11.60$, $P < 0.01$). The blood glucose levels of rats in each of the groups of WSD1, WSD2, EEP1, EEP2 and the positive control group respectively were on average 15.3% (LSD: $P < 0.05$), 13.2%, 10.4%, 17.1% (LSD: $P < 0.05$) and 6.2% less than that of diabetes mellitus model group at the first week of drug administration.

By the second week of drug treatment, blood glucose levels in each group were not significantly different compared with that of diabetes mellitus model group. The blood glucose levels of rats in each of groups WSD1, WSD2, EEP1, EEP2, and the positive control group respectively were 8.8%, 4.9%, 20.2% (LSD: $P < 0.05$), 4.8% and 12.9% less than that of diabetes mellitus model group at the third week of drug treatment. During the last week, the blood glucose levels of the WSD1, WSD2, EEP1, EEP2, and positive control groups were respectively on average 16.0% (LSD: $P < 0.05$), 16.1% (LSD: $P < 0.05$), 12.0%, 17.2% (LSD: $P < 0.05$), and 16.7% (LSD: $P < 0.05$) less than that of diabetes mellitus model group. It is apparent that after four weeks of propolis treatment that blood glucose levels significantly increased in rats with diabetes mellitus ($F_{4,216} = 50.0$, $P < 0.0001$).

Table 2.1 Changes in blood glucose (mmol/L) in normal rats and those with diabetes mellitus over five weeks ($\bar{x}$ ±sd)

Group	n	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Normal control	10	2.30±0.66**	0.77±0.41**	1.02±1.26**	0.78±0.57**	2.19±0.76**
Model control	10	21.38±3.18	24.72±3.54	23.32±2.55	31.17±6.89	32.66±2.17
WSD1	10	21.86±3.15	20.94±3.92*	21.98±1.97	28.43±6.12	27.43±5.95*
WSD2	10	20.90±2.44	21.47±4.58	22.79±2.54	29.63±7.11	27.41±3.93*
EEP1	10	20.57±2.84	22.14±2.05	23.12±2.83	24.86±3.47*	28.74±5.87
EEP2	10	19.45±3.52	20.49±4.81*	23.90±4.03	29.68±3.43	27.04±4.12*
Positive control	10	21.04±2.68	23.19±4.23	23.30±2.88	27.15±7.01	27.20±6.02*

LSD: *$P<0.05$, ANOVA: ** $P < 0.01$ compared with diabetes mellitus model control group.

2.4.2 Effects of propolis on the levels of glycosylated haematoglobin (HbAlc), triglycerol (TG), total cholesterol (TC), total protein (TP), and albumin (ALB) in rats with diabetes mellitus

The effects of propolis on the levels of HbAlc, TG, TC, TP, ALB in rats with diabetes mellitus are given in table 2.2. The results show that glycosylated haematoglobin protein in the EEP2 and WSD2 groups decreased by 13.3% (LSD: $P < 0.05$) and 12.0% (LSD: $P < 0.05$) respectively compared with that of diabetes mellitus model; the WSD1, EEP1, and positive control groups showed no significant differences compared with the diabetes mellitus model group. TG in the WSD2, EEP1, EEP2 groups decreased respectively by 47.7% (LSD: $P < 0.05$), 51.0% (LSD: $P < 0.05$), 51.9% (Tukey: $P < 0.05$) compared with that of the diabetes mellitus model group.

In the WSD1 and positive control groups there was no significant difference compared with diabetes mellitus model group. TC in the EEP1 and EEP2 groups decreased by 18.5% (LSD: $P < 0.05$) and 17.6% (LSD: $P < 0.05$) respectively compared with that of diabetes mellitus model group. The WSD1, WSD2 and positive control groups did not significantly differ from the diabetes mellitus model group. TP in the WSD1, WSD2, EEP1, EEP2, positive control groups increased 9.1%, 5.5%, 9.1%, 11.3% and 18.0% (LSD: $P < 0.05$) compared with that of diabetes mellitus model group. ALB in WSD1, WSD2, EEP1, EEP2, and positive control groups increased 9.1%, 0.2%, 3.9%, 3.4%, 3.2% respectively compared with that of diabetes mellitus model group.

Table 2.2 Effects of propolis on the levels of HbAlc, TG, TC, TP, ALB in rats with diabetes mellitus ($\bar{x}$ ±sd)

Group	n	HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
Normal control	10	0.050±0.012**	0.64±0.17*	1.27±0.34	66.7±8.5**	30.1±3.5**
Model control	10	0.075±0.005	3.97±2.14	1.48±0.25	49.4±9.1	23.2±3.6
WSD1	10	0.069±0.011	3.40±1.90	1.54±0.37	53.9±5.4	25.3±2.4
WSD2	10	0.066±0.010*	2.07±0.65*	1.26±0.22	52.1±9.9	23.3±4.0
EEP1	10	0.070±0.011	1.94±0.96*	1.21±0.20	53.9±8.9	24.1±4.0
EEP2	10	0.065±0.011*	1.91±0.75*	1.22±0.21	55.0±7.2	24.0±1.3
Positive control	10	0.072±0.008	3.83±2.11	1.57±0.31	58.3±5.1*	23.5±3.9
ANOVA		$F_{6,63}$ = 6.93**	$F_{6,63}$ = 7.22**	$F_{6,63}$ = 3.25**	$F_{6,63}$ = 4.99**	$F_{6,63}$ = 5.29**

LSD: *$P < 0.05$, ANOVA: ** $P < 0.01$ compared with diabetes mellitus model control group.

2.4.3 Effects of propolis on the levels of fructosamine (FRU), creatinine (CREA), blood urea nitrogen (BUN), uric acid (UA), and malonaldehyde (MDA) in rats with diabetes mellitus

The effects of propolis on the levels of FRU, CREA, BUN, UA, MDA in rats with diabetes mellitus are shown in table 2.3. The results show that FRU in WSD1, WSD2, EEP1, EEP2 and positive control groups were 6.78%, 10.50% (LSD: $P < 0.05$), 12.42% (LSD: $P < 0.05$), 9.78% (LSD: $P < 0.05$), 6.70% less than that of diabetes mellitus model group. CREA in EEP2 decreased 7.8% (LSD: $P < 0.05$) compared with that of diabetes mellitus model. The WSD1, WSD2, EEP1 and positive control groups did not significantly differ from the diabetes mellitus model group. BUN in WSD1 was 21.2% (LSD: $P < 0.05$) less than that of diabetes mellitus model group while there was no significant difference between the WSD2, EEP1, EEP2, positive control groups and the diabetes mellitus model group. UA in WSD1, WSD2, EEP1, EEP2 were 5.8%, 13.0% (LSD: $P < 0.05$), 15.5%

(LSD: $P < 0.05$), 7.1% less than that of diabetes mellitus model group. MDA in the WSD1, WSD2, EEP1, EEP2 and positive control groups respectively were 12.8%, 19.9% (LSD: $P < 0.05$), 9.1%, 22.1% (LSD: $P < 0.05$) and 12.2% less than that of diabetes mellitus model group.

Table 2.3 Effects of propolis on the levels of FRU, CREA, BUN, UA, MDA in rats with diabetes mellitus ($\bar{x}$ ±sd)

Group	n	FRU (μmol/L)	CREA (μmol/L)	BUN (mmol/L)	UA (μmol/L)	MDA (nmol/L)
Normal control	10	73.36±5.27**	60.43±5.93	5.64±0.39**	159.42±19.92**	9.3±0.9**
Model control	10	104.79±13.72	67.16±5.61	19.87±6.36	238.06±50.76	18.1±5.2
WSD1	10	96.67±8.41	65.57±2.77	15.67±2.20*	224.75±39.73	15.8±2.3
WSD2	10	93.78±15.38	67.42±5.46	17.39±5.14	207.92±9.97*	14.5±1.6*
EEP1	10	91.76±10.72*	66.67±7.07	19.33±4.29	201.67±32.21*	16.4±5.5
EEP2	10	94.54±6.64*	60.43±4.74*	18.11±2.17	221.60±26.24	14.1±3.1*
Positive control	10	97.46±7.741	66.25±7.16	17.09±3.61	224.70±25.73	15. 9±4.4
ANOVA		$F_{6,63} = 8.84$**	$F_{6,63} = 2.59$*	$F_{6,63} = 15.23$**	$F_{6,63} = 6.59$**	$F_{6,63} = 5.74$**

LSD: *$P < 0.05$, ANOVA: ** $P < 0.01$ compared with diabetes mellitus model control group.

2.4.4 Effects of propolis on the level of kidney weight/body weight in rats with diabetes mellitus

The effects of propolis on the levels of kidney weight/body weight in rats with diabetes mellitus are shown in table 2.4. The results show that kidney weight/body weights in the WSD1, WSD2, EEP1, EEP2 and positive control groups were lower, but not significantly so, than that of diabetes mellitus model group.

Table 2.4 Effects of propolis on the levels of kidney weight/body weight in rats with diabetes mellitus

Group	n	Body weight (g)	Kidney weight (g)	Kidney weight/body weight ×100
Normal control	10	335.0±18.4**	1.25±0.08	3.73±0.21**
Model control	10	202.3±28.4	1.30±0.16	6.47±0.52
WSD1	10	228.8±22.3	1.39±0.18	6.11±0.76
WSD2	10	241.3±43.2*	1.47±0.21*	6.23±1.21
EEP1	10	221.7±18.8	1.35±0.14	6.12±0.62
EEP2	10	220.5±36.0	1.33±0.19	6.14±0.11
Positive control	10	232.1±33.4*	1.43±0.13	6.21±0.49

LSD: *$P < 0.05$, ANOVA: ** $P < 0.01$ compared with diabetes mellitus model control group.

2.5 Discussion

2.5.1 Effects of propolis on blood glucose

During their 15-year study, The United Kingdom Prospective Diabetes Study (UKPDS) group demonstrated that type-2 diabetes mellitus was an acute and progressive disease. They found that the levels of glycosylated haematoglobin protein and fasting blood glucose levels persistently increased, which demonstrated that β cells of the pancreas decayed with time. Similarly, the UKPDS study also demonstrated that in patients with type-2 diabetes mellitus, it was very important to improve blood glucose levels, which could decrease the danger of diabetes mellitus syndrome (UKPDS Group, 1998). Moreover, propolis enhances cell activity, accelerating tissue regeneration and ultimately the repair of damage to the pancreatic cell system (Burdock, 1998), further lessening the effects of diabetes mellitus.

Considering the results of analyses of fructosamine, glycosylated haematoglobin protein and fasting blood glucose, it is evident that the concentration of fructosamine and glycosylated haematoglobin protein among the different propolis-treated groups was significantly lower than that of diabetes mellitus model, indicating that propolis effectively controls blood glucose levels. In addition, the varying concentrations of fasting glucose levels observed weekly demonstrate that propolis is associated with a decrease in rising blood glucose levels, and the effect was enhanced with time. Fructosamine levels derive as a by-product of the reaction between glucose and albumin, which occurs in serum with keto-amine. The concentrations of fructosamine in serum were relatively steady and were not affected by diet. Fructosamine levels very likely reflect the changes in blood glucose over several weeks just as occurred in the case of glycosylated haematoglobin protein (Grey *et al.*, 1995). Against this, levels of fasting blood glucose reflected instantaneous blood glucose concentration.

2.5.2 Effects of propolis on blood lipid

Diabetes mellitus is often associated with blood lipid abnormalities, mainly the levels of TC which are associated with increases in high blood pressure, atherosclerotic and coronary heart disease. A relationship between blood lipid abnormalities and atherosclerotic conditions has been previously predicted (Hu *et al.*, 2001).

Propolis could well increase the contractibility of heart, deepen breath and modulate blood pressure, purify blood, as well as modulate blood lipid. Much experimental data supports the interpretation that propolis modulates high blood lipid, high total cholesterol, high blood viscosity and also functions as a prophylactic against atherosclerosis as well as enhancing blood circulation in the heart and blood vessels in the brain. Because patients with diabetes mellitus often exhibit high levels of blood lipid and total cholesterol, the activity of blood vessel scavengers would be conducive to reducing the severity of diabetes mellitus (Havsteen, 2002).

By testing TC and TG in this experiment, we found that the levels of diabetes mellitus in rats after treatment with propolis were lower compared to those of the diabetes mellitus model. Moreover, EEP had a greater effect than did WSD. So it is inferred that propolis modulates the metabolism of lipid thus reducing the syndrome associated with blood lipid abnormalities.

2.5.3 Effect of propolis on renal function

Another acute syndrome of diabetes mellitus is diabetes mellitus nephropathy, the obvious symptoms of which are increases of creatinine, blood urea nitrogen and uric acid concentrations in the serum, and increased kidney weight/body weight (UKPDS Group, 1998). From the results of the experiment, it was found that the diabetes mellitus SD rats treated with propolis, the serum levels of creatinine, blood urea nitrogen, uric acid and kidney weight/body weight were lower than that of diabetes mellitus SD rats. Based on these results, it appears that propolis could protect the kidney in diabetes mellitus.

2.5.4 Effects of propolis on protein metabolism

Diabetes mellitus causes abnormalities of protein metabolism in addition to increases in blood glucose and blood lipid levels. The major symptom is the greater consumption of total protein and albumin (UKPDS Group, 1998). From the results of this experiment it is evident that propolis decreased the consumption of total protein and albumin leading to the inference that propolis modulates the metabolism of protein.

2.5.5 Effects of propolis on lipid peroxidation

That there is a relationship between the increase of free radicals and an increase in blood glucose, as well as changes of free radicals and lipid peroxidation under low density, lipoprotein conditions driving the progress of diabetes mellitus is well established. Oxygen

radicals would otherwise lead to cell damage, while MDA, as a kind of lipid peroxidation mechanism, would reflect the degree of oxidation in the body (Chen, 1998; Hilmi & Serdar, 1999; Muruganandan *et al.*, 2002). Whereas propolis is a natural anti-oxidant, at low concentrations, it leads to increases in the activity of SOD thus reducing the output of lipid peroxidation and protecting the body. The results of the present experiments show that the level of MDA decreased after treatment with propolis, which is evidence that propolis decreases the products of lipid peroxidation.

CHAPTER 3

3. EFFECTS OF PROPOLIS ON BLOOD GLUCOSE, BLOOD LIPID AND FREE RADICALS IN RATS WITH DIABETES MELLITUS

3.1 Summary

The effects of ethanol (EEP) and water (WSD) extracts of propolis collected from North China on blood glucose, blood lipid and free radicals in rats with diabetes mellitus were studied. The results show that EEP and WSD led to decreased levels of blood glucose (FBG), fructosamine (FRU), malonaldehyde (MDA), nitric oxide (NO), nitric oxide synthetase (NOS), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), very-low density lipoprotein cholesterol (VLDL-C) in the serum of fasting rats; and to increased serum levels of high-density lipoprotein cholesterol (HDL-C) and superoxide dismutase (SOD). This suggests that propolis can control blood glucose and modulate the metabolism of glucose and blood lipid, leading to decreased outputs of lipid peroxidation and scavenge free radicals in rats with diabetes mellitus.

3.2 Introduction

Recent articles on propolis credit it with curing diabetes mellitus (Stefano and Francesco, 2002), but detailed studies are few and no uniform criteria for the extraction and preparation of propolis solutions exist nor do standard methods for the preparation of propolis in the treatment of diabetes mellitus in rats. The effects of propolis on blood glucose, blood lipid and free radicals in rats with diabetes mellitus were studied and are reported here.

3.3 Materials and methods

3.3.1 Drugs and reagents

Propolis was obtained from colonies of honeybees, *Apis mellifera* L., in North China in 2001 and the main plant origin was poplar (*Populus sp.*). Water soluble derivatives (WSD) of pulverized propolis were obtained by extraction of a 30 g sample at 80°C for 12 hours. Similar samples of propolis were also extracted in 80% ethanol. Alloxan was obtained from the Sigma Chemical Company. Blood glucose reagents were obtained from the Shanghai Bio-product Research Institute of the Ministry of Health, P. R. China. Fructosamine reagent was obtained from Shanghai Fu-dan- zhangjiang Bio-pharmaceutical Company Ltd. Shanghai, China. Reagents for total cholesterol, triglycerides, low-density lipo-protein cholesterol, high-density lipo-protein cholesterol were obtained from Cicheng Biochemistry Reagent Company, Ningbo, China. Reagents for superoxide dismutase, malonaldehyde, nitric oxide and nitric synthetase were obtained from Jiancheng Biology Engineering Research Institute, Nanjing, China.

3.3.2 Laboratory animals

Male rats (strain SD) of about 350 ± 30 g were provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of the Laboratory of Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. The rats were allowed a standard pellet diet and free access to water and maintained at 25°C under a 12/12 hr light /dark cycle. The animals were maintained in keeping with the Helsinki accord and the experimental protocol was approved by the Animal Ethics Committee of Zhejiang University.

3.3.3 Induction and treatment of diabetes mellitus

Diabetes mellitus was induced in rats by the intravenous injection of alloxan (40 mg/kg) dissolved in physiological saline through the tail vena. Normal control rats were

given only physiological saline intravenously. Seven days later, blood was taken from the cut tip of the tail and the serum centrifuged to test for blood glucose. 72 rats with a blood glucose concentration of about 20 mmol/L were randomly divided into 6 groups including a diabetes mellitus (model) control group and WSD1, WSD2, EEP1, EEP2 groups and a positive control group. An additional 12 rats were used as the normal control group.

3.3.4 Method of drug administration

WSD group: the concentration of dry material of WSD was about 15 mg/ml, and the WSD1 solution was prepared by a three-fold dilution of WSD; the WSD2 solution was prepared by a six-fold dilution of WSD. Rats in the WSD1 and WSD2 groups were each given intragastrically the WSD1 and WSD2 solutions at a rate of 1 ml/100 g respectively.

EEP group: the concentration of dry material of EEP was 100 mg/ml, and the EEP1 solution was prepared by a 20-fold dilution of EEP; the EEP2 solution was prepared by a 40-fold dilution of EEP. Rats in EEP1 and EEP2 groups were each given intragastrically the EEP1 and WSD2 solutions at the rate of 1 ml/100 g respectively.

Positive control group: acarbose was diluted in physiological saline to a concentration of 1mg/ml. Rats in the positive control group were given acarbose solution intragastrically at a rate of 1 ml/100 g.

The normal control and the model groups were given 0.9% physiological saline (1 ml/100 g) intragastrically and treated twice.

All rats were injected intragastrically twice daily (09:00 and 15:00) continuously for 7 weeks during the course of the eight week long experiment.

3.3.5 Methods of measurement

Blood was collected from the cut tip of the tail of all rats each week and the serum centrifuged at 3000 rpm/min to test blood glucose. At the end of the experiment, blood was collected from the eyes of the rats to test other biochemical indexes. The test methods were

done according to the reagent protocols prepared by the manufacturing firms.

3.3.6 Data analyses

Results are presented as means ± standard deviations. Statistical analyses were performed using regression and ANOVA procedures and Tukey multiple pairwise comparison tests with a probability level of $P < 0.05$ being considered as significant.

3.4 Results

3.4.1 The effects of propolis on blood glucose in rats with diabetes mellitus

The experiment lasted 8 weeks: the first week involved the induction of diabetes mellitus and the remaining 7 weeks the period of drug administration. The effects of propolis solutions on blood glucose in rats with diabetes mellitus are given in table 3.1. The results show that blood glucose concentration in the diabetes mellitus model group increased with time ($F_{1,94} = 29.77$, $P < 0.0001$), while the small changes in the rats with diabetes mellitus treated with propolis were not significant (WSD1: $F_{1,94} = 2.08$, $P = 0.1524$; WSD2: $F_{1,94} = 0.05$, $P = 0.8285$; EEP1: $F_{1,94} = 0.84$, $P = 0.3619$; EEP2: $F_{1,94} = 2.33$, $P = 0.1299$; Positive group: $F_{1,94} = 0.96$, $P = 0.3296$). Blood glucose levels in rats with diabetes mellitus of the different treatment groups showed no significant differences at the end of the first week of drug administration ($P > 0.05$).

The blood glucose levels of rats in WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 22.2% ($P < 0.05$), 17.9%, 15.1%, 4.3% and 16.7% respectively compared with that of diabetes mellitus model at the second week of drug administration. The blood glucose levels in the WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 22.2% ($P < 0.01$), 28.0% ($P < 0.01$), 20.8% ($P < 0.01$), 2.6% and 14.0% ($P < 0.01$) respectively compared with that of diabetes mellitus model group by the end of the third week of drug administration. The blood glucose levels in the WSD1, WSD2, EEP1, EEP2, and positive control groups decreased 18.2% ($P < 0.05$), 12.7%, 6.8%, 10.6% and

14.8% ($P < 0.05$) respectively compared with that of diabetes mellitus model group by the end of the fourth week of drug administration.

The blood glucose levels in the WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 25.7% ($P < 0.01$), 13.6%, 18.4% ($P < 0.05$), 19.0% ($P < 0.05$) and 24.7% ($P < 0.01$) respectively compared with that of diabetes mellitus model group by the end of the fifth week of drug administration. The blood glucose levels in the WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 34.8% ($P < 0.01$), 26.4% ($P < 0.01$), 22.6% ($P < 0.01$), 11.4% and 18.4% ($P < 0.01$) respectively compared with that of diabetes mellitus model group by the sixth week of drug administration. The blood glucose levels in the WSD1, WSD2, EEP1, EEP2, and positive control groups decreased 29.5% ($P < 0.01$), 25.9% ($P < 0.01$), 20.4% ($P < 0.01$), 18.2% ($P < 0.05$) and 22.9% ($P < 0.01$) respectively compared with that of diabetes mellitus model group by the seventh week of drug administration (table 3.1).

3.4.2 The effects of propolis on blood lipid in rats with diabetes mellitus

The effects of propolis on blood lipid in rats with diabetes mellitus are given in table 3.2. The results show that TC in the WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 18.4% ($P < 0.01$), 20.1% ($P < 0.01$), 15.8% ($P < 0.05$), 7.7% and 19.2 ($P < 0.01$) compared with that of diabetes mellitus model group. The increases of HDL-C in the WSD1, WSD2, EEP1, EEP2 and positive control groups (6.2%, 11.1%, 3.7%, 13.6% and 16.0%) compared with that of diabetes mellitus model are not significant ($P > 0.05$). LDL-C in the WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 42.7% ($P < 0.01$), 46.3% ($P < 0.01$), 58.5% ($P < 0.01$), 52.4% ($P < 0.01$) and 53.7% ($P < 0.01$) respectively compared with that of diabetes mellitus model group. VLDL-C in the WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 34.9% ($P < 0.05$), 36.8% ($P < 0.01$), 25.0% ($P > 0.05$), 28.9% ($P > 0.05$) and 44.1% ($P < 0.01$) respectively compared with that of diabetes mellitus model group. TG in the WSD1, WSD2, EEP1, EEP2 and positive control groups decreased 10.7%, 23.7%, 27.5%, 38.2% ($P < 0.01$) and 30.5% ($P < 0.05$) respectively compared with that of diabetes mellitus model group.

Table 3.1 Changes in blood glucose in rats with induced diabetes mellitus and normal rats for eight weeks ($\bar{x} \pm$ sd)

Group	n	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
Normal control	12	3.80±0.90**	4.32±0.56**	4.46±0.56**	3.85±0.50**	3.43±0.41**	4.51±0.62**	4.91±0.38**	5.15±0.80**
Model control	12	21.91±2.00	22.69±4.72	22.45±5.87	22.54±1.77	23.49±2.50	25.36±3.48	26.99±1.72	27.57±3.60
WSD1	12	21.94±2.01	19.64±3.63	17.46±2.97*	17.54±2.61**	19.22±4.88	18.84±3.43**	17.60±5.41**	19.44±5.31**
WSD2	12	21.93±2.20	20.12±2.41	18.43±2.46	16.22±1.55**	20.50±0.95*	21.92±3.81	19.87±4.51**	20.43±6.28**
EEP1	12	21.90±1.20	19.72±4.93	19.06±3.19	17.84±1.85**	21.90±2.52	20.70±5.58*	20.91±6.90**	21.95±5.15**
EEP2	12	22.06±1.82	19.10±3.29	21.47±1.45	21.96±1.23	21.01±5.45	20.54±5.47*	23.92±6.40	22.55±4.03*
Positive control	12	21.95±1.85	19.04±3.57	18.70±4.24	19.39±2.61*	20.02±3.32*	19.09±2.07**	22.04±3.24**	21.26±3.25**

* $P < 0.05$, ** $P < 0.01$ compared with the diabetes mellitus model control group.

Table 3.2 Effects of propolis on the level of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), triglycerol (TG) in rats with induced diabetes mellitus ($\bar{x}$ ± sd)

Group	n	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	VLDL-C (mmol/L)	TG (mmol/L)
Normal control	12	$2.01 \pm 0.19^{*}$	0.91 ± 0.07	$0.49 \pm 0.22^{*}$	$0.49 \pm 0.30^{**}$	1.24 ± 0.32
Model control	12	2.34 ± 0.44	0.81 ± 0.09	0.82 ± 0.39	1.52 ± 0.24	1.31 ± 0.35
WSD1	12	$1.91 \pm 0.23^{**}$	0.86 ± 0.14	$0.47 \pm 0.29^{**}$	$0.99 \pm 0.33^{*}$	1.17 ± 0.28
WSD2	12	$1.87 \pm 0.23^{**}$	0.90 ± 0.13	$0.44 \pm 0.12^{**}$	$0.96 \pm 0.35^{**}$	1.00 ± 0.30
EEP1	12	$1.97 \pm 0.28^{*}$	0.84 ± 0.23	$0.34 \pm 0.16^{**}$	1.14 ± 0.25	0.95 ± 0.37
EEP2	12	2.16 ± 0.20	0.92 ± 0.18	$0.39 \pm 0.09^{**}$	1.08 ± 0.44	$0.81 \pm 0.34^{**}$
Positive control	12	$1.89 \pm 0.25^{**}$	0.94 ± 0.17	$0.38 \pm 0.13^{**}$	$0.85 \pm 0.58^{**}$	$0.91 \pm 0.26^{*}$
ANOVA		$F_{6,77} = 4.82^{**}$	$F_{6,77} = 1.18$	$F_{6,77} = 6.19^{**}$	$F_{6,77} = 8.40^{**}$	$F_{6,77} = 4.07^{**}$

* $P < 0.05$, ** $P < 0.01$ compared with diabetes mellitus model control group.

3.4.3 The effects of propolis on the levels of fructosamine, superoxide dismutase, malonaldehyde, nitric oxide and nitric oxide synthetase in rats with induced diabetes mellitus

The effects of propolis on the levels of fructosamine (FRU), superoxide dismutase (SOD), malonaldehyde (MDA), nitric oxide (NO) and nitric oxide synthetase (NOS) in rats with induced diabetes mellitus are given in table 3.3.

Table 3.3 Effects of propolis on the levels of FRU, SOD, MDA, NO, NOS in rats with induced diabetes mellitus ($\bar{x}$ ± sd)

Group	n	FRU (μ mol/L)	SOD (NU/ml)	MDA (nmol/L)	NO(μ mol/L)	NOS (U/ml)
Normal control	12	224.83±13.46**	152.70±14.05**	5.65±1.85**	5.43±0.89**	9.36±2.23**
Model control	12	288.25±6.97	116.08±8.89	9.14±1.80	20.44±4.65	13.98±2.97
WSD1	12	222.58±16.41**	143.18±13.85**	4.87±1.38**	16.63±2.22	9.92±3.12**
WSD2	12	232.92±22.69**	143.67±5.87**	6.31±1.60**	16.65±3.90	12.30±4.10
EEP1	12	215.25±25.06**	136.33±10.95**	6.02±1.51**	18.09±9.19	10.98±1.32
EEP2	12	257.00±22.20**	126.24±14.57	5.67±2.66**	16.64±5.03	10.41±1.22*
Positive control	12	239.50±23.57**	138.12±19.59**	5.43±1.90**	15.10±6.89	11.71±1.76
ANOVA		$F_{6,77}$ = 19.85**	$F_{6,77}$ = 10.15**	$F_{6,77}$ = 6.75**	$F_{6,77}$ = 9.58**	$F_{6,77}$ = 4.48**

* $P < 0.05$, ** $P < 0.01$ compared with diabetes mellitus model control group.

The results of table 3.3 show that FRU in the WSD1, WSD2, EEP1, EEP2 and positive control groups significantly decreased 22.8% ($P < 0.01$), 19.2% ($P < 0.01$), 25.3% ($P < 0.01$), 10.8% ($P < 0.01$) and 16.9% ($P < 0.01$) respectively compared with that of diabetes mellitus model group and this result was consistent with that of fasting blood glucose levels.

SOD in the WSD1, WSD2, EEP1 and positive control groups significantly increased 23.3% ($P < 0.01$), 23.8% ($P < 0.01$), 17.4% ($P < 0.01$), and 18.9% ($P < 0.01$) respectively compared with that of diabetes mellitus model group. MDA in each experimental group, WSD1, WSD2, EEP1, EEP2 and positive control significantly decreased 46.7% ($P < 0.01$), 31.0% ($P < 0.01$), 34.1% ($P < 0.01$), 38.0% ($P < 0.01$) and 40.6% ($P < 0.01$) compared with that of diabetes mellitus model group.

