Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(11): 879-892

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Medicinal plants offer multimechanistic approaches in management of Alzheimer's disease

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ABSTRACT

This study aimed to investigate anti-inflammatory effects of Ruta graveolens and Pegenum harmala extract in management of neuroinflammatory insults characteristic for Alzheimer's disease (AD) in adult male rat. Rats were classified into (1), control group; (2), AD group, orally administered with AlCl3 (17 mg/kg b.wt.) daily for one month; (3), AD group, treated with rivastigmine (0.3 mg/kg b.wt.) daily for three months; (4), AD group divided into two subgroups each one treated with 750 mg/kg b.wt. of Ruta graveolens and 375 mg/kg b.wt. of Pegenum harmala daily for three months and (5), AD group divided into two subgroups each subgroup treated with 375 mg/kg b.wt. of Ruta graveolens and 187.5 mg/kg b.wt. of Pegenum harmala daily for three months. Brain acetylcholine (Ach) & serum and brain acetycholinesterase (AchE) activity, C-reactive protein (CRP), total NF Kappa B_{65} (NF-kappa B_{65}) and Cyclooxygenase 2 (COX 2) levels were estimated. The results showed that administration of AlCl3 revealed significant elevation in AchE, CRP, NF-κB, and COX 2 levels and significant depletion in Ach level. Treatment with the selected extracts caused marked improvement in the measured biochemical parameters. In conclusion, Ruta graveolens and Pegenum harmala have a potent anti-inflammatory effect against neuroinflammation characterizing AD.

Keywords: Alzheimer's disease, *Ruta graveolens*, *Pegenum harmala*, anti-inflammatory.

INTRODUCTION

Alzheimer's disease (AD) is currently a major public health problem and will presumably be the most important disorder of this century in developed/developing countries**,** and it is considered as the fourth most common cause of death in developed nations [1]. In 1906 at the 37th Assembly of the Society of Southwest German Psychiatrists in Tubingen, Alois Alzheimer, Director of the Cerebral Anatomical Laboratory of the Ludwig-Maximilians University Munich, presented for the first time his observations about amyloid plaques and neurofibrillary tangles the neuropathological hallmarks of what later was termed AD, as he found in the postmortem brain of his 55-year old patient Auguste D. Yet [2]. Clinically AD is characterized by progressive memory loss and other cognitive abilities include impairment of behavior, visual-spatial skills, speech and motor ability, depression, delusions, hallucinations, aggressive behaviour and, ultimately, increasing dependence, neuronal dysfunction and subsequent dementia upon others before death [3]. Other essential abnormalities resulted secondary to AD such as gliosis, chronic inflammation, excitotoxicity and oxidative stress [4].

Deficient in acetylcholine (ACh) "cholinergic hypothesis" was stated on cognitive, functional and behavioral

dysfunction associated with AD may be caused by an inability to transmit nerve cell impulses across cholinergic synapses. A deficit in central cholinergic transmission induced by degeneration of the basal forebrain nuclei is an important pathological and neurochemical feature of AD [5].

AD is pathologically characterized by genetic alterations, neuronal apoptosis-like processes leading to premature neuronal death and brain dysfunction. β-amyloid protein (Aβ) deposition in senile plaques and brain vessels, neurofibrillary tangles due to hyperphosphorylation of proteins and synaptic loss [3]. neuroimmune dysfunction, neuroinflammatory processes, accelerated neuronal death due to excitotoxic reactions and cerebrovascular dysfunction [6].

