

# **Potential Neuroprotective Effect of a Neurosteroid in an Experimental Animal Model of Huntington's Disease**

*A thesis submitted in the partial fulfillment of the requirements for the  
master degree in Pharmaceutical Sciences  
(Pharmacology and Toxicology)*

Presented by

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3. Clinical toxicology
4. Selected topics in pharmacology and toxicology

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## List of Abbreviations

°C	Celsius
μl	Microliter
μM	Micromolar
3-NP	3-Nitropropionic acid
5-LOX	5-Lipoxygenase
A	Absorbance
A%	Area percent
AAV2/1	Adeno-associated virus 2/1
ABC	Avidin-Biotin-Peroxidase Complex
ADIOL	5-Androstene-3β, 17β-diol
AIF	Apoptosis-Inducing Factor
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis Of Variance
AP-1	Activator Protein-1
ATP	Adenosine triphosphate
Bax	Bcl2-associated x protein
BDNF	Brain-Derived Neurotrophic Factor
bp	Base pair
BSA	Bovine Serum Albumin
CAG	Cytosine-Adenine-Guanine
ChAT	Choline Acetyl Transferase
cm	Centimeter
CNTF1	Ciliary Neurotrophic Factor 1
COX-2	Cyclooxygenase 2
CtBP	C-terminal Binding Protein
DA	Dopamine
dB	Decibel
DHBS	3, 5-Dichloro-2-hydroxybenzenesulfonic acid
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
DTNB	5,5' Dithiobis (2-nitrobenzoic acid)
EGP	External segment of Globus Pallidus

ELISA	Enzyme-Linked Immunosorbent Assay
ETC	Electron Transport Chain
FDA	Food and Drug Administration
g	Gram
GABA	Gamma-Amino Butyric Acid
GDNF	Glial-cell-line Derived Neurotrophic Factor
GFAP	Glial Fibrillary Acidic Protein
GLT1	Glutamate Transporter 1
GP	Globus Pallidus
GSH	Reduced Glutathione
h	Hour
H & E	Hematoxylin and Eosin
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
HD	Huntington's Disease
HDAC	Histone Deacetylase
Hdh	Huntington's disease gene homolog
HRP	Horseradish Peroxidase
HSD	Hydroxysteroid Dehydrogenase enzyme
Hsp	Heat shock protein
Htt	Huntingtin
i.p.	Intraperitoneal
i.v.	Intravenous
IGP	Internal segment of Globus Pallidus
IKK	Inhibitor of Kappa B Kinase
IL-6	Interleukin-6
iNOS	Inducible Nitric Oxide Synthase
InP <sub>3</sub>	Inositol 1,4,5-triphosphate
IR	Infra Red
IT-15	Interesting Transcript 15
kb	Kilobases
KDa	Kilo Dalton
kg	Kilogram
L	Liter
LDH	Lactate Dehydrogenase

M	Molar
MDA	Malondialdehyde
mg	Milligram
min	Minute
ml	Milliter
mM	Millimolar
MPTP	Mitochondrial Permeability Transition Pore
mRNA	Messenger Ribonucleic Acid
ms	Milliseconds
MSNs	Medium-sized Spiny Neurons
mTOR	Mammalian Target Of Rapamycin
muHtt	Mutant Huntingtin
N	Normal
NADH	Nicotinamide Adenine Dinucleotide, reduced form
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
NF- $\kappa$ B	Nuclear Factor kappa B
ng	Nanogram
NIs	Neuronal Intranuclear Inclusions
nM	Nanomolar
nm	Nanometer
NMDA	N-Methyl-D-Aspartate
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
Nrf2	Nuclear factor E2-related factor 2
NRTN	Neurturin
O <sub>2</sub> <sup>-</sup>	Superoxide anion
OD	Optical Density
ONOO <sup>-</sup>	Peroxynitrite anion
OPG	Osteoprotegerin
PCOS	Polycystic Ovary Syndrome
pg	Picogram
PGC-1 $\alpha$	Peroxisome Proliferator-Activated Receptor Gamma, Coactivator 1 Alpha

PPAR- $\alpha$	Peroxisome Proliferator-Activated Receptor- Alpha
PPAR- $\gamma$	Peroxisome Proliferator-Activated Receptor-Gamma
PPI	Prepulse Inhibition
QA	Quinolinic Acid
R.T	Room Temperature
RANKL	Receptor Activator of Nuclear Factor Kappa B Ligand
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
Rpm	Round per minute
s.c.	Subcutaneous
SDH	Succinate Dehydrogenase enzyme
SEM	Standard Error of the Mean
siRNA	Small Interfering Ribonucleic Acid
Smac/DIABLO	Second mitochondria-derived activator of caspases/Direct Inhibitor of Apoptosis-Binding Protein with a Low isoelectric point
SN	Substantia Nigra
SSRIs	Selective Serotonin Reuptake Inhibitors
STN	Subthalamic Nucleus
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TBS	Tris Buffered Saline
TCA	Trichloroacetic Acid
TGase	Transglutaminase
TMB	3, 3', 5, 5'-Tetramethylbenzidine
TNF- $\alpha$	Tumor Necrosis Factor alpha
U	Unit
UV	Ultra Violet
YAC	Yeast Artificial Chromosome

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## **Abstract**

Huntington's disease (HD) is a progressive neurodegenerative disorder with a wide spectrum of cognitive, behavioral and motor abnormalities. The mitochondrial toxin 3-nitropropionic acid (3-NP) effectively induces specific behavioral changes and selective striatal lesions similar to that observed in HD. Some neurosteroids, synthesized in neurons and glial cells, previously showed neuroprotective abilities. 5-androstene-3 $\beta$ -17 $\beta$ -diol (ADIOL) is a major metabolite of dehydroepiandrosterone (DHEA) with previously reported anti-inflammatory, anti-apoptotic and neuroprotective activities. The neuroprotective potential of ADIOL in HD was not previously investigated. Therefore, the present study investigated the neuroprotective effects of ADIOL against 3-NP-induced behavioral changes, oxidative stress, inflammation and apoptosis. Intraperitoneal administration of 3-NP (20 mg/kg) for 4 consecutive days in rats caused significant loss in body weight, reduced prepulse inhibition (PPI) of acoustic startle response, locomotor hypoactivity with altered brain histological structure, increased brain oxidative stress, inflammation and apoptosis. Administration of ADIOL (25 mg/kg, s.c.) for two days before 3-NP significantly attenuated the reduction in body weights and PPI, increased locomotor activity and restored brain histological structure nearly to normal. Moreover, it displayed anti-oxidant, anti-inflammatory and anti-apoptotic activities as evidenced by the elevation of cortical and striatal reduced glutathione levels, reductions of cortical and striatal malondialdehyde, striatal tumor necrosis factor alpha and interleukin-

6 levels. Only a small number of iNOS and caspase-3 positive cells were detected in sections from rats pretreated with ADIOL. This study suggests a potential neuroprotective role of ADIOL against 3-NP-induced Huntington's disease-like manifestations. Such neuroprotection can be attributed to its anti-oxidant, anti-inflammatory and anti-apoptotic activities.

**Key words:**

3-nitropropionic acid; ADIOL; Prepulse inhibition; Neuroinflammation; Apoptosis.