The decreases of NO in the WSD1, WSD2, EEP1, EEP2 and positive control groups (18.6%, 18.5%, 11.5%, 18.6% and 26.1% respectively) compared with that of diabetes mellitus model group are not significant ($P > 0.05$). NOS in each of the groups, WSD1, WSD2, EEP1, EEP2 and positive controls decreased 29.0% ($P < 0.01$), 12.0% ($P > 0.05$),

21.5% ($P > 0.05$), 25.5% ($P < 0.05$) and 16.2% ($P > 0.05$) respectively compared with that of diabetes mellitus model group.

3.5 Discussion

3.5.1 The effects of propolis on blood glucose

The United Kingdom Prospective Diabetes Study (UKPDS) recognized that type-2 diabetes mellitus is an acute, progressive disease and during their 15-year study found that the level of glycosylated haematoglobin protein and fasting blood glucose level persistently increased. This demonstrated that the β cell system of the pancreas becomes impaired with time. This study also confirmed that in the patients with type-2 diabetes mellitus, it was very important to improve blood glucose levels, decreasing the danger of the diabetes mellitus syndrome (UKPDS Group, 1998). It has now also been demonstrated that while propolis enhances cell activity and accelerates the regeneration of tissue and repair among damaged pancreatic cell (Burdock, 1998), so that it is clearly a therapeutic adjunct for the cure of diabetes mellitus.

Fructosamine is a by-product of the reaction between glucose and albumin, which co-occur with keto-amines in serum. The concentration of fructosamine in serum was relatively steady and could not be affected by diet. Fructosamine could reflect blood glucose changes for 2-3 weeks in a manner similar to glycosylated haematoglobin protein (Grey *et al.*, 1995) whereas fasting blood glucose reflects instantaneous blood glucose concentration.

From the results of fructosamine and fasting blood glucose, it is evident that the concentrations of fructosamine in different propolis-treated groups were lower than that of diabetes mellitus model group, which demonstrates that propolis can efficaciously control blood glucose for some time. In addition, given the weekly concentrations of glucose in fasting animals, it is probable that propolis could decrease levels of blood glucose and that the effect is amplified over time.

3.5.2 The effects of propolis on blood lipid in rats with induced diabetes mellitus

Diabetes mellitus is often associated with blood lipid abnormalities, mainly increased levels of TC, TG, LDL-C and decreased levels of HDL-C, which lead to high blood pressure, atherosclerosis and coronary heart disease. At present a relationship between blood lipid abnormalities and atherosclerosis remains hypothetical. However, it seems likely that propolis could increase the contractibility of heart, deepen breath and modulate blood pressure, purify blood, as well as modulate blood lipid (Havsteen, 2002; Lin *et al.*, 1999). Numerous studies have shown that propolis could modulate high blood lipid, high total cholesterol, high blood viscosity and reduce atherosclerosis as well as to improve blood circulation (Stefano & Francesco, 2002; Burdock, 1998; Lin *et al.*, 1999; Fang *et al.*, 2000; Karsten, 2001). Because patients with diabetes mellitus often have high blood lipid, high total cholesterol, the activity of blood vessel scavengers would contribute to the cure of diabetes mellitus.

By testing LDL-C, VLDL-C, TC, TG, HDL-C in this experiment, it was found that after propolis treatments of rats with induced diabetes mellitus, the levels of LDL-C, VLDL-C, TC, TG were lower compared with that of diabetes mellitus model, but the level of HDL-C was higher than that of diabetes mellitus model group. So, it is reasonable to conclude that propolis could modulate lipid metabolism and reduce the syndrome caused by blood lipid abnormalities.

3.5.3 The effects of propolis on free radicals in rats with induced diabetes mellitus

Several studies have documented the relationship between the increase of free radicals and the increase of blood glucose, as well as changes of free radicals, lipid peroxidation and low-density lipoprotein in the progress of diabetes mellitus. Oxygen radicals would harm cells and MDA, as a kind of lipid peroxidation, would reflect the degree of oxidation in the body. SOD is a scavenger of free radicals, which has important effects in the control of oxidation reactions in the body. The concentration of SOD in type-2 diabetes mellitus was significantly higher than that of normal (Akgul *et al.*, 1997). The cause was probably decreased activity of SOD because higher blood glucose could combine with SOD. In low concentrations propolis is a natural anti-oxidative, and increases the activity

of SOD thus reducing the output of lipid peroxidation (Marcucci, 1995; Isla *et al.*, 2001; Song *et al.*, 2002; Krol & Czuba, 1990). From these experiments, it appears that the levels of SOD increased and that of MDA decreased after treatment with propolis. This suggests that propolis has effective anti-oxidative properties and could well scavenge excess free radicals.

3.5.4 The effects of propolis on NO and NOS in rats with induced diabetes mellitus

NO is an endothelial-enlarging factor produced by NOS, catalysing L-arginine. NO is also associated with many physiological and pathological changes in the body. NO enlarges blood vessels, reduces blood pressure and the multiplication of nonstriated-muscle cells in blood vessels as well as the agglutination of haematoblasts; nonetheless, excessive NO is pathogenic. The changes characteristic of the micro-cycle in type-2 diabetes mellitus includes damage to the endothelial cells and incrassation of the blood vessel matrix. This results in the reduction of blood vessel roughness, diameter and obstructions (Nomara *et al.*, 2000).

Moreover, these pathological changes are relative to increased levels of NO. From the results, we conclude that the levels of NO and NOS in the diabetes mellitus group was higher than that of normal rats. However, following treatment with propolis, the levels of NO and NOS in diabetes mellitus decreased compared with that of diabetes mellitus model. This suggests that propolis decreases the level of NO by decreasing the output of NOS thus protecting the endothelial cells of blood vessels and reducing neuronal toxicity. Propolis exerts its pharmacological effects by decreasing the action of NO and PGE_2 and reducing the activation effect of protein kinase to the diabetes mellitus or tumor model (Burdock, 1998).

3.5.5 The active components of EEP and WSD need further research

Although the main pharmacological constituents of the crude extracts of propolis were flavonoids (fagoyprol, quercetin, kaempferol, isohammetin, etc.), there may be other water-soluble substances in WSD because the anti-inflammatory mechanisms were different between the WSD and EEP (Hu *et al.*, 2003). Because the extracts of propolis

were crude and not purified it is not possible to precisely attribute specific effects to specific constituents. Further research on the full complement of active components of EEP and WSD are clearly required.

CHAPTER 4

4. EFFECTS OF POLLEN AND PROPOLIS ON DIABETES MELLITUS IN SD RATS

4.1 Summary

Diabetes mellitus SD rats were given pollen, water and ethanol extracts of propolis intragastrically twice a day for four weeks and were tested for indexes of serum and kidney weight/body weight. After four weeks treatment, pollen had the ability to protect the kidney from the complications of diabetes mellitus. Pollen and propolis treatments decreased the levels of fructosamine, triglyceride, cholesterol, creatinine, malonaldehyde, blood urea nitrogen, and the consumption of total protein and albumin. It can be concluded that pollen and propolis have a positive effect on diabetes mellitus in SD rats and may enhance the metabolism of glucose, fat and protein, and decrease the danger of diabetes mellitus affixation disease.

4.2 Introduction

Diabetes mellitus is a common incretion disease, caused by the absolute or relative absence of insulin, and results in deregulated metabolism. Diabetes mellitus remains an acute disease that continues to endanger human health (UKPDS Group, 1998). Articles on the effects of propolis and bee pollen curing diabetes mellitus (Song *et al.*, 1991b; Jiang & Kong, 1996; Wang & Jia, 1998; Gao & Liu, 2000) have recently been published, but detailed studies are few. Moreover, there are no uniform criteria for the extraction of propolis, and the analysis of different extraction methods of propolis on diabetes mellitus remains lacking. In this experiment, I studied the effects of bee pollen and propolis on the metabolism of blood glucose, blood lipid and protein from a series of biochemical indexes

including blood glucose, blood lipid, kidney weight/body weight to provide experimental data to test the efficacy of bee pollen and propolis in curing diabetes mellitus.

4.3 Materials and methods

4.3.1 Materials

Male rats (strain SD) about 300 ± 20 g birth weight were provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of Laboratory of Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. The animals were maintained and the experiments performed according to the principles of the Helsinki accord. The experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, Hangzhou, China.

Propolis and bee pollen were purchased from Lingzhi Apiary, Hangzhou, China. The propolis was produced in North China in 2001 and the main plant of origin was poplar (*Populus* sp.). Alloxan was purchased from the Sigma chemical Company. The reagents for blood glucose, fructosamine, total cholesterol, triglycerides, creatinine, total protein and albumin were supplied by Fudan-Zhangjiang Bio-pharmaceutical Company Ltd. Shanghai, China. The reagent for malonaldehyde was provided by Jiancheng Biology Engineering Research Institute, Nanjing, China.

4.3.2 Induction and treatment of diabetes mellitus in SD rats

Diabetes mellitus was induced in rats by the intravenous injection of alloxan (40 mg/kg) dissolved in physiological saline. Control rats were injected with physiological saline alone. 7 days later, blood samples from the cut tip of the tails of all SD rats were taken and the serum centrifuged to test blood glucose. Rats with a blood glucose

concentration of about 20 mmol/L were selected and then randomly divided into 4 groups of 10 diabetes mellitus rats, each: (1) diabetes mellitus control rats, (2) those given bee pollen, (3) those given WSD (water-soluble derivatives of propolis), (4) those given EEP (ethanol extract of propolis) and (5) a group of 10 healthy SD rats as normal controls.

4.3.3 Method of drug administration

Bee pollen: the concentration of bee pollen solution was about 0.375 g/ml, and administered intragastrically at a rate of 1 ml/100 g body weight.

WSD (water-soluble derivative of propolis): WSD was prepared according to the following method: 30 g of raw propolis was pulverized in a mill, extracted in water at 80°C for 12 hours, sonicated for 30 minutes at 25 khz, cooled and filtered at room temperature, and the dry material was concentrated to about 150 mg/ml and then diluted 5 times for the group of WSD and administered intragastrically at a rate of 1 ml/100 g body weight.

EEP (ethanol extract of propolis): EEP was extracted by 80% ethanol and the dry material content was about 750 mg/ml. It was then diluted 25 times for the group of EEP and administered intragastrically at a rate of 1 ml/100 g body weight.

Positive control group: acarbose was diluted in physiological saline to a concentration of 1 mg/ml and administered intragastrically.

SD rats in the normal control group and the diabetes mellitus model group were given 0.9% physiological saline intragastrically at 1 ml/100 g body weight.

All SD rats were treated intragastrically twice a day for about four weeks of the five week-long experiment.

4.3.4 Methods of measurement

Blood was taken from the cut tip of the tails of all SD rats each week after they had fasted 12 hours. The serum samples were centrifuged at 3000 rpm/min for 10 min to test blood glucose. At the end of the experiment, blood samples were taken from the eyeball and the serum centrifuged to conduct the other biochemistry indexes.

4.3.5 Statistical analyses

Data were reported as means ± standard deviations. Comparisons between groups were made using ANOVA and repeated measures ANOVA procedures. Tukey and Fisher LSD multiple pairwise comparisons tests were used to test for differences between groups and the model control group.

4.4 Results

4.4.1 Effects of propolis and bee pollen on blood glucose in diabetes mellitus SD rats

The effects of propolis on blood glucose in diabetes mellitus SD rats are shown in table 4.1. The blood glucose level of SD rats in each group of bee pollen, WSD, EEP decreased by 13.39%, 13.15%, 17.10% (LSD: $P < 0.05$) respectively compared with that of diabetes mellitus model at end of the first week of drug administration. The blood glucose level in the group of bee pollen and WSD rats decreased 1.29% and 2.30% respectively compared with that of diabetes mellitus model at the end of the second week of drug administration. The blood glucose level in each of the groups of bee pollen, WSD, and EEP decreased 6.90%, 4.94%, 4.78% respectively compared with that of diabetes mellitus model at the end of the third week of drug administration. The blood glucose level in each group of bee pollen, WSD, EEP decreased 16.04% (LSD: $P < 0.05$), 16.07% (LSD: $P < 0.05$), 17.21% (LSD: $P < 0.01$) respectively compared with that of diabetes

mellitus model on completion of the experiment at the end of the fourth week of drug administration. From the results, we deduced that bee pollen and propolis could control increases in blood glucose in diabetes mellitus to some degree.

Table 4.1 Changes in blood glucose of diabetes mellitus and normal SD rats for five weeks ($\bar{x} \pm$sd)

Group	n	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Normal	10	2.30±0.66**	0.79±0.41**	1.02±1.26**	0.78±0.57**	2.19±0.76**
Model	10	21.38±3.18	24.72±3.54	23.32±2.55	31.17±6.89	32.66±2.17
Bee pollen	10	21.14±3.20	21.41±3.34	23.02±2.14	29.02±4.44	27.42±4.97*
WSD	10	20.90±2.44	21.47±4.58	22.79±2.53	29.63±7.11	27.41±3.93*
EEP	10	19.45±3.52	20.49±4.81*	23.90±4.03	29.68±3.43	27.04±4.12**

*$P < 0.05$, ** $P < 0.01$ compared with diabetes mellitus model control group.

4.4.2 Effects of bee pollen and propolis on the levels of fructosamine, triglyceride, total cholesterol, creatinine, total protein, albumin and malonaldehyde in diabetes mellitus SD rats.

The effects of bee pollen and propolis on the levels of fructosamine (FRU), triglyceride (TG), total cholesterol (TC), creatinine (CREA), total protein (TP), albumin (ALB) and malonaldehyde (MDA) in diabetes mellitus SD rats are shown in table 4.2.

Table 4.2 Effects of bee pollen and propolis on the level of FRU, TG, TC, CREA, TP, ALB and MDA in diabetes mellitus SD rats ($\bar{x}$ ±sd)

Group	n	FRU	TG (mmol/L)	TC (mmol/L)	CREA (μmol/L)	TP (g/L)	ALB (g/L)	MDA (nmol/L)
Normal	10	73.36±5.27**	0.64±0.17**	1.27±0.34	60.43±5.93*	66.7±8.5**	30.1±3.5**	9.3±0.9**
Model	10	104.79±13.72	3.97±2.14	1.48±0.25	67.16±5.61	49.4±9.1	23.2±3.6	18.1±5.2
Pollen	10	97.37±15.06	2.22±0.73**	1.36±0.36	66.13±12.56	53.0±5.7	23.1±1.2	14.7±2.5
WSD	10	93.79±15.38	2.07±0.65**	1.26±0.22	67.42±5.46	52.1±9.9	23.3±4.0	14.5±1.6
EEP	10	94.54±6.64	1.91±0.75**	1.22±0.20	60.43±4.74*	55.0±7.2	24.0±1.3	14.1±3.1*

*$P < 0.05$, ** $P < 0.01$ compared with diabetes mellitus model control group model.

The FRU level of SD rats in each group of bee pollen, WSD, EEP decreased 17.1%, 10.5%, 9.8% respectively compared with that of diabetes mellitus model at end of four weeks of drug administration. The TG level of SD rats in each group of bee pollen, WSD, EEP decreased 44.2% (P < 0.01), 47.8% ($P < 0.01$), 51.9% ($P < 0.01$) respectively compared with that of diabetes mellitus model at end of four weeks of drug administration. The TC level of SD rats in each group of bee pollen, WSD, EEP decreased 7.8%, 15.1%, 17.6% (LSD: $P < 0.05$) respectively compared with that of diabetes mellitus model at end of four weeks of drug administration. The CREA level of SD rats in EEP group decreased 10.0% (LSD: $P < 0.05$) compared with that of diabetes mellitus model at end of four weeks of drug administration. The TP level of SD rats in each of the groups of bee pollen, WSD, EEP increased 7.29%, 5.52%, 11.34% respectively compared with that of diabetes mellitus model at end of four weeks of drug administration. The MDA level of SD rats in each group of bee pollen, WSD, EEP decreased 18.5%, 19.9%, 22.1% ($P < 0.05$) respectively compared with that of diabetes mellitus model at end of four weeks of drug administration.

4.4.3 The effects of bee pollen and propolis on body weight, kidney weight and kidney weight/body weight.

Table 4.3 Effect of bee pollen and propolis on body weight, kidney weight and kidney weight / body weight ($\bar{x}$ ±sd)

Group	n	Body weight (g)	Kidney weight (g)	Kidney weight/body weight X 100
Normal control	10	335.0±18.4**	1.25±0.08	3.73±0.21**
Model control	10	202.3±28.4	1.30±0.16	6.47±0.52
Bee pollen	10	227.6±22.1	1.37±0.17	6.00±0.80
WSD	10	241.3±43.2**	1.47±0.21	6.23±1.21
EEP	10	220.5±36.0	1.33±0.19	6.14±0.11

** $P < 0.01$ compared with diabetes mellitus model control group model.

The effects of bee pollen and propolis on body weight, kidney weight and kidney weight/body weight are shown in table 4.3. From the result of kidney weight/body weight, we could see that the effect in the groups of bee pollen and EEP was better than others although not significant.

4.5 Discussion

4.5.1 Effects of bee pollen and propolis on blood glucose

As is well known, the level of fasting blood glucose concentration shows instantaneous changes of blood concentration. From the results of five weeks of treatment, it was found that the level of blood glucose decreased significantly by the end of the first week and the fourth week after drug administration compared with that of model group. It is necessary to further investigate the effects of bee pollen and propolis on blood glucose in diabetes mellitus rats over a more extended time period.

Fructosamine was a consequence of the reaction between glucose and albumin, which occurs in serum with keto-amines. The concentration of fructosamine in serum was relatively steady and was not affected by diet. Fructosamine could reflect the blood glucose changes for 2-3 weeks just like glycosylated haematoglobin protein (Li *et al.*, 2001b). It could reflect blood glucose changes better than instantaneous changes of blood concentration, so that the results on fructosamine further support an interpretation that bee pollen and propolis may control the level of blood glucose to some degree.

4.5.2 Effects of bee pollen and propolis on the metabolism of blood lipid

Diabetes mellitus often co-occurs with blood lipid abnormalities, mainly resulting in higher levels of TC and TG than normal, and which could lead to high blood pressure, atherosclerotic and coronary heart disease. At present, the relationship between blood lipid abnormalities and atherosclerotic conditions has been confirmed.

Propolis could increase the contractibility of the heart, deepen respiration, modulate blood pressure, purify blood, as well as modulate blood lipid. Numerous reports have shown that propolis could modulate high blood lipid, high total cholesterol, high blood viscosity and protect against atherosclerotic conditions and protect the blood vessels of the heart and brain to improve blood circulation. Because patients with diabetes mellitus often have high blood lipid and high total cholesterol, the activity of blood vessel scavengers would contribute to the cure of diabetes mellitus.

From the statistical analysis of the results it was found that the levels of the triglycerides in the groups bee pollen and propolis treated animals were lower than that of the model control group. Furthermore, the difference in the groups of WSD and EEP were significant compared with that of model control. I deduce that the effect of propolis to decrease the level of triglyceride was better than that of bee pollen.

From the statistical analysis of the results for total cholesterol, it was found that the

level in the group of bee pollen and propolis was lower than that of model control group. Furthermore, the difference in the group of EEP was significant compared with that of model control. It is inferred that bee pollen and propolis could control increases in blood lipid and the effectiveness of propolis was better than that of bee pollen.

4.5.3 Effects of bee pollen and propolis on diabetes mellitus nephropathy

Another acute syndrome of diabetes mellitus is nephropathy, the main symptom of which is damage to the kidney. From the results of kidney weight/body weight analysis, it was found that the ratio in the group of bee pollen was smallest, the effect in the group of propolis was no better than that of bee pollen, but the effect in the group of EEP was better than that of WSD. From the results for creatinine, it was found that the difference in the EEP group was significant compared with that of model control group. Bee pollen produced more effective protection for the kidney than did propolis.

4.5.4 Effects of bee pollen and propolis on fat metabolism

Diabetes mellitus often causes disturbances of protein metabolism. From the statistical analysis of the results for total protein and albumin, it was found that the wastage of total protein and albumin in the groups of bee pollen and propolis was lower than that of model control group, which showed that bee pollen and propolis could modulate the disturbances of protein metabolism in diabetes mellitus.

4.5.5 Effects of bee pollen and propolis on free radical in diabetes mellitus

The relationship between the increase of free radicals and the increase of blood glucose, and the change of free radicals, lipid peroxidation and low-density lipoprotein are associated with progressive diabetes mellitus. Oxygen radicals would harm cells and DA, as a result of lipid peroxidation and would reflect the oxidation degree of body.

From the experiment, it can be seen that the level of MDA decreased after treatment with bee pollen and propolis, which indicates that bee pollen and propolis have effective anti-oxidative properties and could scavenge much of the free radical pool in the body. Furthermore, the effect of propolis was better than that of bee pollen.

Absence of insulin in diabetes mellitus is related to the function of β cells. Malnutrition inducing the absence of vitamins and some mineral elements such as vitamin B_6, Cr, Zn, Fe, Mg, Ca, P also could lead to damaging β cells. Vitamin B_6 is abundant in pollen and some micro-mineral elements in pollen to protect β cells and aid in the secretion of insulin.

Besides these, propolis is a complex material, and the chemical components of different sources are different, as is the biological activity of different extraction methods. From this experiment, it is evident that the biological activity of WSD and EEP was not the same, but they were useful in the control of diabetes mellitus. It is necessary to study the mechanism of propolis and bee pollen on curing diabetes mellitus more extensively.

CHAPTER 5

5. ANTI-INFLAMMATORY EFFECTS OF ETHANOL AND WATER EXTRACTS OF PROPOLIS

5.1 Summary

Propolis solutions extracted by ethanol (EEP) and water (WSD) were assessed for their anti-inflammatory effects using Freund's complete adjuvant (FCA) induced arthritis in rats. Both EEP and WSD showed inhibitory effects on swelling induced by FCA and decreased the degree of local inflammatory responses. EEP and WSD significantly inhibited the increase of interleukin-6 (IL-6) in inflamed tissues, but had no significant effect on levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ). The results are consistent with the interpretation that EEP and WSD may exert these effects by inhibiting the activation and differentiation of mononuclear macrophages.

5.2 Introduction

Propolis has a very low water solubility and studies on such derivatives are few indeed. Its anti-complementary activity *in vitro* and complementary activity *in vivo* have been documented (Ivanovska *et al.*, 1995a). Previous results indicated that the action of WSD depends on the route of WSD administration and not on relative differences in the effects of WSD and EEP. In this experiment, the effects of propolis solutions extracted by ethanol (EEP) and water (WSD) on Freund's complete adjuvant (FCA) induced arthritis in rats were studied to further assess the pharmacological properties of propolis. Multiple-arthritis is an important character of the arthritic rat model induced by FCA and exhibits important similarities to human rheumatoid arthritis. The model is also sensitive to anti-inflammatory and immune-inhibiting medicines (Ivanovska *et al.*, 1995a; Hu *et al.*, 2003).

5.3 Materials and methods

5.3.1 Laboratory animals

Male Wistar rats of about 200 ± 20 g were provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of Laboratory Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. Standard international animal ethical procedures were followed with respect to housing at five animals per cage and feeding the animals.

5.3.2 Drugs and reagents

Propolis was obtained in North China in 2001 and the main plant origin was poplar (*Populus sp.*). The samples were stored at -20°C prior to analysis and before experimentation samples were assayed for flavonoid content to obtain a uniform batch of experimental propolis.

5.3.2.1 Determination of total contents of flavonoids in propolis

Instrument

UV-Vis Spectrophotometer

Reagents

Preparation of standard rutin solution

Weigh out 200 mg rutin dried to constant weight in a negative pressure drier with 120°C, put it into a 100-ml volumetric flask, add 70 ml methanol (AR) to the same volumetric flask. Heat up this volumetric flask slightly to dissolve the mixtures through water bath, shake the solution up after cooling. Absorb 10 ml of this solution to another 100-ml volumetric flask; add distilled water into the second volumetric flask to 100ml and shake up the new solution. The new solution is the standard rutin solution with a concentration of 0.2 g rutin per ml.

Preparation of 5% $NaNO_2$

Weigh 5g $NaNO_2$, dissolve it with distilled water and then add distilled water to 100 ml.

Preparation of 10% Al $(NO_2)_3$

Weigh 10g Al $(NO_2)_3$, dissolve it with distilled water and then add distilled water to 100 ml.

Preparation of NaOH

Weigh 4.3 g NaOH, dissolved it with distilled water and add to 100 ml.

Determination methods

Preparation of standard curve

Absorb standard rutin solution respectively 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, 6.0 ml, put them respectively into 25-ml volumetric flasks, all are added water to 6.0ml, then add 1 ml 5% $NaNO_2$ to every volumetric flask. Shake these solutions equably and stable for 6 minutes, then add 10% Al $(NO_2)_3$ solution 1 ml to all volumetric flasks. Six minutes later, add 10 ml NaOH to every volumetric flask and then add distilled water to all volumetric flasks to 25 ml. Shake these solutions equably and stable for 5 minutes, determine their absorbance at the wavelength of 550 nm and draw the standard curve.

Determination of total contents of flavonoids in samples

Weigh 5 g propolis and put it into Soxhlet apparatus, add 120 ml ether to the apparatus and heat up for recycling until the extraction solution become colourless. Cool the apparatus and take away the ether solution, then add 90 ml methanol into the apparatus. Heat up until the extraction solution becomes colourless. Transfer methanol solution to a 100-ml volumetric flask, clean the former volumetric flask with a little methanol and transfer the cleaning methanol into the same 100-ml volumetric flask. Add methanol to this volumetric flask to 100 ml and shake and stable the solution. Absorb 3 ml this kind of solution and put it into a 25-ml volumetric flask. According the methods of the preparation of standard curve (refer to step 2.1), to determine the absorbance of propolis solution, conduct the following experiment since the step of adding distilled water to the 25-ml volumetric flask to 6 ml.

Calculations

$$X = \frac{A}{m \times \frac{10.0}{100} \times \frac{3.00}{100} \times 1000}$$

where **X** = the total contents of flavonoids (calculated through the contents of rutin), mg/kg, **A** = the recovery contents of rutin in sample solution, and **m** = the weight of propolis, g.

5.3.2.2 Preparation of WSD and EEP

The WSD was prepared according to the following method: 30 g of raw propolis was pulverized in a mill, extracted in water at 80°C for 12 hours, sonicated for 30 minutes at 25 khz, cooled and filtered at room temperature. EEP was obtained as follows: 30 g propolis powder was extracted in 100 ml 80% ethanol, sonicated for 30 minutes at 25 khz, and filtered. WSD and EEP were prepared to obtain a solution of 1 g/l of propolis extract. Pharmaceuticals included prednisone acetate (Xianju Pharmacy Ltd. Company), Freund's complete adjuvant (Sigma Company, Lot: 68H8504); ELISA reagents for endogenous rat interleukin-2 (IL-2) (RND Company, Lot: R2000), the endogenous rat interleukin-6 (IL-6) (Bender Company, Lot: BMS625) and the endogenous rat interferon-γ (IFN-γ) (Hyzult Company, Lot: Hk010) were obtained from JINGMEI Biotech Company.

5.3.3 Protocols

The experimental protocol was designed in terms of the Helsinki convention and approved by the Animal Ethics Committee of Zhejiang University. The general methodology followed was that of Tang *et al.* (2000). 50 male Wistar rats were randomly divided into 5 groups, and treated once daily intragastrically with (1) EEP, (2) WSD, (3) sterile saline (normal control group), (4) sterile saline (model control group), and (5) prednisone acetate (positive control group) respectively. Both right and left hind paw cubages of all rats were measured manometrically before treatment. On day 1 of the experiments the animals were treated with prednisone acetate and the propolis solution. 3 days later, all the rats were treated with FCA (0.1 ml) by intradermal injection on the pad of the right hind paw (except for the group 3). On the 1st through the 5th, and on the 7th,

11th, 13th 16th, 19th day after treatment, the cubages of both right (site of primary infection) and left (site of secondary infection) hind paws were measured and assessed for any anti-inflammatory effects which were measured as the degree of swelling. On the 19th day, all the rats were weighed and then killed. The right hind paw, thymus and the adrenal glands were excised and weighed separately. The right hind paws were placed in 5 ml sterile saline at 4°C over night. The samples were then centrifuged at 3000 rpm, and the supernatant used to measure the levels of prostaglandin-E_2 (PGE_2), IL-2, IL-6 and IFN-γ. Quantitative measurements of rat IL-2, IL-6 and IFN-γ were determined with an enzyme linked Immunosorbent assay method (Ossege *et al.*, 1998; Gould *et al.*, 1998) and the levels of PGE_2 were measured by absorption spectrometry (Jiang & Geng, 1998; Naderali & Poyser, 1997). Both the thymus and adrenal gland indices were measured as the weight of the individual organ divided by total animal body weight and expressed as mg organ/100 g total body weight.