Aluminum exposure is proposed to be involved in the development of Alzheimer's disease [7]. It produced clinical and pathological features which were strikingly similar to those seen in Alzheimer's disease [8]. Aluminum was detected in both senile plaques and neurofibrillary tangle bearing neurons in the brains of patients with Alzheimer's disease [9]. Animal studies showed that aluminum exposure caused neuropathological and neurobehavioral changes resulting in impaired learning ability [10]. Al acting as a cholinotoxin and and a pro-oxidant**,** affects neuronal function by causing changes in neurotransmission and oxidative stress, such as, a reduction in cholinergic activity [11], enhanced activity of glutamate decarboxylase [12], increases the activity of the glutamine synthetase [13], changes in GABA transport [14], altered membrane associated proteins (Na–K ATPase and protein kinase C (PKC), is a regulator of transmembrane signal transduction) and endogenous antioxidant enzyme activity [15]. Al exposure induces expression of inflammatory genes [16]. Levels of the inflammatory cytokines, such as TNF-α and IL-1a were elevated in response to Al compounds in a dose-dependant manner and for TNF- α was associated with greater expression of the corresponding mRNA in mouse brains [17].

The inflammatory changes that we have found could result in cognitive deficits and promoting neurodegenerative disease [18]. Cerebral inflammation as well as systemic immunological alterations has been reported in the pathogenesis of AD [19]. As very large proportion of the genes whose expression are significantly increased with age, are related to immune function [20]. The neurons themselves seem to play a role in the inflammatory process of AD and have been implicated in the production of inflammatory products [21]. Inflammatory changes include activation of microglia and astrocytes, and infiltrating inflammatory cells in the cerebral inflammation with increased levels of proinflammatory cytokines [22]. Activation of glial cells is well linked to several neurodegenerative diseases involving AD [23]. Stress as well as acute or chronic brain injuries stimulate the generation of free radicals and glutamate, triggering inflammatory pathways that leads to increase chemokines and cytokines from glial cells [24]. The pro-inflammatory mediators such as peripheral blood mononuclear cell (PBMC), cytokines such as interleukin-1β (IL-1β) [25], interleukin-6 (IL-6) [26], tumor necrosis factor-α (TNF- α) [27] and interferon- γ (IFN-γ) as well as the pleiotropic monocyte chemotactic protein-1 (MCP-1) and RANTs (*r*egulated on *a*ctivation, *n*ormal *T*-cell *e*xpressed and *s*ecreted) may be produced by glia and certain central nervous system neurons and showed a biphasic release pattern over time in AD. Cytokines initially assist lymphocytic activation to stimulate immune cells to fight Aβ and to restore homeostasis perturbed by this toxic peptide; however, after chronic deposition of Aβ, (The early and focal glial activation, in conjunction with upregulated beta-site amyloid precursor protein cleaving enzyme (BACE1) mRNA, protein and activity in the presence of its substrate APP [28].

Rivastigmine hydrogen tartrate (S)-*N*-ethyl-3-[(1-dimethyl amino)ethyl]-*N*-methyl-phenylcarbamate hydrogen tartrate is an acetylcholinesterase inhibitor of the carbamate type approved for the treatment of Alzheimer's disease [29]. It is licensed for use in the UK [30] and US Food and Drug Administration for the symptomatic treatment of mild-to-moderately severe AD [31] and it was received FDA approval in 2000. Rivastigmine is absorbed rapidly and completely after oral administration; reaching peak plasma concentration in about 1 h. Inhibition of AchE in the cerebrospinal fluid is maximal at 2.4 h after drug intake in healthy volunteers [32]. Rivastigmine administration modulates the acetylcholine system [33]. The AChEI rivastigmine can significantly reduce agitated behavior in patients suffering from dementia [34].

Many herbal treatments have been tested and demonstrated beneficial effects in different AD related models as well as in clinical trials [35]. Additionally, support for the inflammatory hypothesis suggests that the nonsteroidal antiinflammatory drugs (NSAID) slow the progression of AD [36].

