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Faculty of Science
Biochemistry Department

Biological Evaluation of Some Medicinal Plant Extracts Against Neuroinflammation Characterizing Alzheimer's Disease In Experimental Rat Model

Thesis Submitted by

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Abstract

The current study was designed to explore the potent role of four medicinal plants namely *Salvia triloba, Piper nigrum, Ruta graveolens* and *Pegenum harmala* in management of neuroinflammatory insults characterizing Alzheimer's disease (AD) in experimental rat model. This aim was achieved by performing acute and chronic toxiciological study for the selected medicinal plant extracts. The preclinical toxicological study for the selected medicinal plant extracts (Part I) was conducted from one hundred and sixty eight adult *Sprague Dawley* rats (eighty four male and eighty four female). On the other hand, the pharmacological study (Part II) was conducted from one hundred and ten adult male *Sprague Dawley* rats were classified into seven main groups: (1), control group; (2), AD-induced group in which the rats were orally administered with aluminum chloride ($AlCl_3$) (17 mg/kg b. wt) daily for one month (3), AD-induced group treated orally with Rivastigmine, the conventional therapy for AD (0.3 mg/kg b. wt) daily for three months; (4), AD-induced group which was further divided into two subgroups, the first subgroup was treated orally with *S. triloba* methanolic extract (750 mg/kg b. wt) and the second subgroup was treated orally with *S. triloba* (375 mg/kg b. wt) daily for three months; (5), AD-induced group which was further divided into two subgroups the first subgroup was treated orally with *P. nigrum* methanolic extract (187.5 mg/kg b. wt) and the second subgroup was treated orally with *P. nigrum* (93.75 mg/kg b. wt) daily for three months; (6), AD-induced group which was further divided into two subgroups, the first subgroup was treated orally with *R. graveolens* methanolic extract **(**750 mg/kg b. wt.) and the second subgroup was treated orally with *R.*

graveolens (375 mg/kg b. wt.) daily for three months and (7), AD-induced group which was divided into two subgroups the first subgroup was treated orally with *P. harmala* methanolic extract (375 mg/kg b. wt.) and the second subgroup was treated orally with *P. harmala* (187.5 mg/kg b. wt.) for daily for three months. Brain acetylcholine (ACh), brain and seum acetycholinesterase (AChE) activities, C-reactive protein (CRP), total nuclear factor Kappa B_{65} (NF-kB_{65}), monocyte chemoattractant protein-1 (MCP-1), cyclooxygenase-2 (COX-2), leukotriene B_4 (LTB_4) and B-cell lymphoma 2 (Bcl-2) levels were estimated. Histological investigation of brain sections of all studied groups were also carried out. The present results revealed that administration of $AlCl_3$ resulted in significant elevation in brain and serum AChE, CRP, NF kappa B, MCP-1, COX-2 and LTB_4 levels accompanied with significant depletion in brain Ach as well as brain and serum Bcl2 levels. Histological investigation of the brain of rats administered $AlCl_3$ showed the appearance of β-amyloid (Aβ) plaques characterizing AD. However, treatment of rats with the selected extracts produced marked improvement in the measured biochemical parameters as well as in the histological feature of the brain. The present study suggested that the studied medicinal plant extracts have a different degree of potentiality in alleviating AD. This promising effect was achieved through their powerful anti-cholinesterase activity, anti-inflammatory property and anti-apoptotic capacity. The current study represented good therapeutic approach for intervention against progressive neurological damage associated with AD.

Keywords: Alzheimer's disease, *Salvia triloba, Piper nigrum, Ruta graveolens, Pegenum harmala,* Inflammation, Apoptosis, Rat.