5.3.4 Statistical analyses

Data are reported as means ± standard deviations. Levene's test and Kolmogorov Smirnov test were used to test for homogeneity of the variances and normality of the response variables respectively. Comparisons between groups were made using ANOVA and repeated measures ANOVA procedures. Tukey's multiple pairwise comparisons tests were used to test for differences between groups and the model control group.

5.4 Results

Effects of WSD and EEP on body weight, thymus index, and adrenal gland index in FCA-induced arthritic rats are given in table 5.1. There was no significant difference in the mean initial weight of the rats between groups ($F_{3,28} = 0.93$, $P = 0.4389$), whilst there was a significant difference in mean weight on the 19th day between the groups ($F_{3,28} = 4.2$, $P = 0.0139$). The results showed that both WSD and EEP could prevent body weight loss (Tukey: WSD: P = 0.0232, EEP: $P = 0.1851$), but had no effect on the thymus index or adrenal gland index. Weight, thymus index and adrenal index passed Levene's test of homogeneity ($P > 0.05$) and Kolmogorov Smirnov test of normality ($P > 0.20$).

Table 5.1 Effects of WSD and EEP on body weight, thymus index and adrenal gland index in FCA-induced arthritic rats ($\bar{x}$ ±sd)

Group	Dose (ml/100g)	n	Weight of normal rats (g)	On the 19th day after injecting FCA		
				Weight (g)	Thymus index (mg/100g)	Adrenal index (mg/100g)
Model control	-	8	205.5±5.3	266.5±19.7	156.8±25.0	17.8±2.4
Prednisone acetate	10mg/kg	8	200.4±6.8	269.5±20.3	140.3±20.9	12.9±2.5**
WSD	1	8	204.8±5.6	294.0±11.7*	159.5±17.3	16.7±2.1
EEP	1	8	205.0±9.4	285.1±18.8	163.9±12.9	16.0±1.1

*P < 0.05, **P < 0.01 compared with model control group.

The results of WSD and EEP on paw swelling of the primary (right paw) and secondary (left paw) sites in FCA-induced arthritic rats are given in tables 5.2 and 5.3. The results of a repeated measures ANOVA showed a significant difference in mean FCA-induced paw swelling of the primary infection between the groups ($F_{3,20} = 7.42$, $P = 0.0016$), days ($F_{10,200} = 69.08$, $P < 0.0001$) and interactions of groups x days ($F_{30,200} = 1.89$, $P = 0.0053$). EEP and WSD inhibited swelling in the primary affection but the decrease in swelling was not significant for all the different days (table 5.2). The decrease, however, was significant on day 7 for EEP and WSD and day 13 for EEP when compared to the model group. Swelling passed Levene's test of homogeneity for each of the 20 time periods ($P > 0.01$) and Kolmogorov Smirnov test of normality ($P > 0.10$).

Table 5.2 Effects of WSD and EEP on swelling of the right hind paw in FCA-induced arthritic rats ($\bar{x} \pm$sd) (the primary affection)

Group		Model control	Prednisone acetate	WSD	EEP
Dose (ml/100g)		—	10mg/kg	1	1
n		6	6	6	6
Cubage before inflammation (ml)		1.01±0.05	0.98±0.08	1.02±0.07	1.02±0.07
Cubage of right hind paw after inflammation (ml)	1d	1.43±0.10	1.37±0.08	1.33±0.10	1.37±0.04
	2d	1.89±0.15	1.68±0.15	1.71±0.07	1.72±0.18
	3d	1.84±0.17	1.68±0.09	1.80±0.18	1.76±0.19
	5d	1.70±0.13	1.57±0.16	1.78±0.15	1.64±0.14
	7d	1.82±0.23	1.44±0.06*	1.52±0.15*	1.47±0.08*
	9d	1.75±0.22	1.46±0.10*	1.58±0.13	1.62±0.12
	11d	1.57±0.11	1.54±0.16	1.37±0.05	1.41±0.15
	13d	1.70±0.08	1.43±0.08*	1.61±0.12	1.52±0.09*
	16d	1.52±0.08	1.43±0.23	1.49±0.11	1.53±0.10
	19d	1.74±0.21	1.53±0.16	1.53±0.21	1.65±0.13

*$P < 0.05$ compared with model control group.

Table 5.3 Effects of WSD and EEP on swelling of left hind paw ($\bar{x} \pm sd$) (the secondary affection)

Group		Model control	Prednisone acetate	WSD	EEP
Dose (ml/100g)		—	10 mg/kg	1	1
n		6	6	6	6
Cubage before inflammation (ml)		1.00±0.04	0.98±0.08	1.03±0.04	1.01±0.12
Cubage before inflam-mation of left hind paw (ml)	1d	1.04±0.10	0.97±0.06	1.04±0.08	0.98±0.07
	2d	1.08±0.13	0.94±0.08	0.98±0.13	1.02±0.09
	3d	1.04±0.18	1.13±0.06	0.98±0.17	1.00±0.13
	5d	1.08±0.09	1.10±0.10	1.07±0.20	1.01±0.10
	7d	1.25±0.18	1.07±0.10	1.03±0.17	0.98±0.15
	9d	1.20±0.16	1.15±0.06	1.14±0.17	1.22±0.07
	11d	1.13±0.12	1.21±0.13	1.07±0.15	1.09±0.07
	13d	1.19±0.14	1.10±0.14	1.16±0.08	1.22±0.14
	16d	1.02±0.05	0.94±0.09	1.03±0.12	1.08±0.14
	19d	1.30±0.09	1.15±0.12	1.23±0.04	1.22±0.13

The results of a repeated measures ANOVA showed no significant difference in FCA-induced paw swelling of the secondary infection between the groups ($F_{3,20} = 1.11$, $P = 0.3693$). There were significant differences between days ($F_{10,200} = 12.75$, $P < 0.0001$) and interactions of groups x days ($F_{30,200} = 1.71$, $P = 0.0168$) EEP and WSD had some inhibitory effect on swelling but the results when compared to the model control group were not significant.

The effects of WSD and EEP on the level of PGE_2, IL-2, IL-6 and IFN-γ in the extravasate of the right paw of FCA-induced arthritic rats are given in table 5.4. The transformation Log_e(IL-6) was used in the ANOVA because IL-6 failed Levene's test of homogeneity of the variances. The results showed significant differences in the mean levels of PGE_2 between the groups ($F_{4,25} = 15.4$, $P < 0.0001$), of IL-6 ($F_{4,25} = 8.68$, $P =$

0.0002) and of IFN-γ ($F_{4,25}$ = 5.87, P = 0.0018) (table 5.4). EEP had a significant inhibitory effect on the level of PGE_2 in FCA-induced arthritic rats (Tukey: $P < 0.05$). WSD and EEP had significant inhibitory effects on the level of IL-6 (Tukey: $P < 0.01$), but had no significant effects on the levels of IL-2 and IFN-γ. PGE_2, IL-2, IL-6 and IFN-γ passed Kolmogorov Smirnov test of normality ($P > 0.20$). PGE_2, IL-2, Log_e(IL-6) and IFN-γ passed Levene's test of homogeneity ($P > 0.05$).

Table 5.4 Effects of WSD and EEP on the levels of PGE_2, IL-2, IL-6 and IFN-γ in the extravasate of FCA-induced arthritis rats ($\bar{x} \pm$sd) (n = 6 for each group)

Group	Dose(ml/100g)	PGE_2	IL-2 (pg)	IL-6 (pg)	IFN-γ (pg)
Normal control	-	0.011±0.006**	19.75±5.27	106.67±16.33	733.33±85.71*
Model control	-	0.047±0.011	12.35±5.14	138.67±37.80	623.33±73.12
Prednisone acetate	10mg/kg	0.030±0.010*	17.17±4.67	66.00±23.66**	586.67±45.90
WSD	1	0.034±0.005	14.58±5.89	78.33±11.69**	695.00±96.69
EEP	1	0.026±0.007**	15.27±4.06	88.33±19.41*	565.00±45.06

*P < 0.05, **P < 0.01 compared with model control group.

5.5 Discussion

The characteristics of inflammation can be divided into acute, chronic, irritability and immunity related inflammation. The arthritic rat model induced by FCA is associated with the immunity inflammation reaction in this experiment. Cytokines play an important role in immune and inflammatory responses in vivo, being one group of low molecular weight proteins secreted by immune cells (lymphocyte and mononuclear-macrophage) and to fibroblasts and endothelial cells. The cytokines were divided into two groups: (1) lymphokines (such as IL-2, IL-4, IL-10 and IFN-γ etc.) secreted by T-cells with an immune function and (2) monokines (such as IL-1, IL-6, IL-8 and TNF-α etc.) secreted by mononuclear-macrophages with moderate inflammatory activity. The results of the present experiment show that EEP and WSD had significant inhibitory effects on the levels of IL-6 in FCA-induced arthritis rats, but not on the levels of IFN-γ and IL-2. This suggests that, in

the course of the anti-inflammatory effects of WSD and EEP, the humoral immune system (not cell system) plays an important role. This suggests that inhibiting the activation and differentiation of mononuclear macrophages is one of the possible mechanisms for the anti-inflammatory and immune effects of WSD and EEP.

Any factor that induces tissue damage could be described as the pathogenesis of an inflammation. There are two kinds of induced inflammation factors: the inflammation stimulation factor, which mainly includes physical (e.g., bruises, burns, frostbite, radial damage etc.) and chemical factors (acid, alkali, digestive liquid, allergens, mineral oil etc.), and biochemical factors (microorganisms, parasites, endotoxins, transplant heterogeneity and animal toxins). Other inflammatory media include histamine, bradykinin, prostaglandin, platelet activation factor, neutrophils hydrolase, inflammation pre-stimulation factors (TNF-α, IL-1, IL-6, cell chemotaxis factor etc.), adherence cell (select element, conformity element, adherence cell between cells, blood vessel cell adherence cell etc.), cruor system stimulation factor, acute reaction protein (C reaction protein, LPS combined protein, serum starched protein A etc.) (Akarasereenont *et al.*, 1995). EEP and WSD could inhibit the increase of PGE_2, which was perhaps the mechanism underlying the anti-inflammatory properties of propolis.

The main characteristic of rheumatoid arthritis (RA) is the ongoing damage in arthrosis of cartilage and bone, and at the same time with a disturbance of immune function. In the context of RA, there exist neutrophils, activation macrophages, lymphocytes and other elements associated with the abduction, activation and releasing of cytokine, which is perhaps one of the mechanisms of RA. In the case of rheumatoid arthritis, the concentration of cytokine derived from T cells was generally low, whereas that of mononuclear macrophages was significantly higher (Xu, 1996). WSD and EEP can inhibit the increase of inflammatory medium and decrease the activation and inducing effects of cytokines, which indicates that both extracts exhibit the same anti-inflammatory effects. WSD is processed in a simple manner, at low cost and of lower allergenic effects and olfactory irritability. Likewise, it differs from the glucocorticosteroids, which have the side effects of weight loss and partial inhibition of the immune system.

Further study and analysis of the absorption, metabolism, distribution and excretion

and the effective components of WSD should be performed, especially to isolate and fully characterize the active principles of propolis (Tan, 2001). Likewise, different solvents may change the conformation of the ingredients and hence influence their effects.

CHAPTER 6

6. EFFECTS OF ETHANOL AND WATER EXTRACTS OF PROPOLIS ON ACUTE INFLAMMATORY ANIMAL MODELS

6.1 Summary

The anti-inflammatory effects of ethanol (EEP) and water (WSD) extracts in ICR mice and Wistar rats were analyzed. The results showed that both WSD and EEP exhibited significant anti-inflammatory effects in animal models with respect to thoracic capillary vessel leakage in mice, Carrageenan-induced oedema, Carrageenan-induced pleurisy and acute lung damage in rats. The mechanisms for the anti-inflammatory effects probably involve decreasing PGE_2 and NO levels.

6.2 Introduction

Propolis is generally insoluble in water, so that pharmacological studies on water-soluble derivatives of propolis are few indeed. Thus, the effects of propolis solutions extracted by ethanol (EEP) and water (WSD) on acute inflammatory conditions were studied to further assess its solubility properties in relation to the biological activity of this beekeeping by-product.

6.3 Materials and methods

6.3.1 Laboratory animals

Experiments were performed using male ICR mice of about 18-22 g and male Wistar rats about 180-220 g provided by the Shanghai Laboratory Animal Center of the Chinese

Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003) at The Research Center of the Laboratory of Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. The animals were maintained and the experiments performed according to the principles of the Helsinki accord. The experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, Hangzhou, China.

6.3.2 Drugs and reagents

Propolis was obtained in North China in 2001 and the main plant origin was poplar (*Populus sp.*). Water-soluble derivatives (WSD) of propolis were obtained after it was comminuted in water at 80°C for 12 hours. Ethanol extracted propolis (EEP) was extracted in 80% ethanol. The concentrations of WSD and EEP were 1 g pure propolis per L solution. Prednisone acetate and dexamethasone acetate were obtained from Xianju Pharmacy Ltd.Zhejiang, China; carrageenan, lipopolysaccharide (LPS) and Evans Blue were obtained from Sigma Company. Total protein reagent was obtained from Cicheng Biochemistry Reagent Company, Ningbo, China. Other reagents, such as malodialdehyde (MDA), superoxide dismutase (SOD) and glutathione-peroxidase (GSH-PX), nitric oxide (NO), and lysozyme, were produced by the Institute of Jiancheng Biology Engineering, Nanjing, China. Instruments used were standard laboratory equipment: YP600 electronic microbalance and FA1004 electron analysis scale (Shanghai Second Balance Instrument Plant, Shanghai, China), Micro half-automatic biochemistry instrument (VITALAB Company, Holland), TCL-16G frozen centrifugation and 80-2B centrifugation (Shanghai ANTING Science Instrument Plant, Shanghai, China), 723 Spectrophotometer (Shanghai Second Analytical Instrument Plant, Shanghai, China).

6.3.3 Thorax capillary vessel leakage in mice

The methodology used was that of Lu (1999). 40 male ICR mice were randomly divided into 4 groups, and given intragastrically (1) EEP, (2) WSD, (3) sterile saline

(normal control group), or (4) prednisone acetate (positive control group) respectively and treated once daily continuously for 5 days. One hour after the last dose was given, each mouse was given intravenously 2% Evans Blue (0.1 ml/10 g) through the tail vena, and given intraperitoneally 0.6% acetic acid (0.2 ml/each) at the same time. Twenty minutes later the mice were killed, and each thorax was washed with 5 ml distilled water. The washing liquid was centrifuged to obtain a supernatant for the measurement of optical density (OD) and read spectrophotometrically at 590 nm so that the OD value could serve as a test for any capillary vessel leakage.

6.3.4 Carrageenan-induced paw oedema in rats

The methodology used was that of Wei (1999). Thirty-two male Wistar rats were randomly divided into 4 groups, and given intragastrically (1) EEP, (2) WSD, (3) sterile saline (normal control group), or (4) dexamethasone acetate (positive control group) respectively and treated once daily continuously for 5 days. The right hind paw cubage of all rats was measured before treatment. Thirty minutes later, each rat was treated (intradermal injection) with 1% Carrageenan (0.1 ml) on the pad of the right hind paw, and given intraperitoneally sterile saline (4 ml/100 g avoirdupois). Inflammation, measured as paw cubage, was measured hourly for six hours to assess possible anti-inflammatory effects on oedema.

6.3.5 Carrageenan-induced pleurisy in rats

The methodology used was that of Geng (2002). 60 male Wistar rats were randomly divided into 5 groups, and given intragastrically (1) EEP, (2) WSD, (3) sterile saline (model control group), (4) sterile saline (normal control group), or (5) prednisone acetate (positive control group) respectively and treated once daily continuously for 7 days. Thirty minutes after the initial treatments, all the rats (except those in normal control group) were injected with 1% Carrageenan (0.2 ml/100 g) in the right thorax after light anaesthesia with ether, while rats in the normal control group were treated with sterile saline. Inflammation

was induced within 4 hours. Eight hours later, all the rats were killed by decollation and the extravasate in the thorax collected by aspiration. Each thorax was then washed with 2 ml sterile saline, after which the extravasate and washing solution were mixed. One hundred micolitres of the solution was used to count WBC, the rest was centrifuged and stored at -20°C. An aliquot of 0.5 ml of supernatant was used to measure protein levels; while 0.15 ml solution and 1 ml 0.5N KOH-methanol was used to measure optical density (OD) at 278 nm with a TU-1001 ultra-spectrophotometer after incubation in water at 50°C for 20 minutes, and the OD value measured to calculate the content of prostaglandin-E_2 (PGE_2). Another solution sample of 200 µl was used to measure nitric oxide (NO) levels.

6.3.6 Acute lung damage in rats

The methodology used follows that of De Jongh (1997). 60 male Wistar rats were randomly divided into 5 groups, and given intragastrically (1) EEP, (2) WSD, (3) sterile saline (normal control group), (4) sterile saline (model group), or (5) dexamethasone acetate (positive control group) respectively and treated once daily continuously for 7 days. All the rats were given oleic acid (0.2 ml/kg) intravenously through the tail vena. Four hours later, all the rats were given lipopolysaccharide (LPS) (2 mg/kg) intravenously through another tail vena, and 2 hours later, all the rats were killed to count WBS, and the left lung weighed to calculate the lung index.

6.3.7 Statistical analyses

Data are reported as means $\pm$ standard deviations. Levene's test and the Kolmogorov Smirnov test were used to test for homogeneity of the variances and normality of the response variables, respectively. Comparisons between groups were made using ANOVA and repeated measures ANOVA procedures. Tukey's multiple pairwise comparisons tests were used to test for differences between groups and the model control group.

6.4 Results

6.4.1 The effect of propolis on celiac capillary leakage in mice

A significant effect of propolis on celiac capillary leakage in mice was found between the groups ($F_{3,26} = 3.1$, $P = 0.0442$). The results show that WSD has a significant inhibitory effect on acetic acic-induced celiac capillary leakage (Tukey: $P = 0.0616$). The effect of WSD was similar to that of prednisone acetate, but the effects of EEP and WSD compared with positive drug treatment were not significant (table 6.1). Celiac capillary leakage passed Levene's test of homogeneity ($F = 2.6$, $P = 0.0723$) and Kolmogorov Smirnov test of normality ($d = 0.09$, $P > 0.20$).

Table 6.1 Effects of propolis on celiac capillary leakage induced by acetic acid in mice ($\bar{x} \pm$ sd)

Group	Dose (ml/10g)	n	OD
Normal control	0.2	8	0.621±0.088
Prednisone acetate	10mg/kg	7	0.518±0.060
WSD	0.2	7	0.510±0.114*
EEP	0.2	8	0.533±0.048

*$P < 0.05$ compared with model control group.

6.4.2 The effects of Carrageenan-induced paw oedema in rats

The results of a repeated measures ANOVA showed a significant effect of propolis on carrageenan-induced paw oedema between the groups ($F_{3,28} = 41.8$, $P < 0.0001$), times ($F_{6,168} = 211.5$, $P < 0.0001$) and interactions of groups x time ($F_{18,168} = 14.4$, $P < 0.0001$). The results show that after the second hour, EEP and WSD all had significant inhibitory effects on carrageenan-induced paw oedema (table 6.2). Oedema passed Levene's test of homogeneity for each of the 7 time periods ($P > 0.05$) and Kolmogorov Smirnov test of

normality (d = 0.16, $P > 0.20$).

Table 6.2 Effects of propolis on carrageenan-induced hind paw oedema in rats ($\bar{x} \pm$ sd)

Group		Model control	Dexamethasone acetate	WSD	EEP
n		8	8	8	8
Normal paw cubage (ml)		0.86±0.17	1.11±0.14	1.11±0.10	1.26±0.13
oedema degree (ml)	1 h	0.19±0.15	0.23±0.17	0.12±0.08	0.163±0.09
	2 h	0.59±0.14	0.28±0.17*	0.26±0.14*	0.23±0.11*
	3 h	0.68±0.12	0.19±0.14**	0.34±0.15*	0.30±0.09**
	4 h	0.77±0.12	0.29±0.13**	0.24±0.12**	0.13±0.12**
	5 h	0.61±0.18	0.07±0.08**	0.26±0.02*	0.19±0.10**
	6 h	0.54±0.12	0.08±0.08**	0.18±0.12**	0.16±0.09**

$^{*}P < 0.05$, $^{**}P < 0.01$ compared with model control group.

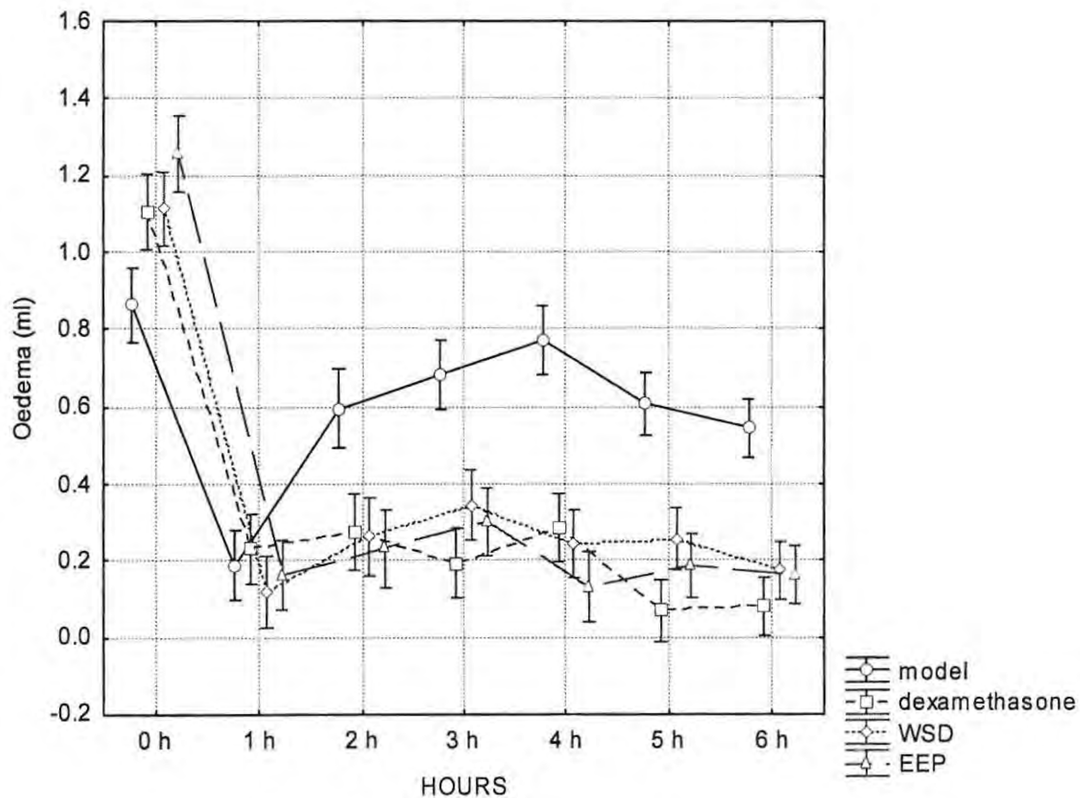

Figure 6.1 Effects of WSD and EEP on paw edema in rats

6.4.3 The effects of Carrageenan-induced pleurisy in rats

The results showed significant effects of propolis on pleurisy extravasate between the groups ($F_{4,34} = 37.4$, $P < 0.0001$), on WBC counts ($F_{4,34} = 15.1$, $P < 0.0001$), on lymphocyte ($F_{4,34} = 25.2$, $P < 0.0001$), and neutrophils ($F_{4,34} = 25.2$, $P < 0.0001$) (table 6.3), and significant effects of propolis on protein level ($F_{4,25} = 27.1$, $P < 0.0001$), NO ($F_{4,25} = 24.5$, $P < 0.0001$) and PGE2 ($F_{4,25} = 21.2$, $P < 0.0001$) in carrageenan-induced pleurisy (table 6.3). The results show that the WBC count in the model control is significantly higher than that of normal control (Tukey: $P < 0.01$), and the ratio of lymphocytes in WBC was significantly lower (Tukey: $P < 0.01$). The ratio of neutrophils is significantly higher (Tukey: $P < 0.01$) than that of the normal control group, typical in acute inflammation, thus indicating that the model was successful. The EEP and WSD had inhibitory effects on the increase of pleurisy extravasate, especially in the group of WSD compared with that of model group (Tukey: $P < 0.01$), the difference was significant. EEP and WSD inhibited the increase of WBC count and neutrophils, which indicated that they could probably alleviate inflammatory reactions but the results were not significant at the 5% level. EEP and WSD of propolis significantly decrease the level of PGE_2 and protein in pleurisy extravasate (Tukey: $P < 0.01$). EEP also significantly decreased the level of NO (Tukey: $P < 0.01$) (table 6.4). WBC, lymphocyte and neutrophils passed Levene's test of homogeneity ($P > 0.05$); pleurisy extravasate failed due to the zero variance in the normal group. All four response variables passed Kolmogorov Smirnov test of normality ($P > 0.20$). Protein, NO and PGE_2 passed Levene's test of homogeneity ($P > 0.05$) and Kolmogorov Smirnov test of normality ($P > 0.10$).

Table 6.3 Effects of propolis on pleurisy extravasate and leucocyte counts in rats ($\bar{x} \pm$ sd)

Group	Dose (ml/100g)	n	Extravas-ate (ml)	WBC ($\times 10^9 \cdot L^{-1}$)	Lymphocyte (%)	Neutrophils (%)
Normal control	-	8	$0.10 \pm 0.00^{**}$	$7.03 \pm 3.66^{**}$	$47.38 \pm 4.57^{**}$	$52.63 \pm 4.57^{**}$
Model control	-	7	1.99 ± 0.35	29.83 ± 7.77	15.14 ± 4.81	84.86 ± 4.81
Prednisone acetate	7.5mg/kg	9	1.62 ± 0.34	25.64 ± 5.31	$27.67 \pm 8.77^{**}$	$72.33 \pm 8.77^{**}$
WSD	1	6	$1.08 \pm 0.34^{**}$	22.07 ± 8.58	25.00 ± 5.62	75.00 ± 5.62
EEP	1	9	1.54 ± 0.44	28.01 ± 7.42	23.67 ± 7.25	76.33 ± 7.25

$^*P < 0.05$, $^{**}P < 0.01$ compared with model control group.

Table 6.4 Effects of propolis on the levels of total protein, NO and PGE_2 in the pleurisy extravasate of rats ($\bar{x} \pm$ sd)

Group	Dose (ml/100 g)	n	Protein (g/L)	NO (umol/L)	PGE_2 (OD)
Normal control	-	6	3.53±0.93**	6.12±2.36**	0.004±0.002**
Model control	-	6	22.73±3.89	32.33±6.68	0.194±0.021
Positive control	7.5mg/kg	6	9.58±3.37**	14.88±4.95**	0.076±0.025**
WSD	1	6	13.64±2.97**	25.15±3.83	0.091±0.067**
EEP	1	6	16.60±4.65*	20.25±5.67**	0.053±0.035**

*$P < 0.05$, **$P < 0.01$ compared with model control group.

6.4.4 The effects of propolis on acute lung damage in rats

The results of propolis on WBC count and lung index in the lung damage model are given in table 6.5. Lung index, neutrophils and lymphocyte response variables failed Levene's test of homogeneity of the variances ($P < 0.01$) but passed Kolmogorov Smirnov test of normality ($P > 0.20$). Kruskal-Wallis nonparametric ANOVA procedures were used since transformation of the variables failed to stabilize the variances between the groups. The results from table 6.5 showed that the WBC count, neutrophils and lung index in lung damage model group were significantly higher than that of normal control, and the ratio of lymphocyte was lower, which indicate that the model was successful (Lung index: $H_{4,30} = 13.7, P = 0.0084$; WBC: $F_{4,25} = 24.9, P < 0.0001$; Neutrophils and lymphocyte: $H_{4,25} = 17.6, P = 0.0014$). EEP and WSD inhibited acute lung damage–induced lung oedema but the results were not significant at the 5% level. EEP had good ability to inhibit the increase of neutrophils (Mann Whitney: $P = 0.024$) and the decrease of lymphocyte (Mann Whitney: $P = 0.024$).

Table 6.5 Effects of propolis on the leucocyte count and lung index of acute lung injury in rats ($\bar{x} \pm$sd)

Group	Dose (ml/100g)	n	Lung index	WBC ($\times 10^9 \cdot L^{-1}$)	Neutrophils (%)	Lymphocyte (%)
Normal control	-	6	0.84±0.06**	5.47±1.43**	17.17±8.18**	82.83±8.18**
Model control	-	6	1.43±0.12	11.03±0.92	58.50±6.83	41.50±6.83
Dexamethasone acetate	7.5 mg/kg	6	1.31±0.22	5.27±1.16**	46.17±22.83	53.83±22.83
WSD	1	6	1.20±0.31	6.10±0.63**	36.67±13.19	63.33±13.19
EEP	1	6	1.14±0.27	10.93±2.43	22.33±10.23*	77.67±10.23*

*$P < 0.05$, **$P < 0.01$ compared with model control group.