Ruta graveolens L. (commonly known as rue) is an herbaceous perennial, a member of Rutaceae family, up to one meter tall, with a characteristic grayish green color and a sharp unpleasant odor, originally native to the

Mediterranean region. It is known as medicinal plant since ancient times [37]. Rue extract has a long history of medicinal usage in homeopathy and traditional medicine worldwide [38]. Phytochemical screening of *Ruta* species has characterized the presence of more than 120 compounds of different classes of natural products such as acridone alkaloids, coumarins, essential volatile oils, terpenes, triterpenes, flavonoids, tannins, glycosides, sterols and furoquinolines [39]. This plant have different established effects like antimicrobial, cytotoxic [40], antibacterial [41], fungicide [42], herbicide [43], anti-inflammatory [44], hypotensive properties [45] and potent female antifertility [46]. *Ruta graveolens L.* traditionally used for the treatment of rheumatism, arthritis and other inflammatory conditions. It has been demonstrated that methanolic extract of *R. graveolens* L. has anti-inflammatory and anti-oxidant effects in rats [47]. In fact, flavonoids, glycosides and tannins are considered potent inhibitors of pro-inflammatory signaling molecules [48]. Rue contains various active compounds like flavonoids, coumarine derivatives, furoquinolines, volatile oils, undecanone and others [49]. The extract of *R. graveolens* contains essential oil with terpenes, coumarins and alkaloids a group of compounds reported to have acetylcholinesterase (AChE) inhibitory Activity [50].

Peganum (*Pegenum harmala*) is a small genus belonging to the family *Zygophylaceae* and mainly distributed in the Mediterranean region. *Pegenum harmala* is the only species found growing wild in the Middle East and northern Africa. The plant is rich in alkaloids (β- carbolines) and contains up to 4% total alkaloids [51]. The principle alkaloids present are harmaline, harmine, harmalol and peganine [52]. It also contains fixed oils. There are several reports which indicated the great variety of pharmacological and biological activities of *Peganum harmala* such as antibacterial, antifungal and monoamine oxidase (MAO) inhibition [51] through prevention of breakdown of neurotransmitters (serotonin, dopamine, norepinepherine), hormones (melatonin) [53], immunomodulatory effects [54] and hypothermic effect [52]. Moreover, it has been reported that the aqueous extract of *peganum harmala* possesses antinociceptive analgesic and anti-inflammatory properties [55]. In addition, Farzin and Mansouri, [56] demonstrated that the β- carbolines (harmane, norharmane and harmine) induce an antidepressant-like effect. Harmaline and harmane are able to lower voltage-gated calcium channel currents at concentrations that are likely to be sufficient for neuroprotective effects *in vivo*. This mechanism is likely to contribute to changes in excitability owing to β-carboline components [57]. Also, β-carbolines (BCs) can be regarded as potential anti-AD drugs as well as endogenous tryptamine- and serotonin-derived neurotoxins. A series of β -carbolines and β -carbolinium salts were synthesized and their inhibitory activity on acetylcholinesterase (AChE) and butyrlcholinesterase (BChE) were documented *in vitro*. All of the carbolinium salts showed moderate to high activity levels in the ChEs reaching those of physostigmine, galantamine, and rivastigmine, compounds which can penetrate the blood–brain barrier [58]. Moura et al., [59] demonstrate that systemic administration of β-carboline alkaloids can improve object recognition memory in mice.

Aim of the work

The current study was to investigate the anti-inflammatory effects of *Ruta graveolens* and *Pegenum harmala* total extract in management of neuroinflammatory insults characteristic for Alzheimer's disease in adult male experimental rat model.

EXPERIMENTAL SECTION

Materials:

A) *Chemical and drug*

- **Aluminium Chloride ($AlCl_3$)** was purchased from Sigma Co. USA. Its M.Wt was 133.34.
- **Rivastigmine,** Exelon, 1.5 mg was purchased from Novartis Co. Germany

Medicinal Plants

- R. graveolens and P.harmala were purchased from local specialized market (Seeds, and the spices and medicinal plants Co., Cairo, Egypt).
- R. graveolens and P. harmala taxonomical features of the plants were kindly confirmed by Prof. M.N. El-Hadidi, Prof. of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University. Voucher specimens were kept in the museum of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

- ***Plant extraction:***

Extraction of *Ruta graveolens* and *Pegenum harmala plant* was carried out according to Kuzovkina et al. [39] and Berrougui et al. [60], respectively. The dried aerial parts of *Ruta graveolens* and seeds of *Pegenum harmala* were

macerated in 500 ml of 70% methanol and left at room temperature for three days, and then filtered. The residue was repeatedly extracted with fresh methanol. The Combined filtrates were evaporated under reduced pressure at 45C in a rotatory evaporator (Heidolph, Germany).