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List of Abbreviations

4-HAD	4-hydroxyalkenals
5-HPETE	5-hydroperoxy eicosatetraenoic acid
5-LOX	5-lipoxygenase
9-me-BC	9-methyl-b-carboline
AA	Arachidonic acid
ACE	Angiotensin converting enzyme
Acetyl-CoA	Acetyl coenzyme A
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChIs	Acetylcholinesterase inhibitors
AD	Alzheimer's disease.
AGEs	Advanced glycation endproducts
AICD	APP intracellular domain
AIF	Apoptosis inducing factor
$AlCl_3$	Aluminium chloride
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
ANOVA	One way analysis of variance
ApoE	Apolipoprotein E gene
ApoE ε4	ε 4 allele apolipoprotein E genotype
APP	Amyloid precursor protein
AST	Aspartate aminotransferase
Aβ	Amyloid β
BACE1	β-site APP-cleaving enzyme
BACE1	Beta-site amyloid precursor protein cleaving enzyme
Bad	B cell lymphoma 2 associated death promoter

Bak	B cell lymphoma 2 homologous antagonist killer
Bax	B cell lymphoma 2 associated x protein
BBB	Blood brain barrier
BChE	Butyrlcholinesterase
Bcl-2	B-cell lymphoma-2
Bcl-xl	B cell lymphoma 2 extra large
BCs	β-carbolines
BDNF	Brain derived neurotrophic factor
BIF	Brain interstitial fluid
C/EBP b	cytidine-cytidine-adenosine-adenosine-thymidine Enhancer Binding Protein Beta
CAA	Congophilic amyloid angiopathy
CAT	Catalase
CCR2	CCL2 (MCP-1) receptors
CDC	Center for Disease Control
Cdk5	Cyclin dependant kinase 5
ChAT	Cholineacetyltransferase
ChAT	Choline acetyl transferase
CMH	Cyanomethemoglobin
CNS	Central Nervous System
COX-2	Cyclooxygenase-2
cPLA2	Cytosolic phospholipase A2
CREB	cAMP response element-binding
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computerized tomography
CT	Cryptotanshinone
α -CTF	α C- terminal fragment

CTS	Cryptotanshinone
CYP4Fs	Cytochrome P450 4Fs
DLA	3,4-Dihydroxyphenyl lactic acid
DNPH	2, 4- Dinitrophenyl hydrazine
DT	15, 16-Dihydrotanshinone I
ECE1	Endothelin converting enzyme 1
EEG	Electroencephalogram
EOAD	Early onset Alzheimer's disease
EOFAD	Early onset famlial Alzheimer's disease
EPA	Environmental Protection Agency
FAβ	Fibrillar Amyloid β
FLAP	5-Lipoxygenase activating-protein
G-6-PD	Glucose-6-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
GR	Glutathione reductase
GSK3β	Glycogen synthase kinase 3 beta
H_2O_2	Hydrogen peroxide
hcCRP	High sensitivity C-reactive protein
HRT	Hormone replacement therapy
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule-1
IDE	Insulin degrading enzyme
IFN-γ	Interferon- γ
IGF-1	Insulin-like growth factor-1
IkB	Inhibitory kappa B
IL-1β	Interleukin-1 β
iNOS	Inducible nitric oxide synthase
JNK	*c-Jun* N-terminal kinase

LDH	Lactate dehydrogenase
LOAD	Late onset Alzheimer's disease
LPS	Lipopolysaccharide
LRP1	Low-density lipoprotein receptor-related protein 1
LSD	Least significant difference
LT	Leukotrienes
LTB_4	Leukotrienes B_4
mAChR	Muscarinic acetylcholine receptors
MAO	Monoamine oxidase
MAP	Microtubule associated protein
MAPK	Mitogen activated protein kinases
MAPK	Mitogen-activated protein kinase
MCI	Mild cognitive impairment
MCI	Mild cognitive impairment
MCP-1	Monocyte chemotactic protein-1
MCP-1 (CCL2)	Monocyte chemoattractant protein-1
MDMs	Monocyte-derived microglia
MLB	Magnesium lithospermate B
MMP9	Matrix metalloprotease 9
MMSE	Mini-Mental Status Examination
MRI	Magnetic resonance imaging
nAChR	Nicotinic acetylcholine receptors
NEP	Neprilysin
NF-kB	Nuclear factor kappa B
NFTs	Neurofibrillary tangles
NGF	Nerve growth factor
NINCDS–ADRDA	National Institute of Neurologic and Communicative Disorders and Stroke– AD