6.5 Discussion

In order to study the effects of EEP and WSD on acute inflammatory performance, laboratory animal models were assessed as follows: Carrageenan-induced paw oedema in rats, acetic acid-induced capillary vessel leakage in the mouse abdomen, carrageenan-induced pleurisy and oleic acid plus LPS-induced acute lung damage in rats. The results showed that EEP and WSD both had high inhibitory activity on leakage, oedema, conglomeration and increase of WBC. These results strongly suggest that EEP and WSD have anti-inflammatory properties and alleviated the extent of the inflammatory reaction.

NO could accelerate an inflammatory reaction by enlarging blood vessels to cause oedema. This could increase the expression of inflammatory reactions and accelerate the development of blood poisoning by activating prostaglandin synthesis as occurs in the progress of rheumatism (Schmidt & Walter, 1994; Akarasereenont *et al.*, 1995). In the experiment, EEP and WSD were demonstrated to inhibit the increase of PGE_2, and had a significant inhibitory effect on NO in carrageenan-induced pleurisy exudation. They also decreased the reciprocity between NO and PGE_2 and decreased the activation ("domino effect") of various enzymes, which in turn could decrease the degree of an inflammatory

reaction (Kujumgiev *et al.*, 1999; Song *et al.*, 2002).

Although the mechanism of WSD and EEP on anti-inflammatory performance appeared similar, there were also some differences. In the Carrageenan-induced pleurisy and oleic acid plus LPS-induced acute lung damage studies, WSD not only inhibited the increase of WBC count, but also inhibited the increase of neutrophils (but not significantly at 5%). This would explain how WSD could inhibit the increase of WBC and alleviate inflammatory reactions during an acute inflammatory period. From the results it is possible that propolis components other than flavonoids exert anti-inflammatory effects. Although EEP did not significantly inhibit WBC, it may possibly alleviate the inflammatory degree synergistically by inhibiting NO.

CHAPTER 7

7. THE EFFECTS AND MECHANISM OF PROPOLIS ON ACUTE LUNG INJURY

7.1 Summary

The mechanism by which propolis protects rats against acute lung injury induced by oleic acid and lipopolysaccharide (LPS) was studied. 40 male Wistar rats were divided into 5 groups: normal group, model control group, positive group (dexamethasone), groups treated by propolis solutions extracted by ethanol and water. Rats were first injected with oleic acid through the tail vein, and then given another injection of LPS 4 hours later. The effects of propolis on acute lung injury was evaluated by counting leukocytes and lung index, changes in the pathological samples examined by microscopy and testing the expression activity of NF-κBp65 immuno-histochemically and *in situ* hybridization. The propolis solutions can counteract lung edema, decrease inflammation and inhibit the expression and activation of NF-κBp65. The anti-inflammatory mechanism of propolis is to restrain the activation of NF-κB p65.

7.2 Introduction

Propolis has wide biological properties such as anti-inflammatory and immuno-modulatory effects, scavenges free radicals, improves blood rheology, protects blood vascular endothelial cells, etc. (Banskota *et al.*, 2000a, b; Moon *et al.*, 2001; Li *et al.*, 2001a; Qi *et al.*, 2001; Santos *et al.*, 2002). There are few reports about the effects of propolis on leukocyte adherence and interference with inflammatory rolling, sticking and expression. In this chapter the effects of propolis on inflammation and its possible mechanisms of action are investigated.

7.3 Materials and methods

7.3.1 Materials

Male Wistar rats of 220 ± 20 g birth weight were provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of Laboratory Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. LPS (Lot: 70k4108, O111: B4) and DEPC were purchased from Sigma Company. Dexamethasone (010235) and oleic acid (OA) was purchased from Xianju Pharmacy Ltd.; Antibody of ICAM-1, CD54, BA0541 and κappa B (BA0610) were purchased from Wuhan Boshide Biology Engineer Company, Wuhan, China. Reagent of NF κappa B p65 used *in situ* hybridization and SABC used in immuno-histochemistry were purchased from Wuhan Boshide Biology Engineer Company, Wuhan, China. The reagent of DAB and poly-l-lysine were purchased from Wuhan Boshide Biology Engineer Company, Wuhan, China. Propolis was purchased from Hangzhou Lingzhi Apiary of China. Propolis was produced in the North China in 2001 and the main plant origin was poplar (*Populus sp.*).

7.3.2 Methods (Zhang *et al.*, 2000)

40 male Wistar rats were randomly divided into 5 groups: a normal control group, a model control group, medicine positive control group, and the groups treated with EEP (ethanol extract propolis) and WSD (water soluble derivative of propolis). Rats in the normal control group, EEP and WSD groups were administrated intragastrically respectively with physiological saline, EEP and WSD at the dose of 300 mg/kg body weight for 7 days. Rats in the medicine positive control group were given dexamethasone i.p. at a dose of 0.75 mg/kg body weight for 7 days prior to beginning the experiment. On the eighth day, all rats were treated with oleic acid at a dose of 0.2 ml/kg body weight through the tail vein. 4 hours later

all rats were given LPS at a dose of 2 mg/kg body weight i.v. Two hours later all the rats were killed, their leukocytes counted, and the left lung taken to calculate lung index, the right lung was preserved with 4% polyformaldehyde, then sectioned and stained with a hematoxylin and eosin (HE) stain for pathological examination with a normal microscope. In addition, NF-κBp65 expression activity was tested immuno-histochemically. The detailed procedure was: de-wax the slice of tissue for hydration then treat with 3% H_2O_2 for 10 min at room temperature and avoiding light in order to destroy the activity of endogenetic enzyme. Then the tissue was washed with distilled water three times to rehabilitate the antigen. Then the histological section was placed in 0.01 $mol \cdot L^{-1}$ citrate buffer (pH 6.0), heated in a microwave oven for 5 min, discontinued for 20 min, then re-heated for 5 min and then allowed to cool. The tissue sections were then washed with 0.1M PBS 3 times for 5 min each. Then normal goat serum blocking liquid (1:10) was added and the tissue incubated for 20 min at room temperature. Then rat p65 monoclonal antibody was added and the tissue incubated for 20 min at 37°C and then held over night at 4°C. The next day, the sections were re-washed with 0.1 $mol \cdot L^{-1}$ PBS three times for 2 min and then biotinylated antibody was added for 1 h at room temperature. Sections were then washed with 0.1 $mol \cdot L^{-1}$ PBS 3 times for 2 min, and placed in streptavidin diluent marked with horseradish enzyme and incubated for 20 min at 37°C. They were then washed with 0.1 mol·L-1 PBS 4 times for 5 min, and colour-enhanced with DAB, then washed with tap water, re-dyed with HE, then dehydrated and sealed with moderate balata. Five histological sections chosen randomly were examined by microscopy and photographs obtained for computer image analysis.

7.3.3 Statistical analyses

Data were reported as means ± standard deviations. Comparisons between groups were made using ANOVA. Tukey's and Fisher LSD multiple pairwise comparisons tests were used to test for differences between groups and the model control group.

7.4 Results

7.4.1 Effects of propolis on the leucocyte count and lung index of rats with acute lung injury

The total leucocyte count and lung index in the animal model group challenged twice with oleic acid and LPS were significantly higher than that of normal rats. The change in leucocytes showed that the ratio of lymphocytes decreased and the ratio of neutrophils increased significantly, which indicated the animal model group was successful in our experiment. The propolis solutions extracted by ethanol and WSD had a significant effect on edema caused by acute lung injury (Fisher LSD: $P < 0.05$). EEP and WSD both prevented an increase of neutrophils, and the difference was significant compared with that of model control (Tukey: $P < 0.05$). The detailed results are shown in table 7.1.

Table 7.1 Effects of propolis solutions on the leucocyte count and lung index of acute lung injury in rats (n = 8, $\bar{x}$ ±sd)

Group	Dose $mg \cdot kg^{-1}$	Lung index	Total WBC $\times 10^9 \cdot L^{-1}$	Neutrophils % change	Lymphocyte % change
Normal control	-	$0.84 \pm 0.05^{**}$	$5.48 \pm 1.42^{**}$	$17.13 \pm 8.13^{**}$	$82.88 \pm 8.13^{**}$
Model control	-	1.43 ± 0.12	11.03 ± 0.94	58.50 ± 6.26	41.50 ± 6.48
Dexamethasone	7.5	1.31 ± 0.22	$5.26 \pm 1.16^{**}$	46.13 ± 22.88	53.88 ± 22.33
WSD	300	$1.20 \pm 0.31^{*}$	$6.10 \pm 0.68^{**}$	$36.63 \pm 13.28^{*}$	$63.25 \pm 13.13^{*}$
EEP	300	$1.18 \pm 0.28^{*}$	10.93 ± 2.29	$22.38 \pm 10.31^{**}$	$77.63 \pm 10.31^{**}$

$^{*}P < 0.05$, $^{**}P < 0.01$ compared with that of model control.

7.4.2 Effects of propolis on pathological changes in the morphology of rats with acute lung injury

From the results of the microscopical observations, the lung colour of rats in the normal control group was red, the lung floccular structure was clear, there was no exudation in the alveolus coelom, no inflammation or incrassation of the alveolus wall, and no inflammatory

exudation in bronchial wall. The lung colour in the model control rat tissues was purplish, and there was significant congestive oedema under the alveolar membrane. Oedema was widespread under the alveolar membrane, there was exudation of inflammatory cells indicative of widespread macrophages, and the exudation in alveolus was filled with red serum and inflammatory cells. Inflammatory cells could also be seen in the group of WSD and EEP rats as well as the incrassation of the alveolus wall, but the degree was significantly less than that of model control. This can be seen in figures 7.1-7.3.

7.4.3 The effects of propolis on the expression and activation of NF-κB p65 of rats with acute lung injury.

The result of immuno-histochemical analyses showed that the cytoplasm in normal lung was brown, with few macrophages in the nuclei. The color of the cytoplasm and nucleus in the lung of the model control rats was brown. Most brown particles had entered the nucleus, which showed that NF-κB p65 had been activated, because p65 is mainly expressed in macrophages, fibroblasts and the bronchial epithelium. Both EEP and WSD significantly inhibited the activation of p65 protein (the brown particles were less than that of model control), the total number of positive cells was significantly less than that of the model control in five histological sections that were analyzed ($P < 0.01$), as can be seen in figures 7.4-7.6 and table 7.2.

Table 7.2 The activation of NF-κB p65 in lungs of rats determined by immuno-histochemistry (n = 8, $\bar{x}$ ±sd)

Group	Dose / mg·kg^{-1}	Total positive cells in 5 sections
Normal control	-	192.81±24.26**
Model control	-	635.81±21.16
Dexamethasone	7.5	399.00±34.16**
WSD	300	346.81±44.12**
EEP	300	323.19±45.86**

$^{**}P < 0.01$ compared with model control group.

7.5 Discussion

The experiment showed that both WSD and EEP could inhibit the increase of neutrophils, which suggests that both materials could inhibit inflammatory cells from spreading to an inflamed region and so reduce the degree of inflammation.

NF-κB is a transcription modulation factor in upstream enhancement elements of the k chain in immunoglobulins that occur in many kinds of cells and tissues and has wide biological properties. When NF-κB is increased, it could accelerate the transcription of cytokine, adhesion factor, chemotactic factor (Natarajan *et al.,* 1996; Baeuerle & Baichwal, 1997) and modulate the gene expression of cytokine in an inflammatory medium. Besides these, it also takes part in immune and inflammatory reactions. NF-κB is intimately associated with inflammatory disease, so that its activation would be enhanced in the disease of asthatics, acute decompensation, and rheumatic arthritis. LPS can lead to activation of monocyte NF-κB and when activated can increase the secretion of TNF-α and IL-1. On the other hand, both cytokines reverse NF-κB and lead to a graded reaction of inflammation inducing further injury to the tissues. NF-κB is a dimer comprised of two subunits of p50 and p65; p65 is the expression product of c-rel-one of the carcinoma genes, whereas Rel protein contains a transcription activation region (Wei, 2001).

The experiment showed that propolis solutions extracted by ethanol and water could both inhibit the expression and activation of NF-κBp65 to reduce the synthesis of p65 protein, and effectively control graded responses of inflammation to reduce further damage in an animal with acute lung injury. The mechanism probably was that caffeic acid phenethyl ester (CAPE) in propolis inhibits the activation and expression of NOS, and further inhibits the combination of NF-kB and NO and transcription of mRNA (Song *et al.*, 2002).

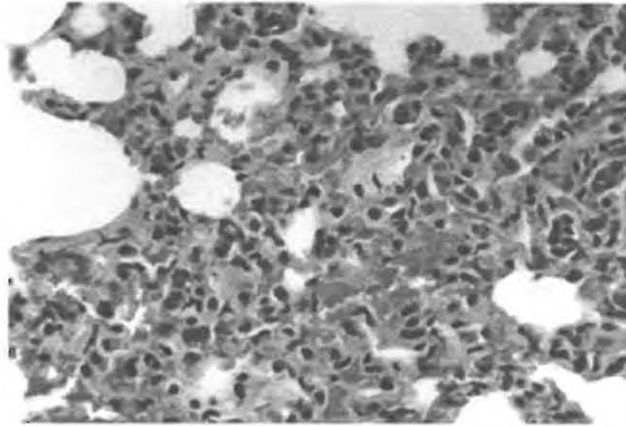

Figure 7.1 HE-stained section of the alveolus in the model group (40×3.3)

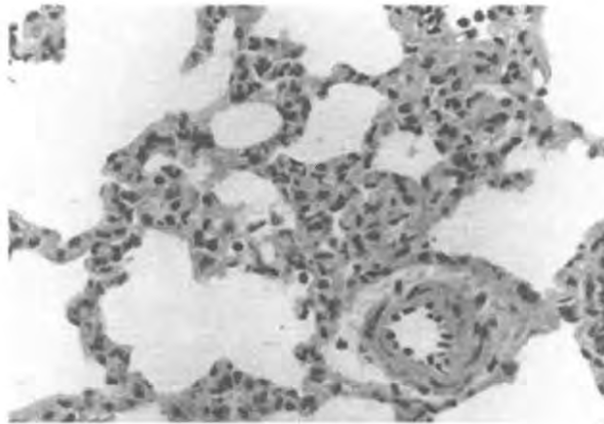

Figure 7.2 HE-stained section of the alveolus in the EEP group (40×3.3)

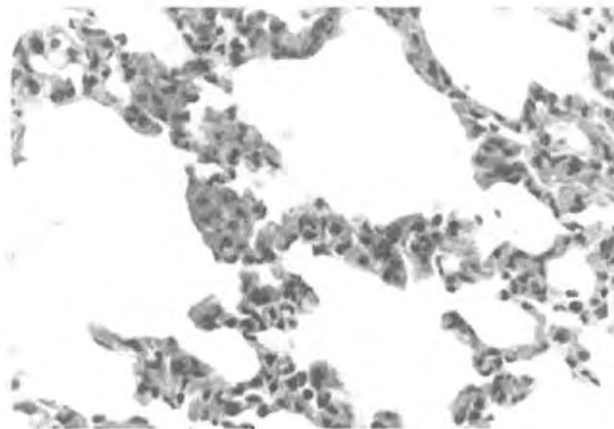

Figure 7.3 HE-stained section of the alveolus in the WSD group (40×3.3)

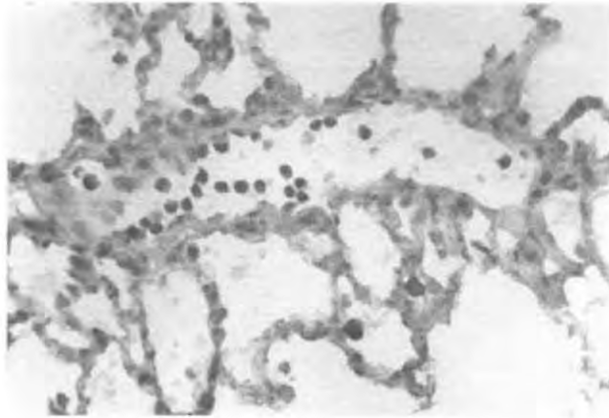

Figure 7.4 Immuno-histochemically stained section of alveolus in model group (100×3.3)

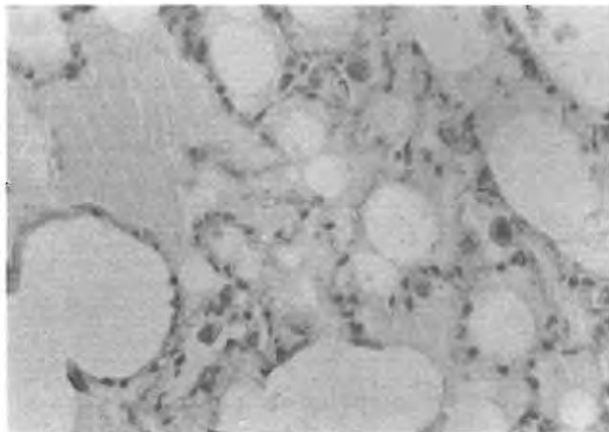

Figure 7.5 Immuno-histochemically stained section of alveolus in EEP group (100×3.3)

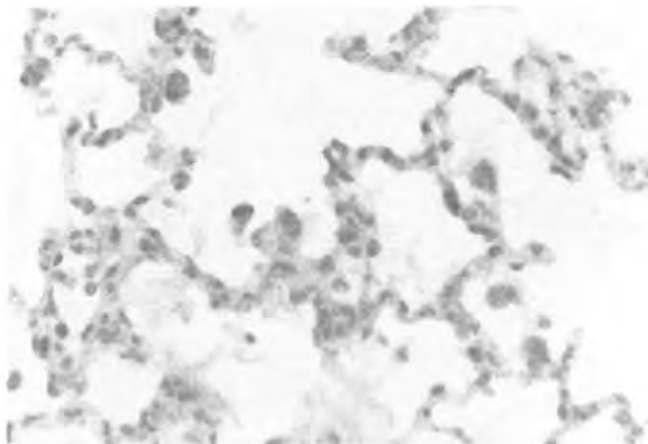

Figure 7.6 Immuno-histochemically stained section of alveolus in WSD group (100×3.3)

CHAPTER 8

8. EFFECTS OF PROPOLIS ON BLOOD LIPID AND LIVER LIPID IN HYPERLIPIDEMIC SD RATS

8.1 Summary

The effects of water and ethanol extracts of propolis on blood lipid and liver lipid of hyperlipidemic SD rats were studied. The results showed that both preparations of propolis had an inhibitory effect on the level of triglyceride (TG), total cholesterol (TC), low density lipo cholesterol (LDL-C), glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) in serum, and TC, TG and malonaldehyde (MDA) in liver; but the two extracts were without effects on high density lipo cholesterol (HDL-C), MDA, superoxide dismutase (SOD) and nitric oxide (NO) in serum. Ethanol extracts of propolis also reduced body weight, liver weight and liver index of hyperlipidemic SD rats, but the water-extracted propolis could not reduce those indexes. The results showed that the two extracts contribute to the improvement of lipid metabolism in hyperlipidemic SD rats and provide them with the required anti-oxidative activity.

8.2 Introduction

Propolis has wide biological properties such as anti-inflammatory, anti-viral, immuno-modulating, softening blood vessels, clearing blood, modulating microcirculation, anti-oxidation and anti-tumor among others (Marcucci, 1995; Isla *et al.*, 2001; Hu *et al.*, 2003; Hu & Xuan, 2003). In fact, hyperlipidemia is a disease of the hyperlipoprotein kind, in which the concentration of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) in blood serum is too high. Hyperlipidemia and hyperlipoprotein are

the major factors associated with the induction of arteriosclerosis. In recent years, there have been some reports on the effect of propolis as a cure for hyperlipidemic disease (Fang, 1998b; Stefano & Francesco, 2002), but the normative experiments are absent. In this chapter, studies on the effects of propolis on hyperlipidemia, liver lipid and anti-oxidation are reported.

8.3 Materials and methods

8.3.1 Materials

8.3.1.1 Animals

Male rats (strain SD) of about 220 ± 20 g were provided by The Shanghai Laboratory Animal Center of The Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of Laboratory Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. The animals were maintained and the experiments performed according to the principles of the Helsinki accord. The experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, Hangzhou, China.

8.3.1.2 Drugs and reagents

Propolis was supplied by Hangzhou Lingzhi Apiary of China. Propolis was produced in North China in 2002 and the main plant origin is poplar (*Populus sp.*); WSD (water soluble derivatives of propolis) was extracted using a natural surface active agent and ultra sonicated at 80°C for 12 h. EEP (ethanol extracted propolis) was extracted in 80% ethanol.

Xuezhikang capsules (a regulator for blood lipid extracted from a Chinese medicine

"red koji") were supplied by Weixin Biology Science and Technique Company Ltd., Beijing, China. Reagents for total cholesterol (TC), triglycerides (TG), high density lipo-cholesterol (HDL-C), and low density lipo-cholesterol (LDL-C) were purchased from Cicheng biochemistry Company Ltd., Ningbo, China. Reagents for malonaldehyde (MDA), nitric oxide (NO) and superoxide dismutase (SOD) were provided by Jiancheng Biology Engineering Research Institute, Nanjing, China. Others reagents were all A.R., made in China.

8.3.2 Methods

8.3.2.1 Replication of hyperlipidemia in SD rats

Blood was taken from the cut tip of the tails of SD rats having fasted 12 hours and the serum centrifuged at 3000 r/min for 10 min to test for TC and TG. According to the level of TC, all SD rats were randomly divided into seven groups of ten rats each, and at the same time, all rats were fed with hyper-lipid feed (0.5% bile, 1% cholesterol, 10% pig fat, 10% yolk, 78.5% common feed). Two weeks later, blood was taken from all SD rats to test TC and TG to determine if all SD rats had become hyperlipidemic. According to the level of TC all SD rats were randomly divided into 10 groups: the normal control group, model control, positive control (Xuezhikang), WSD1 (low dose of WSD), WSD2 (high dose of WSD), EEP1 (low dose of WSD), EEP2 (high dose of WSD) groups and then, all SD rats were treated with different drugs for 28 days according to groups.

8.3.2.2 Treatment methods for hyperlipidemic SD rats

The group of WSD: rats in WSD1 group were given propolis intragastrically at a rate of 50 mg/kg body weight, and the dry material of propolis was 2.5 mg/mL; rats in the WSD2 group were given propolis intragastrically at a rate of 100 mg/kg body weight, and the dry material of propolis was 5 mg/mL.

The group of EEP: rats in the EEP1 group were given propolis intragastrically at a rate of 50 mg/kg body weight, and the dry material of propolis was 2.5 mg/mL; rats in EEP2 group were given propolis intragastrically at a rate of 100 mg/kg body weight, and the dry material of propolis was 5 mg/mL.

The group of positive control: rats in Xuezhikang group were given the drug intragastrically at a rate of 600 mg/kg body weight, and Xuezhikang was diluted to 30 mg/mL.

Rats in the normal and model control groups were given physiological saline intragastrically 0.9% at a rate of 2 ml/100 g body weight.

All rats were treated intragastrically twice a day at 9:00 am and 3:00 pm.

8.3.2.3 Methods of measurement

Blood was taken from the cut tip of the tails of all SD rats after fasting 12 hours every other two weeks and the serum was centrifuged at 3000 r/min for 10 min to test TC and TG. At the end of the experiment, blood was taken from all SD rats from the eyeball and the serum centrifuged to test other biochemical indexes including TC, TG, HDL-C, LDL-C, GOT, GPT, SOD, MDA, NO. The livers were ground in 10% physiological saline to test TC, TG, MDA. The test methods were done according to the specifications for each reagent.

8.3.3 Statistical analyses

Data were reported as means ± standard deviations. Comparisons between groups were made using ANOVA and repeated measures ANOVA procedures. Tukey and Fisher LSD multiple pairwise comparisons tests were used to test for differences between groups

and the model control group.

8.4 Results

8.4.1 Effects of propolis on blood lipid and arteriosclerosis (AS) in hyperlipidemic SD rats

Changes of TC and TG in serum in hyperlipidemic SD rats for six weeks are given in table 8.1. The results of a repeated measures ANOVA showed a significant effect of propolis on TC and TG in serum between the groups (TC: $F_{6,63} = 27.0$, $P < 0.0001$; TG: $F_{6,63} = 12.5$, $P < 0.0001$), times (TC: $F_{3,189} = 211.3$, $P < 0.0001$; TG: $F_{3,189} = 124.4$, $P < 0.0001$) and interactions of groups x time (TC: $F_{18,189} = 9.9$, $P < 0.0001$; TG: $F_{18,189} = 5.9$, $P < 0.0001$). The results show that the level of TC and TG of rats fed hyperlipid feed were higher than that of the normal control group two weeks later ($P < 0.05$). The levels of TC and TG in the control groups WSD1, WSD2, EEP1 and EEP2 were significantly lower compared with that of model control after six weeks (LSD: $P < 0.01$).

The effects of propolis and Xuezhikang on HDL-C, LDL-C and AS of hyperlipidemic SD rats are given in table 8.2. The results show that HDL-C in each group was significantly decreased ($P < 0.05$) and LDL-C was significantly increased ($P < 0.05$) for Xuezhikang, WSD1 and WSD2 groups compared with that of normal control, which demonstrates that the model of hyperlipidemic rats was a success. After six weeks of treatment, the difference in HDL-C in each group was not significant, but did inhibit the increase of LDL-C ($P < 0.05$), especially in the group of Xuezhikang, EEP1 and EEP2; LDL-C respectively decreased from 33.1%, 49.8% and 44.7% compared with that of model group; moreover, each group, except the model control group, decreased AS ($P < 0.01$)

Table 8.1 Blood TC and TG (mmol/L) changes of hyperlipidemic rats for six weeks ($\bar{x} \pm sd$)

Group	n	zero week		2nd week		4th week		6th week	
		TC	TG	TC	TG	TC	TG	TC	TG
Normal control	10	2.09±0.31	1.10±0.27	1.96±0.21**	1.84±0.33**	1.51±0.23**	1.17±0.28*	2.63±0.57**	1.53±0.25**
Model control	10	2.16±0.24	1.01±0.29	3.55±0.42$^{\Delta}$	2.54±0.60	4.24±0.76$^{\Delta}$	1.67±0.49	6.07±1.07$^{\Delta}$	3.19±1.16$^{\Delta}$
Xuezhikang	10	2.14±0.12	1.15±0.16	3.62±0.38$^{\Delta}$	2.89±0.82$^{\Delta}$	3.33±0.74$^{**\Delta}$	0.95±0.19**	4.22±1.57$^{**\Delta}$	1.53±0.35**
WSD1	10	2.12±0.30	1.29±0.39	3.42±0.31$^{\Delta}$	2.87±0.39$^{\Delta}$	3.71±0.54$^{\Delta}$	1.34±0.38	4.81±0.41$^{**\Delta}$	2.51±0.70$^{**\Delta}$
WSD2	10	2.08±0.26	1.16±0.36	3.45±0.49$^{\Delta}$	2.70±0.86$^{\Delta}$	3.35±0.70$^{*\Delta}$	1.36±0.34	4.17±0.62$^{**\Delta}$	1.71±0.74**
EEP1	10	2.19±0.24	1.09±0.20	3.52±0.35$^{\Delta}$	2.62±0.57	3.24±0.53$^{**\Delta}$	1.15±0.29*	3.99±0.61$^{**\Delta}$	1.19±0.50**
EEP2	10	2.00±0.17	1.07±0.28	3.51±0.43$^{\Delta}$	2.65±0.40$^{\Delta}$	3.34±0.38$^{**\Delta}$	1.13±0.24*	3.92±0.77$^{**\Delta}$	1.33±0.39**

Compared with model, * $P < 0.05$, ** $P < 0.01$; compared with normal group, $^{\Delta}P < 0.05$.

Table 8.2 Effects of propolis on HDL-C, LDL-C and AS in hyperlipidemic rats ($\bar{x}$ ±sd)

Group	n	HDL-C(mmol/L)	LDL-C(mmol/L)	AS
Normal control	10	2.20±0.45**	1.09±0.23**	0.63±0.20**
Model control	10	1.24±0.26$^{\Delta}$	2.93±0.64$^{\Delta}$	4.06±1.30$^{\Delta}$
Xuezhikang	10	1.25±0.26$^{\Delta}$	1.96±0.77**$^{\Delta}$	2.37±0.80**$^{\Delta}$
WSD1	10	1.46±0.34$^{\Delta}$	2.40±0.21$^{\Delta}$	2.13±0.39**$^{\Delta}$
WSD2	10	1.53±0.47$^{\Delta}$	2.03±0.37**$^{\Delta}$	2.03±0.56**$^{\Delta}$
EEP1	10	1.39±0.70$^{\Delta}$	1.47±0.52**	2.44±0.93**$^{\Delta}$
EEP2	10	1.36±0.35$^{\Delta}$	1.62±0.63**	2.41±0.84**$^{\Delta}$

Compared with model, * $P < 0.05$, ** $P < 0.01$; compared with normal group, $^{\Delta}$ $P < 0.05$.