C) Experimental Design:

The present study was conducted on one hundred and ten adult male *Sprague Dawley* rats weighing from 150 to 200 gm obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were maintained on standard laboratory diet and water *ad libitum*. After an acclimation period of one week, the animals were distributed into thirteen groups (8 rats/group) and housed in stainless steel cages in a temperature controlled (23 ± 1ºC) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received human care and use according to the guide lines for Animal Experiments which were approved by the Ethical Committee of Medical Research, National Research Centre, Egypt. The animals were inducted with Alzheimer's disease by using $AlCl_3$ orally in a dose of 17 mg/kg b. wt daily for one month [61]. The animals used in the current study were classified into 5 main groups:

Group (1): Normal healthy animals served as untreated negative control group.
Group (2): Animals inducted with AD served as untreated positive control group.
Group (3): Animals induced with AD and treated with the conventional therapy used for AD (Rivastigmine) in a dose of 0.3 mg/kg b.wt [62] as a reference drug for comparison daily for three months.
Group (4): AD-induced group divided into two subgroups the first subgroup was treated orally with *R. graveolens* extract in a dose of 750 mg/kg b. wt and the second subgroup was treated orally with *R. graveolens* extract in a dose of 375 mg/kg b. wt (after stopping $AlCl_3$ administration (1 month, induction of AD)) daily for three months.
Group (5): AD-induced group divided into two subgroups the first subgroup was treated orally with *P. harmala* extract in a dose of 375 mg/kg b. wt and the second subgroup was treated orally with *P. harmala* extract in a dose of 187.5 mg/kg b. wt (after stopping $AlCl_3$ administration (1 month, induction of AD)) daily for three months.

Samples collection:

All Animals were observed for 3 months. Observations were made daily for morbidity and mortality. At the end of the experiment, blood samples were collected after 18 hours fasting using the orbital sinus technique, under light anesthesia by diethyl ether, according to the method Van Herck et al. [63]. Each blood sample was left to clot in clean dry test tubes, and then centrifuged at 3000 rpm for ten minutes to obtain serum. The clear supernatant serum was then frozen at -20 ºC for the biochemical analysis.

At the end of the experimental period, the animals were kept fasting for 12 hours and the rats were killed by decapitation. The whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried and then weighed. One have of each brain was homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containg 50 mM Tris-Hcl (pH 7.4) and 300 mM sucrose [64]. The homogenate was centrifuged at 3000 rpm for 10 min at 4 ºC. The supernatant (10%) was separated for biochemical analysis. Also, brain total protein concentration was measured to express the concentration of different brain parameters per mg protein [65]. The second portion of each brain was fixed in formalin buffer (10%) for histological investigation.

Biochemical Analyses:

Quantitative estimation of total protein level in the brain homogenate was carried out according to the method of Lowry et al. [66] using kit purchased from Biodiagnostic Co., Egypt. Serum and brain acetycholinesterase (AchE) activity colorimetrically according to method of Den Blawen et al. [67] using kit purchased from Quimica Clinica Aplicada S.A Co., Amposta, Spain. Brain acetylcholine (Ach) level was determined using ELISA technique according to Oswald et al. [68] method using choline/acetylcholine assay kit purchased from Biovision Research Products Co., Linda Vista Avenue, USA. Brain high-Sensitivity C-reactive protein (CRP) level was carried out according to Roberts et al. [69] method using ELISA kit purchased from BioCheck, Inc Co., Foster City, USA. Brain total NF Kappa B_{65} (NF-kappa B_{65}) level was estimated using ELISA kit purchased from Invitrogen Co., Camarillo, USA according to method of Adams [70]. Serum and brain Cyclooxygenase 2 (COX 2) level was detected using ELISA technique according to the company method instruction using kit purchased from Immuno-Biological Laboratories Co. (IBL), Japan.

