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"Potential Protective Effects of Phytoestrogen(s) Against Experimentally-Induced Neurotoxicity"

A Thesis Submitted for the Partial Fulfillment of Requirements for the Degree of Doctor of Philosophy in Pharmaceutical Science
(Pharmacology & Toxicology)

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LIST OF ABBREVIATIONS

µl	Microliters
µmol	Micromole
3-NPA	3-Nitropropionic acid
A	Absorbance
A%	Area percent
AAP	4-Aminoantipyrine
Ab	Antibody
ACh	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ATCh	Acetylthiocholine
ATP	Adenosine triphosphate
Bax	Bcl-2 associated x-protein
BBB	Blood brain barrier
BCA	Bicinchonic acid
BSA	Bovine serum albumin
CAG	Cytosine-Adenine-Guanine
ChAt	Choline acetyl transferase
CNS	Central nervous system
COX	Cyclooxygenase
D1	Dopamine receptor 1
D2	Dopamine receptor 2
dB	Decibel
DHBS	Dichloro-2-hydroxy benzene sulfonic acid
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DTNB	Dithionitrobenzoate
DTT	Dithiotheritol
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
ELISA	Enzyme linked immunosorbant assay

ERE	Estrogen responsive element
ERT	Estrogen replacement therapy
g	Gram
GABA	Gamma aminobutyric acid
GluR	Glutamate receptor
GPe	Globus pallidus externa
GPi	Globus pallidus interna
GSH	Reduced glutathione
H_2O_2	Hydrogen peroxide
HD	Huntington's disease
HRP	Horse radish peroxidase
Htt	Huntingtin protein
IgG	Immunoglobulin G
IL	Interleukin
iNOS	Inducible nitric oxide synthase
ip	Intraperitoneal
IR	Infra-red
kb	Kilo base
kDa	Kilo Dalton
kg	Kilogram
M	Molar
mA	Milliampere
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
mg	Milligram
min	Minute
Ml	Milliter
mol	Mole
mRNA	Messenger Ribonucleic acid
ms	Millisecond
MW	Molecular weight
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NF-κB	Nuclear factor- kappa B
nm	Nanometer
NMDA	N-methyl-D-aspartate
nmol	Nanomole

NO	Nitric oxide
NO^-	Nitric oxide radical
NSAIDs	Non-steroidal anti-inflammatory drugs
O_2^-	Superoxide radical
OD	Optic density
OH^-	Hydroxyl radical
$ONOO^-$	Peroxynitrite radical
PBS	Phosphate buffer saline
PD	Parkinson's disease
pg	Picogram
PGE_2	Prostaglandin E_2
PI3K	Phosphatidylinoistol 3-kinase
PVDF	Polyvinylidine fluoride
RNS	Reactive nitrogenous species
ROS	Reactive oxygen species
rpm	Round per minute
s	Second
s.c.	Subcutaneous
SDS	Sodium dodecyl sulfate
SERM	Selective estrogen receptor modulators
STN	Subthalamic nucleous
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive species
TBST	Tris buffered saline tween
TEMED	Tetramethylenediamine
Thal	Thalamus
TNF	Tumor necrosis factor
v	Volt
^oA	Angstrom
^oC	Celsius

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder characterized by motor, cognitive and psychiatric abnormalities. 3-Nitropropionic acid (3-NPA) is a mitochondrial toxin that produces selective lesions in the brain similar to that of HD. Phytoestrogens have well-established neuroprotective and memory enhancing effects with better side effect profile in comparison to estrogens. This study investigated the potential neuroprotective and memory enhancing effects of genistein (5, 10 and 20 mg/kg), a phytoestrogen, in ovariectomized rats challenged with 3-NPA (20 mg/kg). These potential effects were compared to those of 17β-estradiol. Results showed that systemic administration of 3-NPA for 4 days decreased animals body temperature and weight, significantly decreased % PPI, impaired locomotor activity and decreased retention latencies in the passive avoidance task. It also increased striatal, cortical and hippocampal oxidative stress through increasing lipid peroxidation and decreasing catalase activity and reduced glutathione levels. Moreover, 3-NPA significantly increased acetylcholinesterase activity (AChE), cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions also decreased ATP levels and increased prostaglandin E_2 (PGE_2) production and Bax/Bcl-2 ratio. Pretreatment with genistein and 17β-estradiol maintained animals' temperature and weight, restored % PPI, attenuated locomotor hypoactivity, increased retention latencies in the passive avoidance task. They also improved the oxidative stress profile, increased ATP levels and attenuated 3-NPA-induced increase in AChE activity and COX-2 and iNOS expressions. Genistein also decreased PGE_2 production and Bax/Bcl-2 ratio in the striatum. Overall, the highest genistein dose (20 mg/kg) was the most effective. In conclusion, this study suggests a neuroprotective and memory enhancing effects for genistein in a rat model of HD. These effects might be attributed-at least partly- to its antioxidant, anti-inflammatory, antiapoptotic and cholinesterase inhibitory activities.