8.4.2 Effects of propolis on body and liver weight, liver index, TC and TG in liver, GPT and GOT in serum of hyperlipidemic SD rats

The effects of propolis on body weight, liver weight, liver index, TC and TG in liver, GPT and GOT in serum of hyperlipidemic SD rats are given in table 8.3. The results show that the effect of Xuezhikang on TC and TG in liver was not significant compared with that of model control, but decreased GOT ($P < 0.05$) and GPT ($P < 0.01$) in serum; WSD1 significantly decreased TC, and the effect on TG was not significant although the trend was to decline. WSD2, EEP1 and EEP2 significantly decreased the level of TG and TC compared with that of the model control group, and at the same time, they also decreased the level of GOT and GPT. The group of Xuezhikang and EEP significantly decreased liver index ($P <$ 0.05), but the effect of WSD was not significant ($P > 0.05$).

Table 8.3 Effects of propolis on body weight, liver weight, liver index, TC and TG in liver, GPT and GOT in serum of hyperlipidemic rats ($\bar{x} \pm$sd)

Group	n	TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
Normal control	10	$0.33\pm0.08^{**}$	1.08 ± 0.14	$29.7\pm3.9^{**}$	$44.2\pm10.1^{**}$	$358.4\pm28.4^{**}$	$7.75\pm0.89^{**}$	$2.16\pm0.29^{**}$
Model control	10	$2.04\pm0.40^{\Delta}$	$2.90\pm0.59^{\Delta}$	$69.2\pm11.9^{\Delta}$	$71.4\pm11.5^{\Delta}$	$437.9\pm25.4^{\Delta}$	$16.42\pm1.21^{\Delta}$	$3.75\pm0.43^{\Delta}$
Xuezhikang	10	$1.82\pm0.64^{\Delta}$	$2.61\pm0.62^{\Delta}$	$36.9\pm9.4^{**}$	$57.7\pm16.4^{*}$	$410.8\pm27.3^{*\Delta}$	$11.31\pm0.98^{**\Delta}$	$2.75\pm0.33^{**\Delta}$
WSD1	10	$1.63\pm0.44^{*\Delta}$	$2.51\pm0.55^{\Delta}$	$56.4\pm10.9^{*\Delta}$	$43.0\pm5.1^{**}$	$434.2\pm35.7^{\Delta}$	$15.46\pm1.45^{\Delta}$	$3.56\pm0.45^{\Delta}$
WSD2	10	$1.40\pm0.22^{**\Delta}$	$2.28\pm0.43^{**\Delta}$	$46.3\pm8.9^{**\Delta}$	$41.1\pm14.2^{**}$	$418.8\pm22.5^{\Delta}$	$15.29\pm1.32^{*\Delta}$	$3.65\pm0.39^{\Delta}$
EEP1	10	$1.60\pm0.40^{*\Delta}$	$2.39\pm0.53^{*\Delta}$	$35.3\pm5.9^{**}$	$35.6\pm6.0^{**}$	$410.2\pm16.6^{*\Delta}$	$13.95\pm0.96^{**\Delta}$	$3.40\pm0.31^{*\Delta}$
EEP2	10	$1.67\pm0.36^{*\Delta}$	$2.40\pm0.38^{*\Delta}$	$34.0\pm6.5^{**}$	$38.6\pm8.7^{**}$	$406.6\pm29.7^{*\Delta}$	$12.34\pm1.12^{**\Delta}$	$3.03\pm0.34^{**\Delta}$

Compared with model, * $P < 0.05$, ** $P < 0.01$; compared with normal group, $^{\Delta} P < 0.05$.

8.4.3 Effects of propolis on MDA, SOD, NO in serum and MDA in livers of hyperlipidemic SD rats

The effects of propolis on MDA, SOD, NO in serum and MDA in livers of hyperlipidemic SD rats are given in table 8.4. The results show that all groups except WSD1 and Xuezhikang groups significantly decreased the level of MDA ($P < 0.05$) in liver; all groups decreased the level of MDA in serum but the decreases were not significant. The differences in all groups were not significant for SOD compared with that of the model group; Xuezhikang very significantly increased the level of NO ($P < 0.01$), whereas the differences in the propolis groups were not significant.

Table 8.4 Effects of propolis on MDA, SOD, NO in serum and MDA in livers of hyperlipidemic rats ($\bar{x}$ ±sd)

Group	n	MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
Normal control	10	179.9±13.7**	8.26±0.88**	131.4±6.6*	7.80±2.0**
Model control	10	232.5±13.2$^{\Delta}$	11.52±1.23$^{\Delta}$	119.6±9.2$^{\Delta}$	15.5±4.1$^{\Delta}$
Xuezhikang	10	225.3±17.6$^{\Delta}$	12.58±1.48$^{\Delta}$	116.5±8.4$^{\Delta}$	21.3±3.0$^{*\Delta}$
WSD1	10	223.1±18.7$^{\Delta}$	11.70±1.47$^{\Delta}$	116.6±12.2$^{\Delta}$	16.3±4.7$^{\Delta}$
WSD2	10	210.8±18.6$^{*\Delta}$	11.45±2.04$^{\Delta}$	123.4±4.3	18.1±3.4$^{\Delta}$
EEP1	10	213.6±22.3$^{*\Delta}$	10.54±1.45$^{\Delta}$	121.8±8.1	16.2±5.5$^{\Delta}$
EEP2	10	214.9±15.8$^{*\Delta}$	11.28±1.06$^{\Delta}$	121.5±7.5	16.7±3.2$^{\Delta}$

Compared with model, * $P < 0.05$, ** $P < 0.01$; compared with normal group, $^{\Delta}$ $P < 0.05$.

8.5 Discussion

Previous studies showed that increased levels of TC, TG, LDL-C and decreased levels of HDL-C are the major factors in the induction of arteriosclerosis and cardiovascular disease (Zhang & You, 1998). From the results of the present experiments, we found that Xuezhikang, WSD, EEP all inhibited an increase of TC, TG, LDL-C caused by hyperlipid feed, and very significantly decreased the level of AS which showed that they were effectively hyperlilpidemic rats. It is noteworthy that the effect of Xuezhikang and EEP was greater than others; and the level of HDL in hyperlipidemic rats decreased, while Xuezhikang, WSD, and EEP had no significant effect on HDL-C. That propolis might modulate hyperlipidemia is apparently related to the composition of propolis (Fang, 1998b; Stefano & Francesco, 2002), especially the flavonoids, which can exceed 12% of propolis. Flavonoids could modulate hyperlipidemia probably by two different mechanisms: control of HMG CoA deoxidase in the synthesis of cholesterol, or, flavonoids also could inhibit the activity of phosphodiesterase thus delaying the

decomposition of cAMP to inhibit the activity of HMG CoA deoxidase. Such mechanisms could well protect liver and decrease the synthesis of peroxidation (Havsteen, 2002).

Blood lipid disturbance is an important factor for liver fat and damage, when the gastroduodenum takes fat in excess of normal needs, superfluous NEFA, the precursor of TG, is released. If the synthesis of TG is in excess of that of VLDL, this would lead to the sequestration of TG to form liver fat. And, VLDL would transfer TG finally to LDL. From the results of this experiment, hyperlipidemic rats' body weights are greater than that of normal rats, the level of GOT and GPT in serum is increased, and the livers became enlarged and jaundiced. Xuezhikang and EEP significantly decreased the sequestration of fat, the enlargement of the liver and liver index, whereas the effect in WSD was not significant. Xuezhikang, WSD and EEP all decreased the levels of ALT, AST in blood so inhibiting the increase of MDA to protect liver.

Studies showed that there is a relationship between arteriosclerosis and liver damage and lipidoxidation, and that the levels of TC and TG in blood has a positive relationship with lipidoxidation, so that LDL would form OX-LDL under peroxidation, which is the major factor causing the accumulation of lipid and foam producing cells (Chisolm & Marc, 1994). The present experiments showed that the level of MDA in the serum of rats fed hyperlipid feed resulted in enhanced peroxidation. Xuezhikang, WSD, EEP all decreased the levels of MDA in liver, whereas MDA in serum tended to decline. None of the groups exhibited any significant effects on SOD. Besides these, compared with that of normal control, the level of NO increased in hyperlipidemic rats, which was not in accordance with other reports (Wu & Sun, 2003). Previous studies showed that there are at least two components which have anti-oxidative effect, one is the compound with the structure of C_6-C_3-C_6, in which the main mechanism was to chelate metal ions to inhibit the formation of hydroxyl free radicals, which are also important constituents of propolis, the other is caffeic acid phenethyl ester (CAPE), which can form xanthine oxidase to reduce the production of free radicals (Russo *et al.*, 2002).

From these experiments, it was found that propolis extracted in water or ethanol could both decrease hyperlipidemia and protect the liver against oxidation, but the effect of EEP was better than WSD. Besides, propolis has no significant toxic effects (Karsten, 2001), and is an effective, low toxicity material that can protect liver and modulate blood lipid. It provides a broad background for further exploration.

CHAPTER 9

9. DETERMINATION OF THE ANTI-TUMOR AND ANTI-INFLAMMATORY EFFECTS OF DIFFERENT PROPOLIS EXTRACTS

9.1 Summary

The flavonoids of different propolis extracts and their pharmacological effects were assayed for their biological activity on S_{180} entity-tumor mice, chronic tampon granuloma mice, acute pleurisy rats and acute arthritic rats. The 80% ethanol extracts of propolis had the highest flavonoid content (51.36 mg/ml); the flavone content in water extracts of propolis obtained with a natural surface-active agent, under ultrasonic perturbation at 80°C for 12 h, was 6.7 times as that of propolis extracted with water at room temperature. Compared to the ethanol extracts, the water extracts of propolis have the same or a greater anti-tumor or anti-inflammation effect at the same dosage as did the ethanol extracts. Water extracts of propolis show considerable pharmacological potential especially because of its low side effects and ease of preparation.

9.2 Introduction

Recently, there have been several reports on the anti-tumor effects of propolis (Johnson *et al.*, 1996; Sato & Miyataka, 1999; Banskota *et al.*, 2002), but published data on the relationship between extraction methods and effects are wanting. In this chapter, the flavonoid content of propolis extracted by different methods is discussed as well as the effects of WSD (water-soluble derivatives of propolis) and EEP (ethanol extracts of propolis)

on S_{180} entity-tumor mice, chronic tampon granuloma mice, acute pleurisy rats and acute arthritic rats were also studied in order to further assess the pharmacological effect of propolis.

9.3 Materials

9.3.1 Materials

Propolis was purchased from Hangzhou Lingzhi Apiary of China. The propolis was produced in North China in 2001 and the main plant of origin was poplar (*Populus sp.*). Carrageenan (Lot: 117H0151) was purchased from Sigma Company, prednisone acetate (010772) and dexamethasone (010235) were purchased from Xianju Pharmacy Company, Zhejiang, China; the reagent of cholesterol was purchased from Cicheng Biochemistry Company, Ningbo, China.

9.3.2 Animals

Male Wistar rats of about 200 ± 20 g and ICR mice of about 20 ± 2 g were provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of Laboratory for Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China. The animals were maintained in keeping with the Helsinki accord and the experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, China.

9.4 Methods

9.4.1 The preparation of propolis solutions and measurement of flavonoid content

EEP: The crude propolis samples were extracted in ethanol as the solvent at 40%, 50%, 60%, 70%, 75%, 80%, 90% and 95% and then filtered; WSD-1: propolis was extracted with water at room temperature. WSD-2: propolis was extracted with a special method with water using a natural surface-active agent and ultrasonicated at 80°C for 12 h. The methods for measuring the flavonoid content of propolis obtained by different methods were compared against a fagopyrol standard curve (See section 5.3.2.1.). Each sample was measured three times and averaged (cf. table 9.1).

9.4.2 The effect of propolis on S_{180} entity-tumor, chronic tampon granuloma in mice

40 mice were inoculated with S_{180} entity-tumor cells below the axilla under asepsis (Li, 1991). The mice were randomly divided into 4 groups of 10 mice each (5 male and 5 female). Groups included the model control, a group given propolis extracted with 95% ethanol, a group given propolis extracted with 80% ethanol and the WSD-2 group. Each mouse was given intragastrically 600 mg/kg propolis (measured as crude propolis) once a day, and the mice in the control group were given distilled water intragastrically. The experiment ran for 10 days and on the 11th day, all mice were killed, the tumor cell tissue removed and weighed to determine the effective anti-tumor rate.

48 male ICR mice were implanted with a disinfectant tampon 10 ± 1 mg to induce granuloma (Chen, 1993). The surgical wounds of the mice were treated 4 times with two drops of penicillin as an anti-infectant. The mice were then divided into four groups: the model control group, positive control (prednisone acetate), WSD-2 and EEP groups with 80% ethanol. Mice in the propolis groups were given intragastically EEP or WSD at a dosage of 600 mg/kg and treated once daily continuously for 7 days. On the 8th day, all

mice were killed and the tampon with granuloma removed for weighing, then dried at 60°C for 14 hours and then the granuloma weighed.

9.4.3 The effect of propolis on Carrageenan-induced paw oedema in rats (Xu *et al.*, 1991)

32 male Wistar rats were randomly divided into 4 groups, and given intragastrically (1) EEP (propolis extracted with 80% ethanol), (2) WSD-2, (3) sterile saline (normal control group), or (4) dexamethasone acetate (positive control group) respectively and treated once daily continuously for 5 days. The right hind paw cubage of all rats was measured before treatment. 30 minutes later, each rat was treated (intradermic injection) with 1% Carrageenan (0.1 ml) on the pad of the right hind paw, and given sterile saline (4 ml/100 g) intraperitoneally. Inflammation, measured as paw cubage, was measured hourly for six hours to assess possible anti-inflammatory effects on oedema.

9.4.4 The effect of propolis on Carrageenan-induced pleurisy in rats (Xu *et al.*, 1991)

60 male Wistar rats were randomly divided into 5 groups, and given intragastrically (1) EEP (propolis extracted with 80% ethanol), (2) WSD-2, (3) sterile saline (model control group), (4) sterile saline (normal control group), or (5) prednisone acetate (positive control group) respectively and treated once daily continuously for 7 days. 30 minutes after the initial treatments, all the rats (except those in normal control group) were injected with 1% Carrageenan (0.2 ml/100 g) in the right thorax after light anaesthesia with ether, while rats in the normal control group were treated with sterile saline. Inflammation was induced within 4 hours. Eight hours later, all the rats were killed by decollation and the extravasate in the thorax collected by aspiration. Each thorax was then washed with 2 ml sterile saline, after which the residual extravasate and washing solution were mixed. 100 μl of the solution was used to count WBC, the rest was centrifuged and stored at -20°C. An aliquot of 0.5 ml of supernatant was used to measure protein levels; while 0.15 ml solution and 1 ml 0.5 N KOH-methanol was used to measure optical density (OD) at 278 nm with a

TU-1001 ultra- spectrophotometer after incubation in water at 50°C for 20 minutes, and the OD value measured to calculate the content of prostaglandin-E_2 (PGE_2). Another solution sample of 200 μl was used to measure nitric oxide (NO) levels (Tan, 2001).

9.4.5 Statistical analyses

Data were reported as means ± standard deviations. Comparisons between groups were made using ANOVA and repeated measures ANOVA procedures. Tukey and Fisher LSD multiple pairwise comparisons tests were used to test for differences between groups and the model control group.

9.5 Results

9.5.1 The flavonoid content of propolis extracts

The content of flavonoids in propolis extracted with 80% ethanol was 51.36 mg/ml, which was the highest yield. For propolis extracted with 70% and 90% ethanol, the content was 27.10 mg/ml and 26.07 mg/ml respectively; the lowest yield was that extracted with 40% ethanol. The flavonoid content in WSD-1 was only 1.91 mg/ml, whereas the content in WSD-2 was 12.76 mg/m. The detailed results are given in table 9.1.

Table 9.1 The flavonoid content of propolis extracts

Extraction methods	Concentration of ethanol							WSD	
	40% EEP	50% EEP	60% EEP	70% EEP	80% EEP	90% EEP	95% EEP	WSD-1	WSD-2
Flavonoid content mean	4.73	9.23	21.52	27.10	51.36	26.07	22.64	1.91	12.76
Std. Dev.	0.03	0.04	0.03	0.05	0.06	0.04	0.03	0.01	0.04

9.5.2 Effects of propolis extracts on S_{180} tumor growth in mice

The tumor weight of mice in the group, which were administered propolis, was significantly lower than that of model control ($P < 0.01$). The inhibition of the tumor rate was 55.75%-80.78%, and the group administered WSD-2 had the highest inhibition rate, next was the group treated with 80% EEP.

In the experiment of chronic tampon granuloma, mice in groups 80% and 95% EEP and WSD-2 all exhibited an inhibitory effect compared with that of the model control, the difference was significant ($P < 0.01$). The effect in the group of WSD-2 corresponded to that of the prednisone acetate group. The detailed results are given in tables 9.2 and 9.3.

Table 9.2 Effect of propolis extracts on S_{180} tumor growth in mice

Groups	Dose (mg/kg)	n	Average tumor weight (g)	Inhibitory tumor rate (%)
Model control	-	10	1.26±0.03	-
95% EEP	600	10	0.56±0.06**	55.75
80% EEP	600	10	0.41±0.01**	67.36
WSD-2	600	10	0.24±0.01**	80.78

Compared with that of model control, $^{**}P < 0.01$

Table 9.3 Effects of propolis extracts on the weight of granuloma in mice

Group	Dose (mg/kg)	n	Weight of granuloma (mg)
Model control	600	12	34.74±1.30
Prednisone acetate	10	12	21.17±1.03**
WSD	600	12	21.23±1.06**
80% EEP	600	12	24.80±2.18**

Compared with that of model control, $^{**}P < 0.01$

9.5.3 Effects of propolis on Carrageenan-induced paw oedema in rats

Mice in the groups of WSD and EEP exhibited a significant inhibition effect on right hind paw edema caused by carrageenan two hours after administration. The degree of right hind paw edema in the mice of each group was significant, and the degree of edema was highest in the model control and WSD groups four hours later, whereas in the dexamethasone and EEP groups required three hours to reach their highest values. The results are given in table 9.4.

9.5.4 Effects of propolis on Carrageenan-induced pleurisy in rats

The total white blood cells in the model group was significantly higher than that of normal group, and the ratio of lymphocyte decreased, whereas neutrophils increased in the white blood cell fraction, which demonstrated that the pleural model was successful. EEP and WSD had significant antagonistic effects on more pleural exudation (EEP: $P < 0.05$; WSD: $P < 0.01$). EEP had no significant effect on total white blood cells, but it had a trend to significantly inhibit the increase of neutrophils (LSD: $P < 0.05$). EEP and WSD also had a significant effect on reducing the output of NO and PGE_2 in the pleural exudation, and could significantly reduce the output of protein. The results are given in tables 9.5 and 9.6.

Table 9.4 Effects of propolis solutions on edema of the right hind paw induced by Carrageenan

Group	n	Paw cubage of normal mice (ml)	The degree of edema (ml)					
			1h	2h	3h	4h	5h	6h
Model control	8	0.86±0.17	0.19±0.15	0.59±0.14	0.68±0.12	0.77±0.12	0.61±0.18	0.54±0.12
Dexamethasone	8	1.11±0.14**	0.23±0.17	0.28±0.17**	0.19±0.14**	0.29±0.13**	0.07±0.08**	0.08±0.08**
WSD	8	1.11±0.10**	0.12±0.08	0.26±0.14**	0.34±0.15**	0.24±0.12**	0.26±0.02**	0.18±0.12**
EEP	8	1.26±0.13**	0.16±0.09	0.23±0.11**	0.30±0.09**	0.13±0.12**	0.19±0.10**	0.16±0.09**

Compared with that of model control, $^{**}P < 0.01$

Table 9.5 Effects of propolis on pleurisy extravasate and leucocyte counts in rats

Group	Dose (mg/kg)	n	Exudation (ml)	Total WBC ($\times 10^9 \cdot L^{-1}$)	Neutrophils/ %
Normal control	-	8	0.10±0.00**	7.03±3.66**	52.63±4.57**
Model control	-	7	1.99±0.35	29.83±7.77	84.86±4.81
Prednisone acetate	7.5	9	1.62±0.34*	25.64±5.31	72.33±8.77**
WSD	300	6	1.08±0.34**	22.07±8.58*	75.00±5.62*
EEP	300	9	1.54±0.44*	28.01±7.42	76.33±7.25*

Compared with that of model control, LSD: *$P < 0.05$, **$P < 0.01$

Table 9.6 Effects of propolis on the levels of total protein, NO and PGE_2 in the pleurisy extravasate of rats

Group	Dose (mg/kg)	n	Protein (g/L)	NO (umol/L)	PGE_2 (OD)
Normal control	-	6	3.53±0.93**	6.12±2.36**	0.004±0.002**
Model control	-	6	22.73±3.89	32.33±6.68	0.194±0.021
Positive control	7.5	6	9.58±3.37**	14.88±4.95**	0.076±0.025**
WSD	300	6	13.64±2.97**	25.15±3.83*	0.091±0.067**
EEP	300	6	16.60±4.65*	20.25±5.67**	0.053±0.035**

Compared with that of model control, LSD: *$P < 0.05$, **$P < 0.01$

9.6 Discussion

9.6.1 The anti-tumor effects and the flavonoid contents of propolis

There are many studies on the anti-tumor effects of propolis (Matsuno *et al.*, 1997; Scheller *et al.*, 1989; Kimoto *et al.*, 1998, 2000; Kawabe *et al.*, 2000). In this experiment,

propolis was proved to have good anti-tumor effects according to S_{180} entity-body tumor mice and the edema model. The inhibitory effect on tumor rate in the group of WSD was some 80.78%, which was far greater than that of propolis extracted in 95% ethanol. This result was consistent with that of other findings (Mitamura *et al.*, 1996; Gao *et al.*, 2000). The flavonoid content in propolis extracted by different methods was significantly different. The highest value was that of propolis extracted with 80% ethanol. The flavonoid content in propolis extracted by a special method with water (use natural surface active agent, under ultrasonic, 80°C, 12 h) was 6.7 times than that of extracted by common water at room temperature.

9.6.2 The interaction of the inflammatory medium

WSD and EEP had marked anti-inflammatory effects on acute arthritis and acute pleurisy, as well as inhibitory effects on exudation, edema, collecting white blood cells and reducing inflammatory reactions. NO could accelerate inflammatory reactions by stretching blood vessels to form edema and increasing the exudation of inflammatory materials to accelerate the progress of blood poisoning, and activating prostaglandins in the process of rheumatism (Sun & Xu, 2002). This experiment showed that WSD and EEP could inhibit the increase of PGE_2, and inhibit the output of NO in exudation in pleurisy caused by Carrageenan, decrease the interaction of NO and prostaglandins to decrease activation of a range of enzyme to decrease the degree of inflammation, which probably was one of the main mechanisms in the anti-inflammatory reactions.

9.6.3 Future of WSD propolis products

For many years there was no material progress on the methods of propolis extraction by water simply because propolis was long thought to be insoluble in water. The main types of propolis used in recent years are tinctures, troches and capsules. The main extraction solvent is ethanol, but because of this inevitable alcoholic content, development

in the use of propolis has been limited. This experiment showed that propolis extracted by a special method with water had the same or better anti-inflammation and anti-tumor effects than that of EEP. Moreover, there may be other water-soluble, anti-inflammatory and anti-tumor components besides flavonoids in propolis. Propolis extracted by water has the cultural advantage that solvent is non-alcoholic.

CHAPTER 10

10. COMPARISONS OF THE ANTI-TUMOR EFFECTS AMONG PROPOLIS, BEE POLLEN AND ROYAL JELLY

10.1 Summary

The S_{180} entity-tumor mice were given intragastrically aqueous extracts of propolis, aqueous extracts of bee pollen and royal jelly and treated once daily continuously for 15 days. The tumors were removed and weighed. The results showed that the anti-tumor effectiveness of aqueous extracts of propolis, pollen and royal jelly were 79.9%, 69.0% and 75.8%, when compared to the control group.

10.2 Introduction

Cancer, cardiovascular syndromes and diabetes mellitus are particularly dangerous diseases that threaten human health. Modern medicine suggests that about 35% of the incidence of cancer is related to nutritional patterns (Eddy, 1986). Some studies indicate that cancer can be prevented, and the manipulation of diet may well constitute a preventative measure (Howe *et al.*, 1992; Miller *et al.*, 1994). Today, increasingly more people have become aware of the relationship between nutrition and cancer. Although methods for the treatment of cancer primarily depend on chemotherapy and the analysis of genetic predisposition, manipulation of nutrition holds its importance. Bee products such as propolis, bee-collected pollen and royal jelly are considered good healthy foods, and have been widely used in folk medicine for many years, particularly in Asia. The principal constituents of propolis of contemporary interest are the more than 38 flavonoids. There are also relatively large concentrations of amylose in bee-collected pollen and superoxide

dismutase (SOD), glucose oxidase (GOD), 10-hydroxy-α-dece-noic acid (10-HDA) in royal jelly (Wang, 1982; Huang, 1993; Hu & Xuan, 2003). There are several reports that these components have a degree of anti-tumor efficacy (Bankova *et al.*, 1983; Slaga, 1983; Tamura, 1987; Lfarin, 1993; Huang *et al.*, 2000). Here the results of experimental studies on the effects of extracts of propolis, bee-collected pollen and royal jelly for anti-tumor efficacy are reported.

10.3 Materials and methods

10.3.1 Materials

Mice (strain ICR, 16 males and 16 females) about 18-22 g birth weight were provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003), and used at The Research Center of Laboratory Animal Science, Zhejiang College of Traditional Chinese Medicine, Hangzhou, China.

Propolis, bee-collected pollen and royal jelly were purchased from Hangzhou Lingzhi Apiary of China. Propolis was produced in the North China in 2001 and the main plant of origin was poplar (*Populus sp.*). Other materials included S_{180} entity-tumor, physiological saline and distilled water.

10.3.2 Methods

10.3.2.1 Preparation of experimental materials

WSP (water soluble derivative of propolis): the concentration of pure propolis was 10 g/L. Pollen extraction: 20 g bee-collected pollen were placed in 400 ml of distilled water for about 4 h and then filtered and refrigerated. Royal jelly diluent: the concentration of royal jelly was about 25 mg/ml, and was refrigerated. The animals were maintained and

the experiments performed according to the principles of the Helsinki accord. The experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, Hangzhou, China.

10.3.2.2 Anti-tumor experiments

36 mice were randomly divided into 4 groups of 8 each, four males and four females. S_{180} entity-tumor mice were maintained for ten days. They were then killed and the ascites solution was collected and diluted 4 times to inoculate each mouse with 0.2 ml/20 g body weight under epidermal armpit of the forelimb. All operations were performed under aseptic conditions. Each group was treated respectively with propolis, bee-collected pollen and royal jelly intragastrically, and the control group treated with distilled water with 0.5 ml/20 g·d. The experiment lasted for 15 days and on the sixteenth day, all mice were dissected to recover tumor tissue which was then weighed.

10.4 Results

The results are shown in tables 10.1 and 10.2. The average tumor weights of the propolis, bee-collected pollen and royal jelly groups were significantly lower than that of the control group, and the difference was significant ($P < 0.01$). Comparing the incidence of anti-tumor efficacy against the control group, the anti-tumor rates for propolis, bee-collected pollen and royal jelly groups were 79.9%, 69.0% and 75.8%, respectively.

Table 10.1 The tumor weights of each treatment group

Treatment group	Tumor weight (g)								Average tumor weight (g)
Propolis	0.28	0.28	0.28	0.24	0.21	0.21	0.30	0.29	0.26±0.03**
Bee pollen	0.36	0.43	0.46	0.33	0.45	0.31	0.42	0.49	0.41±0.07**
Royal jelly	0.39	0.38	0.28	0.28	0.34	0.29	0.35	0.25	0.32±0.05**
Control	1.35	1.34	1.28	1.33	1.29	1.24	1.33	1.33	1.31±0.04

Compared with that of control group, $^{**}P < 0.01$

Table 10.2 The anti-tumor rate of each treatment group

Group	Propolis	Bee pollen	Royal jelly	Control
Anti-tumor rate (%)	79.9	69.0	75.8	-

Anti-tumor rate (%) = (the average tumor weight of control - average tumor weight of experimental groups) / the average tumor weight of control

10.5 Discussion

10.5.1 The anti-tumor effects of propolis, bee pollen and royal jelly

In this experiment, we found that propolis, bee-collected pollen and royal jelly exhibited anti-tumor effective rates of 79.9%, 69.0% and 75.8% respectively. These results confirm those of Wang *et al.* (1986) who reported that the anti-tumor effective rates were about 70%. The anti-tumor rates of propolis, bee pollen and royal jelly were all higher than the standard index, which is based on a universal standard that a substance has anti-tumor properties if its anti-tumor effective rate is over 30% (Xu *et al.*, 1991).