Statistical Analysis

In the present study, all results were expressed as Mean ± S.E of the mean. Data were analyzed by one way

analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups [71]. Difference was considered significant when P value was < 0.05.

$$\% \text{ difference} = \frac{\text{Food restricted group value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS AND DISCUSSION

The data in Table (**1**) illustrated the effect of treatment with Rivastigmine and the selected medicinal plants total extracts on cholinergic markers represented by brain and serum AChE activities and brain ACh level in AD-induced rats.

In comparison with the negative control group, $AlCl_3$ administration produced significant elevation ($P< 0.05$) in brain and serum AChE activities (34.3 % and 22.7 % respectively) associated with significant reduction ($P< 0.05$) in brain ACh level (- 31.5%).

The present findings revealed that, $AlCl_3$ administration induced significant elevation in brain and serum AChE activities accompanied with significant reduction in brain ACh levels (Table 1). These results are in agreement with those of Zhang et al. [72]. The study of Zubenko and Hanin [73] showed that Al enhanced the activity of AChE *in vivo* and *in vitro*. This could be attributed to allosteric interaction between Al and the peripheral anionic site of the enzyme molecule to modify the secondary structure and eventually its activity [74]. Several lines of evidence supported the idea that the accumulation of Al in the brain might contribute to the observed cholinergic deficiency [75] as Al could alter the cholinergic transmission *via* impairing the function of cholinergic neurons [76] which was ultimately reflected in neurobehavioral deficits [77]. Moreover, Al could reduce ACh levels in the brain due to the interaction of Al with cholinergic system, by altering cholinergic projection functioning and also by intensifying its inflammation, and this is representing the way by which Al contributes to pathological process in AD [78]. Furthermore, the cholinotoxic effects of Al are exerted perhaps by blocking the provision of Acetyl CoA-which is required for ACh synthesis as well as inhibiting ACh release [75]. Finally, Al has been reported to exert its cholinotoxic effects by impairing the activities of biosynthetic enzyme choline acetyl transferase (ChAT) and hydrolytic enzyme AChE [79]. Moreover, a loss of cholinergic neurons and reduced choline acetyltransferase activity in the cerebral cortex and hippocampus are consistent with the findings in AD [80].

The third suggested mechanism for Al-induced promotion of AChE activity in the brain depends on the neurotoxic effects of Al-induced promotion and accumulation of insoluble Aβ protein [81]. Aβ-induced elevation in AChE activity through induction of lipid peroxidation in neuronal membranes due to the production of H_2O_2 [82]. Hydrogen peroxide (H_2O_2) may have a direct action on AChE, as, it is well to bear in mind that there are several aspects of H_2O_2 action: as a factor of damage inducing oxidative stress and as modulator (may be allosteric) of the activity of functionally important proteins, receptors and enzymes [83]. Additionally, Aβ (1–42) induced enhancement of AChE activity is mediated through the direct inhibitory action of Aβ on nicotinic acetylcholine receptors (nAChR) [84]. Therefore, the accumulation of Al in the brain and the decline of nAChRs functions seemed to contribute to incidence of AD and other forms of dementia [85].

