

**AN INVESTIGATION INTO THE
NEUROPROTECTIVE EFFECTS OF MELATONIN
IN A MODEL OF ROTENONE-INDUCED
NEURODEGENERATION**

Thesis

submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE (PHARMACY)

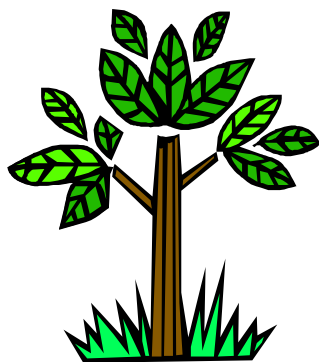
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By

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***ILLNESS IS SO LIMITED...
IT CANNOT
CRIPPLE LOVE,
SHATTER HOPE,
CORRODE FAITH,
DESTROY PEACE,
KILL FRIENDSHIP,
SUPPRESS MEMORIES,
SILENCE COURAGE,
INVADE THE SOUL,
STEAL ETERNAL LIFE,
IT CANNOT CONQUER THE SPIRIT***

- A Patient Suffering from Parkinson's

disease

ABSTRACT

Parkinson's disease, one of the most common neurodegenerative disorders associated with ageing, is characterised by abnormal and profound loss of nigrostriatal dopaminergic neurons. The cause of Parkinson's disease is unknown, but epidemiological studies suggest an association with pesticides and other environmental toxins, and biochemical studies implicate oxidative damage and mitochondrial impairment, particularly at the level of complex I enzyme. Recently, rotenone, a commonly used organic pesticide and a classical inhibitor of mitochondrial complex I has been reported to reproduce the specific features of Parkinson's disease in rodents.

The mitochondrial respiratory chain is one of the most important sites of reactive oxygen species production under physiological conditions. Toxic free radicals have been implicated in a variety of neurodegenerative diseases as well as ageing itself. Melatonin, a secretory product of the pineal gland is a multifaceted free radical scavenger and natural antioxidant. In the present study, the neuroprotective effects of melatonin against the environmental neurotoxin, rotenone was investigated.

Initial studies showed that inhibition of mitochondrial complex I enzyme by rotenone induced superoxide radical generation. Melatonin, administered to the rat *in vivo* and *in vitro* was able to offer neuroprotection by curtailing the production of superoxide radicals induced by rotenone. Mitochondria, being the major target of rotenone, the effects of melatonin were investigated at the mitochondrial level. Melatonin was able to increase the electron transport chain activity thus preventing the respiratory inhibition by

rotenone. The pineal hormone also counteracted the action of rotenone on complex I enzyme. These results suggest melatonin's ability to potentially limit the free radical generation and thereby modulate the mitochondrial functions.

The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in toxicology and in human disease. Melatonin also offered significant protection *in vivo* and *in vitro* against rotenone induced lipid peroxidation. Since iron plays a major role in oxidative damage and in the progression of Parkinson's disease, the effect of melatonin on both rotenone and iron induced lipid peroxidation was investigated, the results of which show that melatonin affords protection and this was suggested to be due to its interaction with the rotenone-iron complex that might have formed. Electrochemical studies were further used to characterise the interactions between melatonin, rotenone and iron (III). Melatonin was shown to bind with iron and thus reducing their toxicity.

Histological studies were undertaken to assess the effects of melatonin on rotenone induced toxicity on the dopaminergic neurons in the rat brain. Rotenone treated brains showed extensive neuronal damage whereas with melatonin less damage was observed. Rotenone induces apoptosis via reactive oxygen species production and apoptotic cell death has been identified in PD brains. Furthermore, the apoptotic cell death was detected and quantified by the TUNEL staining. Rotenone treated sections showed signs of apoptosis whereas with melatonin, less apoptotic damage was observed.

The findings of this study indicate that the neurohormone, melatonin may protect against rotenone-induced neurodegeneration. Since melatonin production falls substantially during ageing, the loss of this antioxidant is theorized to be instrumental in the degenerative processes associated with advanced age. Considering how devastating diseases such as Parkinson's disease, are to a patient and the patient's families, the discovery of protective agents are a matter of urgency. Further investigations using the pesticide model will help to determine the involvement of environmental exposure in the pathogenesis of human diseases as well as to test therapeutic strategies for the treatment of such diseases.

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LIST OF ABBREVIATIONS

μA —Microamperes
 μg —Microgram
 μl —Microlitre
 μM —Micromolar
4-HDA—4-Hydroxyalkenals
5-HT—5-Hydroxytryptamine (Serotonin)
AD—Alzheimer's disease
AIF—Apoptosis-inducing factor
ALS—Amyotrophic lateral sclerosis
ANOVA—Analysis of variance
ASV—Adsorptive stripping voltammetry
ATP—Adenosine triphosphate
BHT—Butylated hydroxytoluene
BSA—Bovine serum albumin
 Ca^{2+} —Calcium (II)
CE—Counter Electrode
CNS—Central nervous system
CV—Cyclic voltammetry
DA—Dopamine
DNA—Deoxyribonucleic acid
DPI—2,6-dichlorophenolindophenol
EAA—Excitatory amino acid
EDTA—Ethylenediaminetetraacetic acid
ETC—Electron transport chain
 Fe^{2+} —Iron II
 Fe^{3+} —Iron III
FMN—Flavin mononucleotide
GCE—Glassy carbon electrode
GSH—Glutathione
 H_2O_2 —Hydrogen peroxide
i.p.—Intraperitoneal
KCN—Potassium cyanide
LB—Lewy bodies
LC—Locus ceruleus
LHON—Leber's hereditary optic neuropathy
LPS—Lipopolysaccharide

MAO— Monoamine oxidase
MDA—Malondialdehyde
MEL—Melatonin
mg—Milligram
MPTP—1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
MtDNA—Mitochondrial DNA
NAD—Nicotinamide adenine dinucleotide
NADH—Nicotinamide adenine dinucleotide
NAT—N-Acetyltransferase
NBD— Nitroblue diformazan
NBT— Nitroblue tetrazolium
NFT—Neurofibrillary tangles
NMDA—N-methyl-D-aspartate
NO—Nitric oxide
NOS—Nitric oxide synthase
 O_2^- —Superoxide radical
 OH^\cdot —Hydroxyl radical
 $ONOO^\cdot$ —Peroxynitrite
OXPHOS—Oxidative phosphorylation system
PBS—Phosphate buffered saline
PD—Parkinson's disease
Q—Ubiquinone
RE—Reference electrode
ROS—Reactive Oxygen Species
ROT—Rotenone
SCN—Suprachiasmatic nucleus
SEM—Standard error of mean
SN—Substantia nigra
SNpc—Substantia nigra pars compacta
SOD—Superoxide dismutase
TBA—Thiobarbituric acid
TBA-MDA—Thiobarbituric acid-Malondialdehyde
UV—Ultraviolet
WE—Working electrode
WHO— World Health Organisation

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