In the experiment, the increase in body weight in the group of propolis treated animals was significantly lower than that of the group treated with bee-collected pollen and royal jelly. In addition, the tumor multiplication rate in the propolis treated group was also lower than that of other groups. The body weight increases in the bee pollen and royal jelly groups was rapid. While the inhibition effect was initially weak, the effect increased with time. The probable cause was that the immune system strengthened for a few days after administration of bee pollen and royal jelly intragastrically (Wang *et al.*, 1986; Qiu *et al.*, 1993; Li *et al.*, 2000). On the other hand, there could well be several different mechanisms for the effects of the propolis extracts, which contain numerous flavonoids such as mulberrin, quercetin, kaempferol, galangin, etc. Kimoto *et al.* (1998) reported that 3,5-diprenyl-4 hydroxylcinnamic acid had anti-bacterial effects expressed as a cytotoxicity to malignancy, possibly resulting in apoptosis. In addition, propolis could also modulate the reactivity of the immune system (Mitamura *et al.*, 1996; Mahran, 1996; Kimoto *et al.*, 1998; Sharma & Pillai, 1998; Fang, 1998a; Hu & Li, 2002). However, the mechanisms of the extracts of these bee products in exerting anti-tumor effects need further study.

10.5.2 The combined effects of propolis, bee pollen and royal jelly

It is well known that pollen is a mini-type nutrition pool, and includes amino acids, enzymes, vitamins and trace elements. The anti-tumor effects of propolis have been widely studied (Scheller *et al.*, 1989; Mitamura *et al.*, 1996; Kimoto *et al.*, 1998, 2000; Sato & Miyataka, 1999). In addition, it may be that there are some unknown, but active nutritional components in royal jelly. So the combined effects of pollen, propolis and royal jelly may well prove more effective and possibly synergistic than any of these bee products used alone; but this certainly requires further study.

CHAPTER 11

11. REFERENCES

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Appendix 1

Original Experimental Data

Table 2.1: Changes in blood glucose in normal rats and those with diabetes mellitus over five weeks

(1) Normal control group

1 week	2 weeks	3 weeks	4 weeks	5 weeks
3.29	1.41	3.57	0.36	3.29
1.61	0.31	0.43	0.53	1.91
2.98	1.01	0.14	0.36	1.95
2.04	0.65	0.02	2.22	2.23
2.97	0.88	0.57	1.10	2.23
2.46	1.26	1.63	0.45	0.83
1.26	0.20	0.13	0.54	2.78
2.45	1.09	2.89	0.51	1.17
2.13	0.62	0.15	1.04	2.78
1.78	0.43	0.65	0.68	2.70
2.297±0.655	**0.786±0.409**	**1.018 ±1.264**	**0.779 ±0.568**	**2.187±0.760**

(2) Model control group

1 week	2 weeks	3 weeks	4 weeks	5 weeks
19.55	29.66	19.39	30.95	30.26
24.13	29.02	25.52	25.51	34.89
17.08	22.39	22.02	24.60	30.04
21.61	21.76	26.22	34.24	32.00
21.33	25.39	25.06	27.68	33.06
20.28	20.33	22.30	21.56	33.38
24.37	24.33	20.74	34.79	33.06
24.32	24.33	22.26	34.52	32.98
25.13	29.2	27.26	45.74	36.80
16.01	20.79	22.43	32.15	30.17
21.381±3.182	**24.720±3.537**	**23.320±2.551**	**31.174±6.886**	**32.664±2.168**

(3) WSD1 group

1 week	2 weeks	3 weeks	4 weeks	5 weeks
22.81	19.24	23.5	26.65	26.16
15.91	14.36	20.58	30.38	27.41
20.31	17.77	21.19	21.35	24.71
20.22	24.02	19.34	26.62	21.89
27.68	20.99	19.27	21.69	27.42
22.39	16.23	24.82	23.13	30.87
20.79	22.79	23.07	29.59	32.47
21.53	23.24	21.18	28.24	38.72
21.65	24.42	22.52	36.59	27.87
25.35	26.37	24.3	40.06	16.73
21.864± 3.145	**20.943±3.920**	**21.977±1.966**	**28.430±6.120**	**27.425±5.954**

(4) WSD2 group

1 week	2 weeks	3 weeks	4 weeks	5 weeks
20.88	23.94	21.8	30.62	20.36
21	18.97	23.77	28.64	33.65
23.94	29.64	26.82	30.75	32.36
18.15	12.59	21.04	19.88	28.65
20.62	19.09	22.75	23.27	26.26
16.02	23.95	23.84	24.27	28.28
22.21	20.2	19.08	25.32	25.75
20.16	24.87	19.14	42.35	28.38
22.04	22.31	24.44	39.8	27.32
23.96	19.13	25.17	31.43	23.09
20.898±2.441	**21.469±4.584**	**22.785±2.535**	**29.633±7.106**	**27.410±3.932**

(5) EEP1 group

1 week	2 weeks	3 weeks	4 weeks	5 weeks
20.57	22.13	23.85	25.18	38.66
25.42	19.91	22.99	21.73	20.39
23.04	24.1	23.14	24.53	26.44
18.99	23.2	21.87	22.96	22.68
20.09	21.41	25.26	22.76	26.55
22.16	26.08	21.12	19.98	28.34
17.65	19.42	18.03	24.59	26.24
21.74	22.25	22.93	25.87	27.66
15.32	22.78	22.96	31.42	33.86
20.75	20.16	29.08	29.59	36.58
20.573±2.836	**22.144±2.048**	**23.123±2.832**	**24.861±3.470**	**28.740±5.871**

(6) EEP2 group

1 week	2 weeks	3 weeks	4 weeks	5 weeks
16.50	19.76	23.02	27.61	29.20
24.73	30.13	25.14	30.10	30.28
20.43	20.04	27.30	27.70	27.77
20.19	23.27	30.50	26.42	28.15
21.02	22.77	21.25	30.86	24.45
16.79	20.54	21.11	23.14	20.14
15.39	14.23	17.10	33.63	19.99
15.11	12.86	21.11	30.95	30.20
24.82	22.11	28.35	32.70	31.33
19.52	19.23	24.09	33.72	28.93
19.450±3.524	**20.494±4.810**	**23.897±4.029**	**29.683±3.429**	**27.044±4.122**

(7) Positive control group

1 week	2 weeks	3 weeks	4 weeks	5 weeks
21.81	29.70	22.84	17.55	25.42
23.81	24.75	25.87	26.98	16.10
20.45	22.39	22.50	24.03	23.26
19.01	14.78	22.40	23.01	27.08
24.66	26.19	23.52	22.39	29.87
21.02	24.37	18.39	21.01	25.94
23.77	26.51	25.02	38.47	25.97
17.31	18.81	28.91	37.48	31.60
16.90	21.11	20.52	32.30	39.63
21.70	23.24	23.03	28.31	27.14
21.044±2.683	**23.185±4.231**	**23.300±2.880**	**27.153±7.009**	**27.201±6.019**

Table 2.2: Effects of propolis on the levels of HbAlc, TG, TC, TP and ALB in rats with diabetes mellitus

(1) Normal control group

HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
0.04	0.46	1.06	54	27
0.07	0.68	1.30	70	27
0.04	0.73	1.27	69	32
0.05	0.67	1.39	67	25
0.04	0.67	1.52	83	35
0.05	0.65	1.38	72	32
0.05	0.73	1.91	71	34
0.07	0.35	1.30	60	28
0.04	0.53	0.91	65	33
0.05	0.95	0.68	56	28
0.050±0.012	**0.642±0.165**	**1.272±0.34**	**66.700±8.51**	**30.100±3.48**

(2) Model control group

HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
0.08	4.13	1.53	45	19
0.07	2.49	1.25	46	21
0.08	0.54	1.21	41	22
0.08	8.55	1.84	41	20
0.08	2.76	1.44	52	28
0.07	4.90	1.31	44	23
0.07	5.17	1.72	58	25
0.07	3.43	1.49	43	20
0.08	4.85	1.83	69	30
0.07	2.85	1.18	55	24
0.075±0.005	**3.967±2.136**	**1.480±0.25**	**49.400±9.06**	**23.200±3.62**

(3) WSD1 group

HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
0.08	1.40	1.60	54	25
0.06	3.58	1.59	51	26
0.06	5.01	1.96	63	28
0.06	6.53	2.28	53	26
0.09	1.67	1.36	57	28
0.06	2.22	1.24	45	22
0.07	1.70	0.98	47	21
0.06	1.94	1.28	56	27
0.07	3.99	1.44	59	26
0.08	5.93	1.65	54	24
0.069±0.011	**3.397±1.903**	**1.538±0.37**	**53.900±5.36**	**25.300±2.36**

(4) WSD2 group

HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
0.07	2.28	1.45	56	28
0.05	1.83	1.05	46	18
0.06	1.80	1.31	69	28
0.06	1.53	0.96	49	20
0.06	2.68	1.20	59	21
0.08	0.95	1.01	56	25
0.06	3.30	1.25	31	18
0.07	1.82	1.63	55	27
0.08	2.31	1.23	51	26
0.07	2.24	1.47	49	22
0.066±0.010	**2.074±0.646**	**1.256±0.22**	**52.100±9.86**	**23.300±3.97**

(5) EEP1 group

HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
0.07	1.67	1.21	53	21
0.06	0.93	1.13	62	27
0.05	0.32	0.85	57	21
0.07	2.18	1.15	55	26
0.08	2.27	1.23	52	25
0.06	2.45	1.41	47	25
0.08	2.09	1.44	69	30
0.07	2.93	1.36	41	21
0.08	3.48	1.34	61	28
0.08	1.11	0.94	42	17
0.070±0.011	**1.943±0.955**	**1.206±0.196**	**53.900±8.91**	**24.100±3.98**

(6) EEP2 group

HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
0.07	1.81	1.21	56	24
0.06	3.39	1.22	56	23
0.05	1.58	1.19	57	25
0.06	1.23	0.91	44	23
0.06	1.36	1.11	48	24
0.05	1.16	0.95	59	25
0.08	3.09	1.25	55	23
0.07	1.72	1.33	48	22
0.07	1.80	1.58	70	25
0.08	1.96	1.45	57	26
0.065±0.011	**1.910±0.751**	**1.220±0.20**	**55.000±7.23**	**24.000±1.25**

(7) Positive control group

HbAlc	TG (mmol/L)	TC (mmol/L)	TP (g/L)	ALB (g/L)
0.07	1.95	1.59	58	25
0.08	4.15	0.98	57	15
0.08	4.91	1.88	56	25
0.08	2.07	1.40	55	24
0.06	4.47	1.50	58	25
0.07	2.87	1.50	62	30
0.07	6.98	2.00	70	25
0.08	1.23	1.29	60	22
0.07	2.37	1.59	51	20
0.06	7.29	1.94	56	24
0.072±0.008	**3.829±2.109**	**1.567±0.313**	**58.300±5.056**	**23.500±3.923**

Table 2.3: Effects of propolis on the levels of FRU, CREA, BUN, UA and MDA in rats with diabetes mellitus

(1) Normal control group

FRU (μ mol/L)	CREA (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	MDA (nmol/L)
70.33	57.52	4.80	135.48	9
73.40	56.61	5.70	168.32	9
71.15	63.56	5.66	146.62	8
75.77	54.22	5.56	183.86	10
70.25	56.22	5.62	191.43	9
83.36	71.29	6.37	155.73	10
73.89	69.59	5.65	160.42	9
73.11	61.34	5.96	129.28	8
78.63	57.52	5.51	171.51	10
63.75	56.39	5.58	151.50	11
73.364±5.27	**60.426±5.93**	**5.641±0.390**	**159.415±19.917**	**9.300±0.95**

(2) Model control group

FRU (μ mol/L)	CREA (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	MDA (nmol/L)
97.63	66.12	17.63	202.34	29
91.87	65.43	21.05	201.31	14
92.66	60.77	17.94	214.35	11
98.59	71.01	16.97	283.54	24
111.46	71.41	11.12	245.90	19
121.75	67.6	16.17	218.39	17
119.14	71.69	22.29	232.06	19
85.52	58.61	21.66	218.73	17
124.29	62.36	35.48	363.28	15
104.94	76.59	18.41	200.70	16
104.785±13.717	**67.159±5.61**	**19.872±6.357**	**238.060±50.762**	**18.100±5.15**

(3) WSDl group

FRU (μ mol/L)	CREA (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	MDA (nmol/L)
92.6	67.03	13.85	188.05	16
106.36	63.84	19.38	244.76	14
95.44	65.95	18.23	287.29	18
97.02	60.26	13.36	224.06	20
100.81	63.16	17.47	200.30	16
80.90	66.86	14.18	158.82	13
103.56	63.95	14.37	203.99	13
89.38	69.93	14.46	240.34	15
108.45	67.14	13.94	221.12	18
92.20	67.60	17.42	278.77	15
96.672±8.415	**65.572±2.77**	**15.666±2.202**	**224.750±39.734**	**15.800±2.30**

(4) WSD2 group

FRU (μ mol/L)	CREA (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	MDA (nmol/L)
80.36	69.41	19.39	199.14	13
103.6	65.42	15.37	215.89	16
121.15	76.96	26.6	214.25	14
91.74	62.82	10.03	200.9	14
84.97	66.23	17.85	204.56	13
95.23	66.97	18.41	204.61	13
66.5	67.82	13.59	188.73	17
102.09	74.25	24.27	216.9	16
105.96	66.86	12.33	220.65	16
86.25	57.43	16.03	213.57	13
93.785±15.376	**67.417±5.46**	**17.387±5.138**	**207.920±9.973**	**14.500±1.58**

(5) EEP1 group

FRU (μ mol/L)	CREA (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	MDA (nmol/L)
90.76	67.64	19.31	201.63	16
101.48	68.30	26.13	178.36	13
100.61	66.69	14.25	170.81	10
90.24	75.63	18.43	217.15	25
82.70	68.56	16.43	184.31	17
89.23	79.30	22.52	210.45	18
110.19	60.71	12.58	260.96	25
84.22	59.13	18.70	188.05	13
95.43	54.33	24.49	244.43	18
72.77	56.41	20.47	160.54	9
91.763±10.725	**66.670±7.08**	**19.331±4.286**	**201.669±32.205**	**16.400±5.50**

(6) EEP2 group

FRU (μ mol/L)	CREA (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	MDA (nmol/L)
88.91	55.70	15.88	203.82	11
85.14	62.82	21.04	226.96	12
95.09	62.76	16.50	239.43	13
91.41	64.98	21.11	186.47	12
97.25	54.60	18.28	208.38	18
91.10	59.80	16.84	206.28	11
91.27	60.43	16.93	212.89	18
104.04	53.36	21.10	236.88	12
94.50	68.31	17.47	280.96	19
106.65	61.51	15.93	213.91	15
94.536±6.641	**60.427±4.74**	**18.108±2.168**	**221.598±26.243**	**14.100±3.14**

(7) Positive control group

FRU (μ mol/L)	CREA (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	MDA (nmol/L)
93.00	62.42	14.28	260.66	19
81.69	75.29	20.96	224.46	12
98.89	57.97	18.75	241.81	15
94.17	76.97	14.18	181.45	10
98.37	55.59	13.69	222.31	12
101.79	63.44	11.22	180.45	14
108.30	70.38	19.31	244.12	21
106.86	61.96	16.35	233.36	16
99.45	67.51	20.56	224.35	16
92.13	71.01	21.56	234.03	24
97.465±7.741	**66.254±7.158**	**17.086±3.615**	**224.700±25.733**	**15. 900±4.358**

Table 2.4: Effects of propolis on the levels of kidney weight/body weight in rats with diabetes mellitus

(1) Normal control group

Body weight (g)	Kidney weight (g)	Kidney weight/body weight×100
333	1.3495	4.053
305	1.0980	3.600
335	1.3206	3.942
337	1.2670	3.760
310	1.1896	3.837
331	1.1596	3.503
345	1.2771	3.702
345	1.2819	3.716
338	1.2933	3.826
371	1.2398	3.342
335.0±18.4	**1.25±0.08**	**3.73±0.21**

(2) Model control group

Body weight (g)	Kidney weight (g)	Kidney weight/body weight×100
243	1.3667	5.624
205	1.2656	6.174
205	1.2237	5.969
210	1.3590	6.471
209	1.4057	6.726
247	1.6653	6.742
175	1.2838	7.336
172	1.1443	6.653
160	1.1163	6.977
197	1.1914	6.048
202.3±28.4	**1.30±0.16**	**6.47±0.52**

(3) WSD1 group

Body weight (g)	Kidney weight (g)	Kidney weight/body weight × 100
204	1.1789	5.779
244	1.4414	5.907
233	1.2854	5.517
234	1.3127	5.610
273	1.4363	5.261
233	1.8425	7.908
223	1.3045	5.850
240	1.4909	6.212
204	1.3365	6.551
200	1.3120	6.560
228.8±22.3	**1.39±0.18**	**6.11±0.76**

(4) WSD2 group

Body weight (g)	Kidney weight (g)	Kidney weight/body weight × 100
242	1.4770	6.103
241	1.4530	6.029
212	1.3468	6.353
296	1.5448	5.219
270	1.7246	6.387
283	1.7403	6.149
256	1.5659	6.117
191	1.4971	7.838
265	1.0345	3.904
157	1.2894	8.213
241.3±43.2	**1.47±0.21**	**6.23±1.21**

(5) EEP1 group

Body weight (g)	Kidney weight (g)	Kidney weight/body weight × 100
221	1.3562	6.137
210	1.4168	6.747
200	1.2577	6.289
258	1.4364	5.567
226	1.4243	6.302
219	1.5317	6.994
228	1.0827	4.749
243	1.4682	6.042
217	1.3473	6.209
195	1.2039	6.174
221.7±18.8	**1.35±0.14**	**6.12±0.62**

(6) EEP2 group

Body weight (g)	Kidney weight (g)	Kidney weight/body weight × 100
236	1.4280	6.051
201	1.3804	6.868
238	1.2866	5.406
245	1.3267	5.415
289	1.6350	5.657
230	1.5500	6.739
186	1.1517	6.192
218	1.1534	5.291
158	1.3900	8.797
204	1.0165	4.983
220.5±36.0	**1.33±0.19**	**6.14±0.11**

(7) Positive control group

Body weight (g)	Kidney weight (g)	Kidney weight/body weight×100
235	1.4100	6.000
221	1.5271	6.910
225	1.4396	6.398
287	1.4939	5.205
242	1.5513	6.410
240	1.5045	6.269
186	1.1668	6.273
283	1.5873	5.609
205	1.3580	6.624
197	1.2633	6.413
232.1±33.4	**1.43±0.13**	**6.21±0.49**

Table 3.1: Changes in blood glucose in rats with induced diabetes mellitus and normal rats for eight weeks

(1) 1 week

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
4.23	21.95	20.70	23.65	20.90	19.74	20.79
4.72	22.07	24.56	21.56	22.08	21.26	20.75
5.05	24.6	19.31	23.98	18.76	23.11	23.60
4.30	20.00	23.46	21.56	24.01	19.69	20.81
3.45	23.24	19.86	24.95	21.91	24.02	23.27
3.53	21.85	20.19	18.99	20.94	19.17	24.26
3.43	21.56	22.60	18.55	21.34	23.37	22.55
4.51	18.59	22.83	19.61	24.80	20.76	24.20
2.45	24.10	19.00	24.01	24.97	24.01	19.45
4.53	19.48	23.80	22.06	23.17	22.98	20.00
3.16	20.70	24.31	20.26	19.47	22.98	23.90
2.24	24.75	22.66	23.93	20.5	23.66	19.83
3.800± 0.898	**21.908± 2.001**	**21.940± 2.011**	**21.926± 2.198**	**21.904± 1.998**	**22.062± 1.819**	**21.951± 1.850**

(2) 2 weeks

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
4.29	17.04	19.41	24.64	13.00	21.61	10.84
5.29	23.44	24.08	22.32	19.07	22.10	21.91
3.72	34.90	19.84	19.46	23.34	19.60	18.89
5.18	20.60	24.34	20.82	29.05	14.79	18.54
4.80	25.65	16.2	20.49	20.22	20.21	22.16
4.33	25.58	23.27	17.74	18.88	20.66	19.42
4.70	22.06	17.32	17.37	18.64	20.93	23.41
4.06	19.64	22.33	18.14	19.90	13.49	13.88
3.87	21.44	14.40	19.74	10.39	21.85	21.55
3.77	17.29	13.66	23.80	23.60	13.19	18.03
3.64	21.60	21.21	17.74	17.39	20.80	20.09
4.21	23.00	19.66	19.19	23.15	20.02	19.74
4.322± 0.561	**22.687± 4.717**	**19.643± 3.633**	**20.121± 2.406**	**19.719± 4.928**	**19.104± 3.287**	**19.038± 3.571**

(3) 3 weeks

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
4.53	28.19	11.59	19.98	19.06	23.09	14.45
4.54	20.47	21.83	18.97	18.86	22.98	22.21
4.37	33.32	17.30	21.15	15.98	20.83	21.71
4.12	24.74	18.80	16.53	24.42	19.37	14.88
4.63	23.08	16.88	21.17	21.78	22.90	21.64
5.36	24.08	20.41	15.38	19.31	23.18	23.34
5.14	16.12	16.69	16.03	17.77	22.89	24.68
5.05	22.97	19.80	21.60	20.44	19.86	15.56
3.65	20.72	17.88	16.73	13.04	21.13	17.04
3.91	10.06	15.65	21.20	22.37	19.77	14.81
3.62	25.85	13.13	16.37	15.35	20.68	21.77
4.63	19.74	19.51	16.06	20.32	21.01	12.35
4.463± 0.562	**22.445± 5.870**	**17.456± 2.968**	**18.431± 2.463**	**19.058± 3.189**	**21.474± 1.450**	**18.703± 4.237**

(4) 4 weeks

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
4.51	23.66	16.42	17.88	14.86	23.24	23.51
4.63	25.03	20.10	16.18	15.98	20.36	19.17
3.12	24.82	18.38	18.31	19.8	20.27	20.94
3.82	24.22	17.13	15.44	20.25	21.60	17.24
3.71	22.83	12.18	16.28	16.38	22.4	22.81
4.32	20.32	15.09	13.31	16.50	23.42	17.71
3.97	19.73	16.02	16.19	17.79	21.36	23.01
3.33	21.70	17.83	15.53	18.76	23.60	19.00
3.93	20.52	17.04	15.87	16.80	22.41	16.25
3.19	22.3	17.64	18.04	20.64	20.26	16.21
3.57	21.85	21.16	17.50	17.23	22.95	19.31
4.12	23.5	21.5	14.10	19.09	21.70	17.57
3.852± 0.495	**22.540± 1.774**	**17.541± 2.609**	**16.220± 1.545**	**17.840± 1.849**	**21.964± 1.232**	**19.394± 2.613**

(5) 5 weeks

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
3.31	22.04	11.35	20.56	18.68	20.42	18.18
3.83	23.28	19.55	21.39	23.90	21.35	23.01
3.87	24.77	22.81	21.58	19.22	28.83	23.10
3.71	22.53	22.95	20.98	23.13	18.75	17.74
3.18	23.68	26.27	21.94	18.35	15.82	22.52
3.29	27.05	24.45	18.92	25.72	14.76	22.70
4.08	27.02	11.30	20.61	19.78	18.93	23.10
3.06	23.40	20.52	20.69	25.62	28.96	17.23
3.33	22.90	14.22	20.16	21.76	29.51	19.10
2.67	19.71	17.00	20.38	21.89	13.70	14.99
3.14	19.38	19.73	19.88	22.96	20.47	23.57
3.64	26.15	20.48	18.9	21.77	20.61	15.01
3.426±0.405	**23.493±2.496**	**19.219±4.881**	**20.499±0.948**	**21.898±2.518**	**21.009±5.447**	**20.021±3.324**

(6) 6 weeks

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
5.73	19.2	18.50	25.24	21.54	20.33	15.95
5.11	23.62	22.02	21.48	23.77	26.20	20.75
5.05	24.96	14.34	23.83	18.30	22.62	18.47
4.6	23.52	22.11	18.52	20.46	20.27	20.57
4.02	23.05	18.70	15.97	8.05	22.52	18.19
3.76	25.18	24.85	29.75	24.35	11.36	18.15
3.96	34.04	13.87	21.43	24.65	25.49	16.87
4.21	27.11	22.21	23.06	26.86	11.01	20.76
3.77	25.19	16.07	19.33	12.43	27.87	20.97
4.69	26.42	18.10	24.26	21.2	15.32	17.74
4.22	27.22	16.06	22.93	20.53	20.14	23.00
5.01	24.8	19.27	17.25	26.24	23.32	17.71
4.511±0.622	**25.359±3.480**	**18.842±3.431**	**21.921±3.810**	**20.698±5.578**	**20.538±5.468**	**19.094±2.070**

(7) 7 weeks

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
5.22	24.01	10.48	21.13	22.86	29.30	19.30
5.56	27.35	29.00	21.27	25.94	20.30	22.04
4.82	28.21	19.02	27.02	32.13	25.87	24.33
5.41	26.68	18.42	21.64	9.69	18.10	19.41
4.36	27.58	15.39	23.65	12.40	26.63	22.14
5.26	26.76	21.19	16.87	22.88	26.22	23.33
4.76	28.56	14.39	9.15	9.57	29.48	23.41
4.69	28.02	14.38	16.53	24.02	15.16	21.80
4.57	25.6	9.55	22.51	20.97	29.79	18.69
4.66	28.49	16.06	21.44	25.48	10.40	30.44
4.60	28.86	21.80	20.03	23.56	26.95	20.19
5.00	23.8	21.51	17.25	21.37	28.78	19.38
4.909± 0.376	**26.993± 1.716**	**17.599± 5.410**	**19.875± 4.507**	**20.905± 6.899**	**23.915± 6.396**	**22.038± 3.241**

(8) 8 weeks

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
4.27	21.85	15.57	24.45	21.75	20.25	15.16
4.81	22.41	31.28	21.04	23.56	24.17	26.46
3.80	28.17	21.00	24.2	15.80	24.74	26.02
6.74	29.75	17.55	21.76	28.91	16.09	18.81
5.88	24.82	14.98	36.59	12.28	24.99	22.27
5.23	30.87	24.35	12.00	23.22	23.61	20.58
5.28	31.77	11.72	17.43	19.10	26.99	21.91
4.61	32.50	25.06	17.31	21.52	15.98	22.64
4.92	27.70	17.72	15.10	19.71	28.75	22.34
4.79	30.36	16.28	16.04	20.29	18.69	21.38
5.61	25.14	18.29	20.31	27.63	22.71	17.03
5.90	25.50	19.53	18.95	29.68	23.57	20.49
5.153± 0.800	**27.570± 3.604**	**19.444± 5.314**	**20.432± 6.277**	**21.954± 5.153**	**22.545± 4.032**	**21.258± 3.253**

Table 3.2: Effect of propolis on the level of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerol (TG) in rats with induced diabetes mellitus

(1) TC

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
2.06	1.59	1.71	1.76	2.20	2.19	1.48
2.40	2.44	2.03	1.98	1.94	2.04	1.73
1.94	2.82	1.75	2.13	1.86	2.26	2.24
2.18	3.27	1.86	2.27	1.51	2.11	1.61
2.03	1.99	1.44	1.87	2.5	1.91	2.16
2.11	2.05	1.91	1.51	1.85	2.19	1.87
2.16	2.07	2.17	1.74	1.59	2.03	1.97
1.83	2.20	1.79	1.82	1.82	2.46	2.27
1.99	2.69	2.09	1.95	2.30	2.02	1.86
1.80	2.26	2.24	1.97	2.15	2.10	2.02
1.76	2.24	1.79	1.47	1.97	2.05	1.74
1.83	2.44	2.08	1.97	1.96	2.61	1.72
2.007±0.189	**2.34± 0.44**	**1.91±0.23**	**1.870±0.23**	**1.971±0.283**	**2.164±0.199**	**1.889±0.25**

(2) HDL-C

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
0.84	0.81	0.82	0.89	0.82	0.80	0.94
0.90	0.75	0.72	0.87	0.78	0.76	0.81
0.92	0.76	0.80	0.95	0.74	0.90	0.98
0.87	0.83	0.71	1.13	0.64	1.12	0.85
0.97	0.86	1.06	1.02	1.20	0.81	0.91
0.97	0.61	0.82	0.99	0.65	0.94	0.73
1.03	0.86	0.89	0.67	0.66	0.83	0.76
0.85	0.84	0.68	0.75	0.93	1.36	0.99
0.93	0.88	1.06	0.97	0.95	0.72	0.90
0.84	0.70	1.03	0.79	0.75	1.07	1.37
0.82	0.88	0.75	0.90	1.32	0.82	0.89
0.95	0.89	1.01	0.83	0.59	0.96	1.09
0.908±0.068	**0.81±0.09**	**0.86±0.14**	**0.898±0.13**	**0.836±0.228**	**0.924±0.183**	**0.937±0.17**

(3) LDL-C

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
0.69	1.11	0.50	0.20	0.12	0.37	0.32
0.33	1.65	0.70	0.54	0.33	0.55	0.15
0.61	0.56	0.24	0.4	0.45	0.48	0.45
0.44	0.42	0.25	0.52	0.31	0.28	0.53
0.24	1.42	0.09	0.37	0.21	0.19	0.50
0.52	0.53	0.19	0.27	0.04	0.41	0.22
1.07	0.51	0.76	0.58	0.29	0.41	0.30
0.35	0.93	0.39	0.48	0.40	0.42	0.23
0.41	0.60	0.60	0.36	0.35	0.33	0.51
0.45	0.69	0.38	0.49	0.52	0.43	0.50
0.42	0.71	0.45	0.44	0.56	0.45	0.50
0.39	0.68	1.13	0.60	0.45	0.39	0.37
0.491± 0.22	**0.820± 0.39**	**0.475± 0.29**	**0.438± 0.115**	**0.34± 0.16**	**0.394± 0.09**	**0.381± 0.127**

(4) VLDL-C

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
0.43	1.24	1.39	1.07	1.45	1.27	0.55
1.17	1.50	1.01	0.57	0.81	0.95	0.87
0.31	1.30	1.29	1.08	0.94	1.43	0.09
0.30	1.52	1.20	0.62	1.15	1.89	2.29
0.82	1.19	0.89	0.48	1.49	1.31	0.75
0.42	1.63	1.28	0.79	1.31	1.21	1.32
0.06	1.73	0.52	0.99	1.14	1.49	1.11
0.63	1.25	0.72	1.08	0.79	0.96	1.05
0.65	1.69	0.43	1.35	1.02	0.95	0.55
0.21	1.61	0.83	1.67	1.43	0.41	0.15
0.32	1.58	1.38	0.66	0.9	0.47	0.68
0.59	1.99	0.98	1.14	1.28	0.67	0.74
0.494± 0.30	**1.516± 0.24**	**0.994± 0.33**	**0.958± 0.35**	**1.14± 0.25**	**1.08± 0.44**	**0.847± 0.58**

(5) TG

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
1.79	1.93	1.04	1.22	1.38	0.76	0.93
1.10	1.23	0.99	1.06	1.18	1.57	0.86
1.28	1.10	0.93	1.39	1.39	0.58	0.88
1.04	1.47	1.54	1.54	0.73	1.15	0.61
0.93	1.56	1.08	0.58	0.51	0.66	0.55
0.93	0.82	1.33	1.19	0.81	0.80	0.59
1.44	1.49	1.09	0.81	0.66	0.71	0.80
1.49	1.63	1.08	0.85	0.99	1.26	1.20
1.31	0.71	1.15	0.63	1.63	0.49	1.41
1.75	1.07	1.44	0.78	0.90	0.49	0.94
0.94	1.33	0.69	1.14	0.51	0.64	1.21
0.88	1.34	1.66	0.78	0.74	0.55	0.96
1.240±0.322	**1.306±0.35**	**1.162±0.28**	**1.000±0.30**	**0.952±0.367**	**0.805±0.342**	**0.912±0.264**

Table 3.3: Effects of propolis on the levels of FRU, SOD, MDA, NO and NOS in rats with induced diabetes mellitus

(1) FRU

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
250	295	216	235	269	279	190
215	276	256	242	165	257	235
202	291	219	285	214	294	271
209	288	227	248	218	242	219
219	289	184	201	221	277	267
232	281	231	213	219	225	266
233	292	223	218	188	286	241
235	296	225	209	239	233	227
227	295	223	247	204	243	258
216	276	233	242	215	257	226
237	289	213	221	214	250	231
223	291	221	234	217	241	243
224.833±13.456	**288.250±6.969**	**222.583±16.407**	**232.917±22.686**	**215.250±25.060**	**257.00±22.197**	**239.500±23.567**

(2) SOD

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
160.956	128.765	151.445	151.445	148.519	136.447	110.475
173.028	114.499	152.543	142.300	131.326	108.280	160.591
160.956	110.840	145.592	138.642	128.765	139.374	159.493
140.837	122.912	128.034	144.495	134.618	131.326	145.958
145.227	114.499	155.469	140.105	136.447	109.011	146.690
173.760	118.157	150.348	145.227	137.910	112.669	139.008
143.398	103.890	124.375	143.763	147.056	136.813	136.447
155.470	124.375	160.225	139.008	138.276	140.471	148.519
164.980	108.646	138.642	151.811	145.227	143.398	155.469
130.960	103.158	144.861	145.592	141.568	136.447	127.302
139.374	113.767	115.230	149.982	107.182	108.280	131.692
143.398	129.497	151.445	131.692	139.008	112.304	95.8422
152.70±14.05	**116.078±8.89**	**143.218±13.85**	**143.67±5.87**	**136.33±10.95**	**126.24±14.57**	**138.12±19.59**

(3) MDA

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
5.70	9.95	4.15	6.01	5.34	10.77	3.78
5.74	12.17	4.74	6.33	4.25	5.04	5.42
8.27	8.89	5.34	8.64	6.67	2.79	5.75
3.60	6.40	4.59	7.42	4.04	8.59	5.37
5.12	7.31	4.48	7.04	6.01	3.45	4.91
5.57	10.07	4.71	5.07	7.25	3.67	4.83
3.91	10.87	4.13	7.24	4.96	3.86	6.23
8.01	7.14	4.79	3.26	4.96	8.70	9.97
8.48	10.51	8.05	3.61	8.52	4.22	7.27
4.18	7.05	6.88	6.85	8.41	4.65	2.27
6.26	9.28	3.47	7.49	5.12	3.94	5.30
2.96	10.04	3.07	6.70	6.73	8.36	4.09
5.646± 1.85	**9.145± 1.80**	**4.87± 1.38**	**6.306± 1.60**	**6.024± 1.51**	**5.67± 2.66**	**5.430± 1.90**

(4) NO

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
5.90	21.73	16.89	11.63	14.78	17.86	15.72
4.01	15.94	16.03	15.29	13.81	20.14	22.20
6.32	18.49	14.61	11.82	35.94	16.62	21.36
6.06	24.60	18.36	16.53	18.3	14.63	27.63
6.15	23.01	15.10	15.54	12.53	15.41	7.65
5.60	16.07	13.91	14.43	23.17	18.60	9.91
6.61	17.51	14.34	16.36	23.04	16.93	8.34
5.69	22.15	21.42	17.77	24.46	3.12	16.70
4.05	16.69	15.48	22.69	2.44	16.48	3.54
5.50	28.67	18.74	21.54	11.01	16.3	14.71
4.43	14.08	16.34	22.84	9.75	18.72	15.12
4.79	26.35	18.36	13.34	27.86	24.87	18.27
5.43± 0.89	**20.44± 4.65**	**16.63± 2.22**	**16.65± 3.90**	**18.091± 9.19**	**16.64± 5.03**	**15.10± 6.89**

(5) NOS

Normal control	Model control	WSD1	WSD2	EEP1	EEP2	Postive control
5.097	18.970	4.955	8.636	11.892	11.326	12.741
10.0517	16.988	4.813	14.723	11.750	10.193	9.060
9.0607	13.733	9.485	14.015	9.768	9.202	12.033
7.2207	18.263	12.033	8.636	12.033	11.042	13.024
7.362	16.422	13.308	6.371	10.618	10.477	12.033
9.344	12.741	13.874	8.636	9.060	12.741	14.157
12.317	9.627	9.768	8.353	9.060	10.051	10.193
8.494	11.326	10.193	14.865	9.485	8.777	10.759
12.033	12.600	9.627	15.006	11.326	10.051	12.883
8.919	13.308	12.175	11.892	12.175	8.636	13.449
9.768	11.892	12.458	18.404	12.741	11.892	11.750
12.600	11.892	6.371	18.121	11.892	10.476	8.494
9.36±0.23	**13.99±2.97**	**9.92±3.12**	**12.30±4.10**	**10.98±1.32**	**10.41±1.22**	**11.71±1.76**

Table 4.1: Changes in blood glucose of diabetes mellitus and normal SD rats for five weeks

Bee pollen group:

1 week	2 weeks	3 weeks	4 weeks	5 weeks
18.04	24.81	24.71	27.72	22.43
25.16	20.56	21.82	32.28	27.9
22.89	16.92	20.63	30.78	34.15
20.52	20.66	25.44	29.46	29.02
19.24	22.55	25.98	24.45	21.33
25.8	18.76	24.1	34.02	25.55
21.08	26.39	19.89	27.91	35.19
16.37	25.85	22.21	25.73	25.51
23.9	19.47	24.22	21.7	21.91
18.41	18.09	21.17	36.11	31.18
21.141±3.204	**21.406±3.344**	**23.017±2.137**	**29.016±4.439**	**27.417±4.968**

(For the data of other groups see table 2.1)

Table 4.2: Effects of bee pollen and propolis on the levels of FRU, TG, TC, CREA, TP, ALB and MDA in diabetes mellitus SD rats

Bee pollen group:

FRU	TG	TC	CREA	TP	ALB	MDA
102.30	2.94	1.26	69.30	54	25	13
105.56	1.47	1.63	66.69	51	24	16
103.44	2.29	0.91	89.07	64	23	15
97.02	2.12	1.27	68.57	53	22	19
73.00	1.02	1.37	80.60	49	24	19
82.80	1.75	1.51	50.54	44	22	13
103.68	2.03	1.09	52.73	47	21	13
86.14	2.69	1.82	51.10	56	23	14
127.59	2.32	0.86	69.42	58	23	13
92.20	3.54	1.92	63.28	54	24	12
97.373±15.06	**2.217±0.73**	**1.364±0.36**	**66.130±12.56**	**53.000±5.72**	**23.100±1.20**	**14.700±2.54**

(For the data of other groups see tables 2.2 and 2.3)

Table 4.3: Effect of bee pollen and propolis on body weight, kidney weight and kidney weight / body weight

Bee pollen group:

Body weight (g)	Kidney weight (g)	Kidney weight/body weight
203	1.1532	6.747
242	1.4002	6.289
232	1.2879	5.567
233	1.3127	6.302
272	1.4167	6.994
231	1.7802	4.197
223	1.3045	6.042
238	1.4492	6.209
202	1.3086	6.174
200	1.2870	6.058
227.6±22.1	**1.3700±0.17**	**6.00±0.80**

(For the data of other groups see table 2.4)

Table 5.1: Effects of WSD and EEP on body weight, thymus index and adrenal gland index in FCA-induced arthritic rats

(1) Model control group

Weight of normal rats (g)	At the 19th day after injecting Freund's complete adjuvant		
	Weight(g)	Thymus index(mg/100g)	Adrenal index(mg/100g)
212	268	145.19	14.93
213	294	152.38	19.22
210	288	136.18	13.23
203	281	145.12	18.33
199	238	193.95	19.87
202	253	123.44	18.42
203	258	187.98	19.88
202	252	170.52	18.41
205.5±5.3	**266.5±19.7**	**156.8±25.0**	**17.8±2.4**

(2) Prednisone acetate group

Weight of normal rats (g)	At the 19th day after injecting Freund's complete adjuvant		
	Weight(g)	Thymus index(mg/100g)	Adrenal index(mg/100g)
212	309	174.47	13.85
209	256	136.25	13.98
201	270	117.41	15.85
198	262	163.05	7.75
199	281	117.01	13.20
195	263	150.91	15.10
197	275	134.80	12.33
192	240	128.75	11.21
200.4±6.8	**269.5±20.3**	**140.3±20.9**	**12.9±2.5**

(3) WSD group

Weight of normal rats (g)	At the 19th day after injecting Freund's complete adjuvant		
	Weight(g)	Thymus index(mg/100g)	Adrenal index(mg/100g)
213	291	145.22	18.25
210	286	150.45	15.91
206	292	148.70	13.18
202	287	181.01	14.22
207	308	137.14	17.14
205	303	173.56	18.98
199	309	182.46	16.80
196	276	157.68	19.02
204.8±5.6	**294.0±11.7**	**159.5±17.3**	**16.7±2.1**

(4) EEP group

Weight of normal rats (g)	At the 19th day after injecting Freund's complete adjuvant		
	Weight(g)	Thymus index(mg/100g)	Adrenal index(mg/100g)
224	309	156.15	17.96
210	275	184.36	16.22
201	279	165.52	14.44
200	272	148.53	14.67
207	314	161.21	16.56
193	281	177.76	15.23
199	292	168.66	16.23
206	259	149.00	16.37
205.0±9.4	**285.1±18.8**	**163.9±12.9**	**16.0±1.1**

Table 5.2: Effects of WSD and EEP on swelling of the right hind paw in FCA-induced arthritic rats (the primary affection)

(1) Cubage before inflammation

Model control	Prednisone acetate	WSD	EEP
0.95	0.90	1.00	1.05
1.00	0.85	1.05	1.05
1.00	1.05	1.05	0.90
1.10	1.00	1.10	1.00
1.00	1.05	0.90	1.10
1.00	1.00	1.00	1.00
1.01±0.05	**0.98±0.08**	**1.02±0.07)**	**1.02±0.07**

(2) Cubage of right hind paw after inflammation (l day)

Model control	Prednisone acetate	WSD	EEP
1.60	1.35	1.30	1.40
1.45	1.35	1.45	1.35
1.45	1.45	1.45	1.40
1.35	1.25	1.30	1.40
1.40	1.35	1.20	1.30
1.30	1.45	1.30	1.35
1.43±0.10	**1.37±0.08**	**1.33±0.10**	**1.37±0.04**

(3) Cubage of right hind paw after inflammation (2 days)

Model control	Prednisone acetate	WSD	EEP
1.95	1.75	1.65	1.60
1.65	1.95	1.75	1.55
2.05	1.55	1.75	1.55
1.80	1.65	1.65	1.80
1.90	1.65	1.80	2.00
2.00	1.55	1.65	1.80
1.89±0.15	**1.68±0.15**	**1.71±0.07**	**1.72±0.18**

(4) Cubage of right hind paw after inflammation (3 days)

Model control	Prednisone acetate	WSD	EEP
1.85	1.70	2.00	1.45
1.85	1.55	1.65	1.65
2.05	1.75	1.85	1.85
1.70	1.65	2.00	1.80
2.00	1.80	1.60	2.00
1.60	1.65	1.70	1.80
1.84±0.17	**1.68±0.09**	**1.80±0.18**	**1.76±0.19**

(5) Cubage of right hind paw after inflammation (5 days)

Model control	Prednisone acetate	WSD	EEP
1.60	1.45	1.75	1.85
1.75	1.45	1.65	1.65
1.90	1.45	1.90	1.65
1.75	1.55	1.80	1.45
1.55	1.65	1.60	1.70
1.65	1.85	2.00	1.55
1.70±0.13	**1.57±0.16**	**1.78±0.15**	**1.64±0.14**

(6) Cubage of right hind paw after inflammation (7 days)

Model control	Prednisone acetate	WSD	EEP
1.45	1.45	1.45	1.45
1.65	1.40	1.50	1.35
1.80	1.40	1.80	1.45
2.00	1.40	1.40	1.60
2.00	1.45	1.40	1.50
2.00	1.55	1.55	1.45
1.82±0.23	**1.44±0.06**	**1.52±0.15**	**1.47±0.08**

(7) Cubage of right hind paw after inflammation (9 days)

Model control	Prednisone acetate	WSD	EEP
1.55	1.55	1.80	1.55
1.75	1.55	1.45	1.55
2.00	1.40	1.50	1.45
1.45	1.35	1.60	1.75
1.95	1.35	1.50	1.75
1.80	1.55	1.65	1.65
1.75±0.22	**1.46±0.10**	**1.58±0.13**	**1.62±0.12**

(8) Cubage of right hind paw after inflammation (11 days)

Model control	Prednisone acetate	WSD	EEP
1.65	1.65	1.40	1.35
1.55	1.80	1.45	1.20
1.70	1.55	1.35	1.35
1.50	1.45	1.35	1.60
1.40	1.35	1.30	1.55
1.60	1.45	1.35	1.40
1.57±0.11	**1.54±0.16**	**1.37±0.05**	**1.41±0.15**

(9) Cubage of right hind paw after inflammation (13 days)

Model control	Prednisone acetate	WSD	EEP
1.65	1.50	1.65	1.40
1.65	1.45	1.40	1.45
1.80	1.35	1.55	1.50
1.75	1.35	1.70	1.55
1.60	1.40	1.60	1.65
1.75	1.55	1.75	1.55
1.70±0.08	**1.43±0.08**	**1.61±0.12**	**1.52±0.09**

(10) Cubage of right hind paw after inflammation (16 days)

Model control	Prednisone acetate	WSD	EEP
1.45	1.35	1.60	1.45
1.50	1.55	1.35	1.40
1.50	1.35	1.45	1.45
1.45	1.30	1.60	1.60
1.55	1.20	1.40	1.60
1.65	1.85	1.55	1.65
1.52±0.08	**1.43±0.23**	**1.49±0.11**	**1.53±0.10**

(11) Cubage of right hind paw after inflammation (19 days)

Model control	Prednisone acetate	WSD	EEP
1.75	1.65	1.95	1.55
1.85	1.60	1.45	1.65
2.10	1.35	1.45	1.85
1.60	1.40	1.45	1.55
1.60	1.45	1.50	1.75
1.55	1.75	1.35	1.55
1.74±0.21	**1.53±0.16**	**1.53±0.21**	**1.65±0.13**

Table 5.3: Effects of WSD and EEP on swelling of left hind paw (the secondary affection)

(1) Cubage before inflammation (ml)

Model control	Prednisone acetate	WSD	EEP
0.95	0.90	1.05	1.00
0.95	0.85	1.05	0.95
1.05	1.05	1.00	0.90
1.05	1.05	1.00	1.10
1.00	1.00	1.00	1.20
1.00	1.00	1.10	0.90
1.00±0.04	**0.98±0.08**	**1.03±0.04**	**1.01±0.12**

(2) Cubage before inflammation of left hind paw (1 day)

Model control	Prednisone acetate	WSD	EEP
1.00	1.00	1.00	1.05
1.05	0.90	1.05	0.90
1.20	1.00	1.20	0.90
1.10	0.90	1.00	1.00
0.90	0.95	0.95	1.05
1.00	1.05	1.05	1.00
1.04±0.10	**0.97±0.06**	**1.04±0.08**	**0.98±0.07**

(3) Cubage before inflammation of left hind paw (2 days)

Model control	Prednisone acetate	WSD	EEP
0.90	1.00	1.10	0.85
1.00	0.95	1.10	1.10
1.15	0.85	1.10	1.05
1.20	0.95	0.80	1.00
1.20	1.05	0.90	1.00
1.00	0.85	0.90	1.10
1.08±0.13	**0.94±0.08**	**0.98±0.13**	**1.02±0.09**

(4) Cubage before inflammation of left hind paw (3 days)

Model control	Prednisone acetate	WSD	EEP
1.05	1.20	1.15	0.95
1.25	1.10	1.15	1.00
1.25	1.05	1.10	1.05
0.90	1.10	0.90	1.20
1.00	1.15	0.80	1.00
0.80	1.20	0.80	0.80
1.04±0.18	**1.13±0.06**	**0.98±0.17**	**1.00±0.13**

(5) Cubage before inflammation of left hind paw (5 days)

Model control	Prednisone acetate	WSD	EEP
1.10	1.20	1.15	0.95
1.20	1.00	1.10	1.00
1.15	1.00	1.15	1.00
1.00	1.25	1.30	1.20
0.95	1.10	1.00	1.00
1.10	1.05	0.70	0.90
1.08±0.09	**1.10±0.10**	**1.07±0.20**	**1.01±0.10**

(6) Cubage before inflammation of left hind paw (7 days)

Model control	Prednisone acetate	WSD	EEP
1.05	1.15	1.15	1.00
1.05	0.95	1.30	1.00
1.30	1.00	1.05	1.15
1.50	1.10	0.95	1.00
1.20	1.00	0.90	0.70
1.40	1.20	0.85	1.00
1.25±0.18	**1.07±0.10**	**1.03±0.17**	**0.98±0.15**

(7) Cubage before inflammation of left hind paw (9 days)

Model control	Prednisone acetate	WSD	EEP
1.10	1.15	1.35	1.20
1.30	1.15	1.35	1.15
1.45	1.15	1.05	1.25
1.00	1.25	1.00	1.20
1.15	1.15	1.00	1.35
1.20	1.05	1.10	1.20
1.20±0.16	**1.15±0.06**	**1.14±0.17**	**1.22±0.07**

(8) Cubage before inflammation of left hind paw (11 days)

Model control	Prednisone acetate	WSD	EEP
1.15	1.40	1.30	1.00
1.30	1.20	1.20	1.05
1.20	1.20	1.05	1.20
1.10	1.30	0.90	1.10
1.00	1.10	1.00	1.05
1.00	1.05	0.95	1.15
1.13±0.12	**1.21±0.13**	**1.07±0.15**	**1.09±0.07**

(9) Cubage before inflammation of left hind paw (13 days)

Model control	Prednisone acetate	WSD	EEP
1.40	1.05	1.25	1.20
1.30	1.25	1.10	1.05
1.15	1.00	1.15	1.30
1.00	1.00	1.25	1.40
1.10	1.00	1.05	1.30
1.20	1.30	1.15	1.05
1.19±0.14	**1.10±0.14**	**1.16±0.08**	**1.22±0.14**

(10) Cubage before inflammation of left hind paw (16 days)

Model control	Prednisone acetate	WSD	EEP
0.95	1.10	1.05	0.90
1.00	0.90	1.25	0.95
1.00	0.95	0.90	1.05
1.10	0.95	1.00	1.30
1.00	0.90	1.00	1.15
1.05	0.85	1.00	1.10
1.02±0.05	**0.94±0.09**	**1.03±0.12**	**1.08±0.14**

(11) Cubage before inflammation of left hind paw (19 days)

Model control	Prednisone acetate	WSD	EEP
1.25	1.25	1.25	1.15
1.25	1.25	1.25	1.00
1.30	1.15	1.15	1.35
1.45	1.00	1.25	1.25
1.20	1.25	1.25	1.25
1.35	1.00	1.20	1.30
1.30±0.09	**1.15±0.12**	**1.23±0.04**	**1.22±0.13**

Table 5.4: Effects of WSD and EEP on the levels of PGE_2, IL-2, IL-6 and IFN-γ in the extravasate of FCA-induced arthritis rats

(1) Normal control group

PGE_2	IL-2 (pg)	IL-6 (pg)	IFN-γ (pg)
0.009	22.00	110	820
0.010	22.70	80	860
0.004	21.00	110	660
0.022	25.50	130	660
0.012	10.80	100	690
0.011	16.50	110	710
0.011±0.006	**19.75±5.27**	**106.67±16.33**	**733.33±85.71**

(2) Model control group

PGE_2	IL-2 (pg)	IL-6 (pg)	IFN-γ (pg)
0.050	10.70	90	570
0.031	22.70	176	570
0.063	11.50	106	760
0.051	9.30	170	590
0.042	9.40	120	600
0.044	10.50	170	650
0.047±0.011	**12.35±5.14**	**138.67±37.80**	**623.33±73.12**

(3) Prednisone acetate group

PGE_2	IL-2 (pg)	IL-6 (pg)	IFN-γ (pg)
0.017	12.50	60	630
0.029	12.50	40	590
0.028	25.00	80	500
0.026	17.50	50	590
0.046	19.00	106	590
0.034	16.50	60	620
0.030±0.010	**17.17±4.67**	**66.00±23.66**	**586.67±45.90**

(4) WSD group

PGE_2	IL-2 (pg)	IL-6 (pg)	IFN-γ (pg)
0.034	12.50	70	660
0.035	9.80	90	690
0.042	9.20	80	820
0.036	12.00	90	620
0.027	22.50	60	580
0.030	21.50	80	800
0.034±0.005	**14.58±5.89**	**78.33±11.69**	**695.00±96.69**

(5) EEP group

PGE_2	IL-2 (pg)	IL-6 (pg)	IFN-γ (pg)
0.040	17.80	80	520
0.021	12.50	60	510
0.027	10.30	80	570
0.024	12.50	110	560
0.026	20.70	110	610
0.019	17.80	90	620
0.026±0.007	**15.27±4.06**	**88.33±19.41**	**565.00±45.06**

Table 6.1: Effects of propolis on celiac capillary leakage in mice induced by acetic acid

Normal control	Prednisne acetate	WSD	EEP
0.600	0.557	0.571	0.446
0.626	0.561	0.465	0.541
0.544	0.547	0.328	0.606
0.736	0.457	0.422	0.501
0.673	0.586	0.626	0.546
0.535	0.427	0.518	0.542
0.739	0.492	0.643	0.511
0.517			0.573
0.6213±0.088	**0.5181±0.060**	**0.5104±0.114**	**0.5333±0.048**

Table 6.2: Effects of propolis on Carrageenan-induced hind paw oedema in rats

(1) Normal paw cubage (ml)

Model control	Dexamethasone acetate	WSD	EEP
0.95	1.20	1.20	1.30
0.90	1.00	1.20	1.20
0.85	1.15	1.00	1.30
0.55	0.95	1.10	1.00
1.10	1.25	1.25	1.30
0.80	1.20	1.15	1.20
1.00	0.90	1.00	1.30
0.75	1.20	1.00	1.45
0.863±0.169	**1.106±0.135**	**1.113±0.103**	**1.256±0.129**

(2) Oedema degree (1 h)

Model control	Dexamethasone acetate	WSD	EEP
0.10	0.20	0.15	0.00
0.15	0.25	0.15	0.15
0.20	0.20	0.05	0.15
0.35	0.45	0.20	0.25
0.00	0.05	0.05	0.30
0.15	0.05	0.10	0.20
0.45	0.50	0.00	0.15
0.10	0.15	0.25	0.10
0.188±0.146	**0.231±0.167**	**0.119±0.084**	**0.163±0.092**

(3) Oedema degree (2 h)

Model control	Dexamethasone acetate	WSD	EEP
0.50	0.20	0.25	0.15
0.40	0.40	0.20	0.25
0.65	0.45	0.35	0.35
0.85	0.40	0.15	0.35
0.65	0.10	0.15	0.20
0.65	0.05	0.10	0.35
0.55	0.45	0.45	0.05
0.50	0.15	0.45	0.15
0.594±0.137	**0.275±0.167**	**0.263±0.138**	**0.231±0.113**

(4) Oedema degree (3 h)

Model control	Dexamethasone acetate	WSD	EEP
0.50	0.15	0.20	0.25
0.60	0.20	0.35	0.30
0.65	0.15	0.45	0.35
0.90	0.50	0.35	0.40
0.70	0.20	0.10	0.40
0.65	0.05	0.30	0.30
0.70	0.25	0.45	0.15
0.75	0.05	0.55	0.25
0.681±0.116	**0.194±0.143**	**0.344±0.145**	**0.300±0.085**

(5) Oedema degree (4 h)

Model control	Dexamethasone acetate	WSD	EEP
0.75	0.25	0.20	0.05
0.65	0.10	0.35	0.20
0.80	0.45	0.25	0.10
1.00	0.35	0.30	0.25
0.65	0.15	0.15	0.00
0.85	0.30	0.45	0.05
0.65	0.25	0.10	0.35
0.80	0.45	0.15	0.05
0.769±0.122	**0.288±0.127**	**0.244±0.118**	**0.131±0.122**

(6) Oedema degree (5 h)

Model control	Dexamethasone acetate	WSD	EEP
0.45	0.05	0.20	0.15
0.45	0.05	0.30	0.10
0.55	0.05	0.25	0.20
0.85	0.25	0.15	0.40
0.40	0.05	0.20	0.25
0.65	0.00	0.25	0.15
0.85	0.10	0.35	0.15
0.65	0.00	0.35	0.10
0.606±0.176	**0.069±0.080**	**0.256±0.023**	**0.188±0.099**

(7) Oedema degree (6 h)

Model control	Dexamethasone acetate	WSD	EEP
0.45	0.05	0.05	0.10
0.50	0.10	0.30	0.20
0.65	0.10	0.40	0.10
0.80	0.15	0.10	0.35
0.45	0.10	0.15	0.20
0.50	0.15	0.15	0.10
0.55	-0.10	0.05	0.10
0.45	0.10	0.20	0.15
0.544±0.124	**0.081±0.080**	**0.175±0.122**	**0.163±0.088**

Table 6.3: Effects of propolis on pleurisy extravasate and leucocyte counts in rats

(1) Normal control group

Extravasate (ml)	WBC ($\times 10^9 \cdot L^{-1}$)	Lymphocyte (%)	Neutrophils (%)
0.10	5.80	45.00	55.00
0.10	6.00	42.00	58.00
0.10	5.80	46.00	54.00
0.10	9.60	52.00	48.00
0.10	6.00	41.00	59.00
0.10	3.80	53.00	47.00
0.10	15.00	51.00	49.00
0.10	4.20	49.00	51.00
0.10±0.00	**7.03±3.66**	**47.38±4.57**	**52.63±4.57**

(2) Model control group

Extravasate (ml)	WBC ($\times 10^9 \cdot L^{-1}$)	Lymphocyte (%)	Neutrophils (%)
2.10	33.20	8.00	92.00
2.50	20.40	17.00	83.00
1.70	37.00	12.00	88.00
2.40	21.40	15.00	85.00
1.70	39.80	23.00	77.00
1.70	32.80	13.00	87.00
1.80	24.20	18.00	82.00
1.99±0.35	**29.83±7.77**	**15.14±4.81**	**84.86±4.81**

(3) Prednisone acetate group

Extravasate (ml)	WBC ($\times 10^9 \cdot L^{-1}$)	Lymphocyte (%)	Neutrophils (%)
1.70	18.00	26.00	74.00
1.60	28.90	30.00	70.00
1.60	27.00	31.00	69.00
2.10	24.90	38.00	62.00
2.20	21.70	33.00	67.00
1.30	29.50	35.00	65.00
1.20	24.40	20.00	80.00
1.50	35.60	9.00	91.00
1.40	20.80	27.00	73.00
1.62±0.34	**25.64±5.31**	**27.67±8.77**	**72.33±8.77**

(4) WSD group

Extravasate (ml)	WBC ($\times 10^9 \cdot L^{-1}$)	Lymphocyte (%)	Neutrophils (%)
1.50	19.70	32.00	68.00
1.50	14.00	21.00	79.00
0.90	37.80	19.00	81.00
0.90	15.20	29.00	71.00
1.00	23.75	20.00	80.00
0.70	21.95	29.00	71.00
1.08±0.34	**22.07±8.58**	**25.00±5.62**	**75.00±5.62**

(5) EEP group

Extravasate (ml)	WBC ($\times 10^9 \cdot L^{-1}$)	Lymphocyte (%)	Neutrophils (%)
2.10	39.00	28.00	72.00
1.20	22.30	26.00	74.00
1.90	27.90	21.00	79.00
1.70	20.10	18.00	82.00
1.00	30.70	33.00	67.00
1.40	31.15	13.00	87.00
1.70	19.20	15.00	85.00
2.00	23.35	32.00	68.00
0.90	38.40	27.00	73.00
1.54±0.44	**28.01±7.42**	**23.67±7.25**	**76.33±7.25**

Table 6.4: Effects of propolis on the levels of total protein, NO and PGE_2 in the pleurisy extravasate in rats

(1) Normal control group

Protein (g/L)	NO (umol/L)	PGE_2 (OD)
5.00	3.72	0.003
3.28	5.60	0.007
3.24	9.10	0.005
4.28	3.50	0.004
2.67	6.20	0.004
2.69	8.60	0.001
3.53±0.93	**6.12±2.36**	**0.004±0.002**

(2) Model control group

Protein (g/L)	NO (umol/L)	PGE_2 (OD)
18.52	28.28	0.193
23.79	24.43	0.233
24.89	35.99	0.194
28.73	28.65	0.192
18.92	33.52	0.182
21.50	43.09	0.169
22.73±3.89	**32.33±6.68**	**0.194±0.021**

(3) Positive control control group

Protein (g/L)	NO (umol/L)	PGE_2 (OD)
7.48	10.30	0.054
12.30	22.78	0.075
6.96	10.24	0.068
12.37	11.96	0.126
5.31	17.07	0.066
13.05	16.95	0.066
9.58±3.37	**14.88±4.95**	**0.076±0.025**

(4) WSD group

Protein (g/L)	NO (umol/L)	PGE_2 (OD)
14.04	26.30	0.162
14.40	19.51	0.189
8.80	29.65	0.056
14.82	28.79	0.068
12.13	23.59	0.044
17.67	23.06	0.027
13.64±2.97	**25.15±3.83**	**0.091±0.067**

(5) EEP group

Protein (g/L)	NO (umol/L)	PGE_2 (OD)
11.96	16.16	0.118
14.44	29.10	0.028
17.42	18.52	0.040
17.74	22.30	0.030
13.16	12.94	0.071
24.86	22.46	0.033
16.60±4.65	**20.25±5.67**	**0.053±0.035**

Table 6.5: Effects of propolis on the leucocyte count and lung index in rats with of acute lung injury

(1) Normal control group

Lung index	WBC ($\times 10^9 \cdot L^{-1}$)	Neutrophils (%)	Lymphocyte (%)
0.9215	4.20	13.00	87.00
0.7748	4.00	11.00	89.00
0.8466	6.00	16.00	84.00
0.8858	6.00	30.00	70.00
0.7763	7.80	9.00	91.00
0.8274	4.80	24.00	76.00
0.84±0.06	**5.47±1.43**	**17.17±8.18**	**82.83±8.18**

(2) Model control group

Lung index	WBC ($\times 10^9 \cdot L^{-1}$)	Neutrophils (%)	Lymphocyte (%)
1.3666	11.20	52.00	48.00
1.4835	12.00	61.00	39.00
1.3094	10.80	56.00	44.00
1.6376	10.60	62.00	38.00
1.4013	9.60	51.00	49.00
1.3876	12.00	69.00	31.00
1.43±0.12	**11.03±0.92**	**58.50±6.83**	**41.50±6.83**

(3) Dexamethasone acetate group

Lung index	WBC ($\times 10^9 \cdot L^{-1}$)	Neutrophils (%)	Lymphocyte (%)
1.4216	6.00	31.00	69.00
1.5349	5.60	73.00	27.00
1.2209	4.80	68.00	32.00
1.0008	3.80	56.00	44.00
1.5379	7.00	33.00	67.00
1.1479	4.40	16.00	84.00
1.31±0.22	**5.27±1.16**	**46.17±22.83**	**53.83±22.83**

(4) WSD group

Lung index	WBC ($\times 10^9 \cdot L^{-1}$)	Neutrophils (%)	Lymphocyte (%)
1.2605	5.60	45.00	55.00
0.8308	6.40	28.00	72.00
1.3123	5.20	47.00	53.00
1.6152	6.20	53.00	47.00
0.8353	7.00	22.00	78.00
1.3171	6.20	25.00	75.00
1.20±0.31	**6.10±0.63**	**36.67±13.19**	**63.33±13.19**

(5) EEP group

Lung index	WBC ($\times 10^9 \cdot L^{-1}$)	Neutrophils (%)	Lymphocyte (%)
1.3171	13.60	17.00	83.00
1.3868	9.80	15.00	85.00
1.0374	12.00	27.00	73.00
1.3802	10.80	23.00	77.00
1.0268	12.60	12.00	88.00
0.7071	6.80	40.00	60.00
1.14±0.27	**10.93±2.43**	**22.33±10.23**	**77.67±10.23**

Table 7.1: Effects of propolis solutions on the leucocyte count and lung index of acute lung injury in rats

(See Table 6.5)

Table 7.2: The activation of NF-κB p65 in lung determines immuno-histochemically

Normal control	Model control	Dexamethasone	WSD	EEP
204	633	397	344	386
226.5	655	402.5	289.5	345.5
184	613	378	416	328.5
189.5	664	362	369.5	301
201	614	445	348	367
149	660	356	315.5	280.5
214	617	447.5	391	331
174.5	630.5	404	301	246
192.81±24.26	**635.81±21.16**	**399.00±34.16**	**346.81±44.12**	**323.19±45.86**

Table 8.1: Blood TC and TG (mmol/L) changes of hyperlipidemic rats for six weeks

(1) Normal control group

zero week		2nd week		4th week		6th week	
TC	TG	TC	TG	TC	TG	TC	TG
1.56	0.76	1.89	2.20	1.68	1.08	2.04	1.62
1.92	1.03	1.60	1.73	0.98	0.96	1.49	1.44
2.66	0.85	2.19	1.57	1.59	1.19	3.36	1.27
2.17	0.77	2.18	1.45	1.50	1.18	2.55	1.56
2.46	1.63	2.23	1.67	1.55	1.27	2.80	1.74
2.01	1.40	1.79	1.36	1.47	1.69	2.56	1.17
1.96	1.20	2.08	1.92	1.88	1.51	3.03	1.32
2.23	1.13	1.86	2.23	1.54	0.82	2.70	1.95
1.92	1.12	2.01	2.29	1.44	0.82	3.32	1.79
2.01	1.09	1.78	1.94	1.44	1.14	2.44	1.42
2.09±0.31	**1.10±0.27**	**1.96±0.21**	**1.84±0.33**	**1.51±0.23**	**1.17±0.28**	**2.63±0.57**	**1.53±0.25**

(2) Model control group

zero week		2nd week		4th week		6th week	
TC	TG	TC	TG	TC	TG	TC	TG
2.04	1.27	3.67	2.71	3.97	1.16	5.15	1.51
2.00	0.56	3.61	3.27	3.91	1.61	5.83	2.82
1.99	1.52	4.18	1.90	5.28	2.12	6.00	3.21
2.48	0.83	3.35	2.23	5.52	1.58	7.94	2.85
2.25	0.82	3.08	2.27	4.62	1.40	6.76	2.53
2.19	0.90	4.06	3.11	4.34	2.56	7.51	4.38
2.17	0.74	3.50	1.97	4.46	2.04	5.91	5.11
1.72	1.14	3.02	1.99	3.56	1.48	4.77	2.27
2.55	1.12	3.09	2.38	3.19	0.88	6.01	4.67
2.20	1.21	3.96	3.61	3.56	1.84	4.80	2.58
2.16±0.24	**1.01±0.29**	**3.55±0.42**	**2.54±0.60**	**4.24±0.76**	**1.67±0.49**	**6.07±1.07**	**3.19±1.16**

(3) Xuezhikang group

zero week		2nd week		4th week		6th week	
TC	TG	TC	TG	TC	TG	TC	TG
2.26	1.16	3.87	3.81	4.30	0.78	5.42	1.81
2.32	1.09	3.07	2.42	2.66	1.04	5.05	2.04
2.23	1.29	3.10	2.80	2.70	0.84	5.44	1.33
2.15	1.10	3.51	2.65	4.00	1.32	5.63	1.57
2.02	0.86	4.12	3.29	4.57	0.91	6.34	1.95
2.00	1.12	3.50	2.44	2.40	1.20	2.48	1.09
2.24	1.41	3.57	2.22	2.74	0.90	2.01	1.50
2.05	1.02	4.03	4.00	3.21	0.80	2.90	0.92
2.02	1.30	3.44	1.49	3.20	0.98	2.67	1.53
2.14	1.15	4.01	3.82	3.48	0.74	4.22	1.53
2.14±0.12	**1.15±0.16**	**3.62±0.38**	**2.89±0.82**	**3.33±0.74**	**0.95±0.19**	**4.22±1.57**	**1.53±0.35**

(4) WSD1 group

zero week		2nd week		4th week		6th week	
TC	TG	TC	TG	TC	TG	TC	TG
1.87	1.01	3.77	2.32	3.41	1.07	4.93	3.11
2.20	1.62	2.96	2.39	3.39	1.10	5.06	2.23
1.93	1.87	3.28	2.60	4.46	1.70	5.34	3.74
2.13	1.87	2.97	3.30	3.14	1.70	4.25	2.69
2.40	0.86	3.64	3.29	3.81	1.67	5.22	1.96
1.74	0.93	3.78	3.37	4.69	1.26	4.99	1.50
2.31	1.07	3.42	3.08	3.26	0.84	4.98	2.46
1.95	1.18	3.30	2.73	3.45	0.75	4.79	3.20
2.06	1.45	3.38	3.06	3.39	1.70	4.41	1.74
2.59	0.99	3.71	2.59	4.11	1.65	4.12	2.51
2.12±0.30	**1.29±0.39**	**3.42±0.31**	**2.87±0.39**	**3.71±0.54**	**1.34±0.38**	**4.81±0.41**	**2.51±0.70**

(5) WSD2 group

zero week		2nd week		4th week		6th week	
TC	TG	TC	TG	TC	TG	TC	TG
1.91	1.61	3.46	2.07	3.41	1.07	3.29	1.60
1.77	0.65	3.84	2.57	3.39	1.10	4.37	0.95
1.90	1.23	2.95	2.37	4.46	1.70	3.92	0.74
2.26	1.57	3.83	4.47	3.14	1.70	4.94	1.19
2.38	1.56	3.01	2.65	3.81	1.67	4.18	1.56
1.83	1.23	3.12	2.06	4.69	1.16	4.07	3.17
2.16	0.96	3.76	2.65	3.26	0.94	3.74	1.43
2.39	1.18	3.74	4.02	3.45	0.95	5.43	1.63
1.78	0.97	4.14	2.34	3.39	1.70	3.92	2.32
2.38	0.65	2.63	1.82	4.11	1.65	3.87	2.46
2.08±0.26	**1.16±0.36**	**3.45±0.49**	**2.70±0.86**	**3.35±0.70**	**1.36±0.34**	**4.17±0.62**	**1.71±0.74**

(6) EEP1 group

zero week		2nd week		4th week		6th week	
TC	TG	TC	TG	TC	TG	TC	TG
2.25	1.29	3.46	2.25	3.77	1.47	4.64	1.53
2.18	1.17	3.57	2.95	4.19	1.27	4.75	1.64
2.66	1.27	3.51	2.39	2.83	0.77	4.07	0.82
2.32	0.83	3.89	3.60	3.12	1.04	3.55	1.40
2.11	1.34	3.31	2.07	3.58	1.63	3.75	1.98
1.79	0.78	2.96	1.70	2.71	1.17	3.36	0.71
2.17	0.86	3.19	3.25	3.14	1.20	4.18	0.54
1.95	1.05	3.33	2.83	2.43	0.71	2.85	0.99
2.40	1.21	3.79	2.36	3.52	1.27	4.58	0.68
2.04	1.09	4.16	2.84	3.14	0.92	4.18	1.60
2.19±0.24	**1.09±0.20**	**3.52±0.35**	**2.62±0.57**	**3.24±0.53**	**1.15±0.29**	**3.99±0.61**	**1.19±0.50**

(7) EEP2 group

zero week		2nd week		4th week		6th week	
TC	TG	TC	TG	TC	TG	TC	TG
1.84	0.96	3.21	2.89	3.66	1.19	4.27	1.80
1.92	0.91	3.95	2.14	3.36	1.02	5.01	1.66
1.82	1.30	3.43	2.40	3.12	1.01	3.46	1.53
1.87	0.81	3.57	2.09	3.14	1.24	4.56	1.19
2.24	1.51	3.55	2.97	3.53	1.29	4.17	1.10
1.98	1.15	3.39	2.75	3.79	1.68	4.84	1.61
2.24	1.39	3.60	3.15	3.77	0.87	3.13	0.82
2.21	0.64	2.91	2.24	2.93	1.12	3.18	0.76
2.01	0.81	3.08	3.01	2.63	0.83	2.80	1.76
1.86	1.18	4.41	2.90	3.42	1.02	3.82	1.02
2.00±0.17	**1.07±0.28**	**3.51±0.43**	**2.65±0.40**	**3.34±0.38**	**1.13±0.24**	**3.92±0.77**	**1.33±0.39**

Table 8.2: Effects of propolis on HDL-C, LDL-C and AS in hyperlipidemic ratss

(1) Normal control group

HDL-C (mmol/L)	LDL-C (mmol/L)	AS
2.67	1.27	0.58
1.37	0.82	0.47
2.64	0.93	0.66
2.17	0.93	0.58
2.73	1.28	0.30
1.62	1.29	0.64
2.36	1.33	0.51
2.33	1.34	0.69
1.95	0.91	0.83
2.13	0.80	1.03
2.20±0.45	**1.09±0.23**	**0.63±0.20**

(2) Model control group

HDL-C (mmol/L)	LDL-C (mmol/L)	AS
1.56	2.43	2.30
0.76	2.49	6.67
1.51	2.54	2.97
1.29	3.66	5.16
1.32	3.75	4.12
1.52	4.00	3.94
1.17	2.55	4.05
0.98	2.58	3.87
1.01	3.02	4.95
1.31	2.28	2.61
1.24±0.26	**2.93±0.64**	**4.06±1.30**

(3) Xuezhikang group

HDL-C (mmol/L)	LDL-C (mmol/L)	AS
1.56	2.30	2.57
0.76	2.35	2.50
1.51	2.97	2.33
1.29	2.46	1.76
1.32	2.96	2.35
1.52	1.15	1.03
1.17	0.80	2.07
0.98	1.39	3.26
1.01	1.25	3.93
1.33	1.96	1.88
1.25±0.26	**1.96±0.77**	**2.37±0.80**

(4) WSD1 group

HDL-C (mmol/L)	LDL-C (mmol/L)	AS
1.47	2.44	2.18
1.05	2.56	1.84
1.17	2.73	2.79
1.77	2.25	2.00
1.55	2.43	2.73
2.13	2.56	2.01
1.61	2.24	2.30
1.01	2.34	2.07
1.34	2.48	1.51
1.46	2.00	1.86
1.46±0.34	**2.40±0.21**	**2.13±0.39**

(5) WSD2 group

HDL-C (mmol/L)	LDL-C (mmol/L)	AS
1.18	1.31	1.15
1.50	1.91	2.26
0.93	1.85	1.88
1.62	2.16	2.53
1.12	2.16	1.42
1.56	2.18	2.36
2.58	1.91	1.94
1.26	2.63	2.72
1.89	2.41	1.39
1.67	1.76	2.62
1.53±0.47	**2.03±0.37**	**2.03±0.56**

(6) EEP1 group

HDL-C (mmol/L)	LDL-C (mmol/L)	AS
1.46	2.09	2.18
3.17	2.37	2.26
0.85	1.32	3.79
1.54	0.86	1.31
1.52	1.66	1.47
1.07	1.09	2.14
1.36	1.10	2.07
0.74	0.90	2.75
1.43	1.92	2.20
0.80	1.39	4.23
1.39±0.70	**1.47±0.52**	**2.44±0.93**

(7) EEP2 group

HDL-C (mmol/L)	LDL-C (mmol/L)	AS
0.80	1.74	4.34
1.68	2.57	1.98
1.14	0.93	2.04
1.30	1.95	2.51
1.27	1.90	2.28
1.10	2.48	3.40
1.25	0.99	1.50
1.68	1.38	2.12
2.02	0.74	2.12
1.36	1.52	1.84
1.36±0.35	**1.62±0.63**	**2.41±0.84**

Table 8.3: Effects of propolis on body weight, liver weight, liver index, TC and TG in liver, GPT and GOT in serum of hyperlipidemic rats

(1) Normal control group

TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
0.44	1.16	26	38	394	7.92	1.97
0.44	1.20	32	36	371	7.02	1.88
0.26	1.02	29	51	398	6.94	2.29
0.23	0.92	26	33	301	9.02	2.37
0.31	0.89	35	66	339	8.81	2.73
0.23	0.92	28	45	372	6.24	1.98
0.35	1.27	25	36	353	7.93	1.83
0.29	1.01	35	50	339	8.34	2.37
0.44	1.25	27	38	362	7.14	2.28
0.34	1.14	34	49	355	8.11	1.88
0.33±0.08	**1.08±0.14**	**29.7±3.9**	**44.2±10.1**	**358.4±28.4**	**7.75±0.89**	**2.16±0.29**

(2) Model control group

TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
2.56	3.63	63	58	419	17.80	4.47
1.71	2.35	62	70	430	17.94	3.48
1.47	2.05	47	85	449	15.25	3.70
2.31	3.43	88	81	433	17.33	4.31
2.11	3.18	86	73	448	14.72	3.91
2.18	3.26	72	53	472	16.65	3.76
1.37	1.92	71	89	404	15.65	3.89
2.31	3.12	70	62	475	17.57	3.11
2.36	3.20	63	72	401	15.03	3.60
2.04	2.90	70	71	448	16.22	3.23
2.04±0.40	**2.90±0.59**	**69.2±11.9**	**71.4±11.5**	**437.9±25.4**	**16.42±1.21**	**3.75±0.43**

(3) Xuezhikang group

TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
2.31	2.40	43	66	380	10.86	2.96
2.29	3.26	52	60	417	13.46	2.92
0.93	1.81	45	45	406	11.81	2.97
2.88	3.83	42	89	376	10.21	2.46
1.45	1.98	40	31	451	11.45	2.66
1.80	3.09	27	58	394	10.74	3.01
1.95	2.61	25	71	402	11.89	2.42
0.75	2.26	25	40	461	11.57	2.44
1.92	2.27	31	59	410	11.02	3.32
1.92	2.61	39	58	411	10.05	2.34
1.82±0.64	**2.61±0.62**	**36.9±9.4**	**57.7±16.4**	**410.8±27.3**	**11.31±0.98**	**2.75±0.33**

(4) WSD1 group

TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
1.13	2.12	69	40	458	17.75	3.72
2.07	3.07	50	45	471	14.71	3.66
1.77	2.87	62	41	412	14.76	3.21
1.58	2.04	64	54	481	17.70	2.87
1.35	2.41	74	46	428	13.66	3.91
1.46	2.62	52	44	380	15.32	3.57
2.40	3.65	43	38	452	13.79	3.36
1.16	2.02	56	41	386	14.92	4.50
1.24	1.94	54	45	412	16.61	3.22
2.12	2.35	40	36	462	15.40	3.56
1.63±0.44	**2.51±0.55**	**56.4±10.9**	**43.0±5.1**	**434.2±35.7**	**15.46±1.45**	**3.56±0.45**

(5) WSD2 group

TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
1.49	2.17	37	33	413	16.43	3.44
1.22	1.64	53	34	388	16.33	3.48
1.49	2.49	53	35	429	15.31	3.70
1.17	2.07	55	41	415	17.82	4.42
1.56	2.91	50	78	400	14.72	3.96
1.46	2.04	48	32	435	13.18	3.76
1.75	2.71	38	28	419	15.65	3.89
1.56	2.85	30	47	425	14.26	3.11
1.32	1.99	43	41	467	14.49	3.60
1.02	1.97	56	42	397	14.66	3.18
1.40±0.22	**2.28±0.43**	**46.3±8.9**	**41.1±14.2**	**418.8±22.5**	**15.29±1.32**	**3.65±0.39**

(6) EEP1 group

TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
1.26	1.79	33	34	435	13.99	3.51
1.10	2.05	43	32	406	11.79	3.55
1.86	2.18	26	42	390	15.03	3.04
1.26	2.09	32	39	413	14.17	2.88
1.30	1.68	35	47	408	13.11	3.41
1.25	2.25	47	33	409	14.92	3.46
2.09	3.13	32	37	424	14.35	3.96
2.01	3.05	35	31	409	13.55	3.60
1.72	3.01	36	26	380	14.05	3.40
2.12	2.66	34	35	428	14.56	3.16
1.60±0.40	**2.39±0.53**	**35.3±5.9**	**35.6±6.0**	**410.2±16.6**	**13.95±0.96**	**3.40±0.31**

(7) EEP2 group

TC (mmol/L)	TG (mmol/L)	GPT (U/L)	GOT (U/L)	Body weight (g)	Liver weight (g)	Liver index
1.76	2.27	21	31	440	14.23	2.55
0.95	1.99	38	33	373	10.97	3.40
1.62	2.74	41	35	443	13.88	3.11
1.94	2.30	41	34	365	13.21	2.48
2.13	2.31	40	36	431	11.19	2.75
1.62	2.06	34	32	408	11.56	3.13
1.82	3.28	33	55	428	12.19	3.38
2.01	2.16	34	54	367	11.69	3.40
1.22	2.49	31	38	404	11.85	3.04
1.67	2.40	27	38	407	12.60	3.04
1.67±0.36	**2.40±0.38**	**34.0±6.5**	**38.6±8.7**	**406.6±29.7**	**12.34±1.12**	**3.03±0.34**

Table 8.4: Effects of propolis on MDA, SOD and NO in serum and MDA in livers of hyperlipidemic rats

(1) Normal control group

MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
174.87	6.88	130.6	8.43
169.21	8.49	134.7	7.86
200.36	8.30	125.4	7.04
198.31	8.45	127.9	11.27
166.90	7.75	127.5	6.25
171.43	10.15	125.7	9.73
161.2	8.04	123.6	6.61
189.24	8.31	135.4	9.48
177.61	8.85	141.7	4.29
190.63	7.42	141.5	7.02
179.9±13.7	**8.26±0.88**	**131.4±6.6**	**7.80±2.0**

(2) Model control group

MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
207.12	9.39	115.5	14.75
253.46	10.31	144.0	10.05
236.41	14.10	113.4	18.49
234.50	12.12	121.3	18.02
232.14	12.08	110.8	10.56
245.63	11.75	121.7	12.22
219.45	11.38	116.8	22.18
239.62	11.39	119.9	11.92
233.61	11.21	116.4	18.34
223.37	11.49	116.2	18.42
232.5±13.2	**11.52±1.23**	**119.6±9.2**	**15.5±4.1**

(3) Xuezhikang group

MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
236.12	10.43	120.2	16.89
235.70	14.81	121.5	22.31
213.60	12.43	130.0	19.31
246.10	14.60	123.3	25.08
217.30	14.11	117.8	20.02
203.54	12.59	114.4	20.44
231.01	12.40	100.3	18.56
245.20	11.30	118.0	23.90
213.17	11.58	109.5	26.27
189.43	11.58	110.0	20.20
225.3±17.6	**12.58±1.48**	**116.5±8.4**	**21.3±3.0**

(4) WSD1 group

MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
236.12	11.45	116.5	20.06
235.70	12.31	128.2	19.04
213.60	12.66	96.7	13.78
246.10	12.31	117.8	15.83
217.30	10.54	112.0	15.03
203.54	10.87	110.7	13.87
231.01	14.10	125.6	13.83
245.20	12.45	104.8	12.34
213.17	8.63	139.6	11.76
189.43	11.70	114.1	27.44
223.1±18.7	**11.70±1.47**	**116.6±12.2**	**16.3±4.7**

(5) WSD2 group

MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
203.56	9.46	120.4	18.41
213.45	14.26	119.5	23.04
189.10	12.56	119.2	16.47
229.30	9.95	131.5	22.05
226.31	12.03	118.3	17.61
198.32	14.11	123.2	13.62
224.16	7.88	124.6	19.71
239.40	11.31	129.1	18.09
183.45	12.42	123.2	11.99
201.11	10.53	125.0	20.04
210.8±18.6	**11.45±2.04**	**123.4±4.3**	**18.1±3.4**

(6) EEP1 group

MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
237.81	9.66	101.4	6.49
213.45	10.41	130.6	23.76
228.90	12.89	121.6	14.58
212.45	12.16	122.5	12.90
226.30	11.70	128.7	14.41
237.89	7.98	122.1	14.84
189.31	10.87	127.9	14.03
197.2	10.62	122.4	25.58
223.41	9.64	122.5	15.97
169.31	9.50	118.3	19.47
213.6±22.3	**10.54±1.45**	**121.8±8.1**	**16.2±5.5**

(7) EEP2 group

MDA in liver (nmol/mg prot)	MDA in serum (nmol/mL)	SOD (NU/mL)	NO (Umol/L)
213.5	9.99	102.7	18.41
214.21	10.22	125.8	19.48
188.47	10.5	121.6	16.47
239.12	11.89	122.5	11.82
229.4	12.02	121.8	17.61
212.31	12.24	122.1	12.62
193.70	11.26	125.1	19.71
224.13	10.19	122.4	18.09
207.23	13.21	131.9	12.49
227.35	11.28	119.1	20.34
214.9±15.8	**11.28±1.06**	**121.5±7.5**	**16.7±3.2**

Table 9.2: Effects of propolis extracts on S_{180} tumor growth in mice

Average tumor weight (g)

Model control	95% EEP	80% EEP	WSD
1.2796	0.5487	0.4102	0.2488
1.2303	0.5489	0.4322	0.2375
1.3069	0.4879	0.4189	0.2309
1.2563	0.5973	0.4356	0.2580
1.3120	0.6833	0.4008	0.2433
1.2341	0.5392	0.4129	0.2429
1.2794	0.5484	0.3861	0.2451
1.2572	0.5873	0.4097	0.2344
1.2383	0.5480	0.4109	0.2422
1.2302	0.4870	0.4036	0.2434
1.262±0.031	**0.558±0.057**	**0.412±0.014**	**0.243±0.008**

Table 9.3: Effects of propolis extracts on the weight of granuloma in mice

Weight of granuloma (mg):

Model control	Prednisone acetate	WSD	80% EEP
34.91	20.58	20.11	24.76
33.05	20.61	21.29	25.90
34.96	22.92	20.96	23.74
35.06	22.54	21.34	22.89
34.45	22.45	19.70	25.56
37.58	20.91	21.04	23.81
32.57	19.81	22.53	28.97
35.55	19.70	22.65	24.41
34.82	21.09	22.81	25.69
34.01	20.67	19.91	24.93
34.11	21.44	20.64	20.09
35.79	21.32	21.77	26.85
34.738±1.300	**21.170±1.030**	**21.229±1.058**	**24.800±2.180**

Table 9.4: Effects of propolis solutions on edema of the right hind paw of rats induced by Carrageenan.

(See table 6.2)

Table 9.5: Effects of propolis on pleurisy extravasate and leucocyte counts in rats

(See table 6.3)

Table 9.6: Effects of propolis on the levels of total protein, NO and PGE_2 in the pleurisy extravasate in rats

(See table 6.4)