Treatment of AD-induced rats with Rivastigmine or with either one of the selected medicinal plants total extracts resulted in significant decrease ($P< 0.05$) in brain and serum AChE activities (-21.44 %, - 16.22 % for Rivastigmine; - 12.27 %, - 7.85 % for *R. graveolens* (750 mg/kg b.wt) ; - 19.0 %, - 13.72 % for *R. graveolens* (375 mg/kg b.wt); - 16.76 %, - 11.33 % for *P. harmala* (375 mg/kg b.wt); - 16.66 %, - 10.16 % for *P. harmala* (187.5 mg/kg b.wt)) as compared to untreated AD-induced group. The treatment with *R. graveolens* (750 mg/kg b.wt) as well as *P. harmala* (375 mg/kg b.wt) extract caused marked improvement in brain and serum AChE activities but not as did the Rivastigmine.

Treatment of AD-induced rats with Rivastigmine or either of the selected medicinal plants total extracts led to significant increase ($P< 0.05$) in brain ACh level (29.9 % for Rivastigmine; 24.7 % for *R. graveolens* (750 mg/kg b.wt), 21.62 % for *R. graveolens* (375 mg/kg b.wt), 21.62 % for *P. harmala* (375 mg/kg b.wt) and 13.51 % for *P. harmala* (187.5 mg/kg b.wt)) when compared to AD-induced group. In comparing AD-induced group treated with

Rivastigmine, treatment with *R. graveolens* (375 mg/kg b.wt) or *P. harmala* (375 or 187.5 mg/kg b.wt) extract caused a marked improvement in brain ACh levels but not as did the Rivastigmine.

Treatment of AD-induced rats with Rivastigmine, Table (1) produced a significant decrease in brain and serum AChE activities associated with a significant increase in brain ACh levels. These results are in agreement with those of Liang and Tang [86]. Rivastigmine is a novel acetylcholinesterase (AChE) inhibitor that displays specific activity for central AChE over peripheral AChE [87]. It is licensed in the UK for the treatment of AD and memory dysfunction [88]. The inhibition of brain AChE activity increases the amount of available acetylcholine in the brain and this is responsible for improvement in cognitive task with Rivastigmine [89].

Rivastigmine appears to inhibit cholinesterases (ChEs) in plaques and tangles with the same potency as those in neurons and axons by interacting with the esteratic site in ChE molecules [90]. It prevents the hydrolysis of ACh released from surviving nerve terminals and correlates best with increases in steady-state levels of ACh in the brain [91]. The use of Rivastigmine is expected to compensate cholinergic deficits indirectly by inhibiting the destruction of acetylcholine and directly by increasing the expression of cholineacetyltransferase [92]. In general, cholinesterase inhibitors increased the availability of ACh, hence they enhanced the cholinergic transmission in the brain and improved the symptoms of AD [93].

Treatment of AD-induced rats with *R. graveolens* resulted in significant inhibtion in brain and serum AChE activities, Table (1). Anti-acetylcholinesterase (AChE) activity of *R. graveolens* has been reported [94] which may be contributed to its constituent (terpenes, alkaloids, flavonoids, coumarins, furanocoumarins, triacylglycerines, and glycosides) that exhibite inhibitory influence on AChE activity [95]. It has been demonstrated that the treatments which inhibit acetylcholinesterase, retard the catabolism of acetylcholine, and therefore result in increased synaptic availability of Ach [96]. *R. graveolens* could retard the catabolism of brain acetylcholine, and hence inhibit the breakdown of acetylcholine leading to increased synaptic availability of Ach [97].

Treatment of AD-induced rats with *P. harmala* caused significant depletion in brain and serum AChE activities, Table (1). AChE inhibitor activity of *P. harmala* has been previously reported by Schott et al. [58]. The AChE-inhibiting property of this plant is probably contributed to its alkaloids [98], coumarins [99] and β-carbolines which have anti- acetylcholinesterase and anti-butyrylcholinesterase activity [58]. Recent report has been shown that the extract of *P. harmala* inhibits the breakdown of brain acetylcholine *via* decreasing the activity of AChE [97].

Table (1): Effect of treatment of AD-induced rats with the selected medicinal plants total methanolic extracts on brain and serum acetylcholnesterase (AChE) activities and brain acetylcholine (ACh) levels

Groups (n=10)	AChE activity		Brain ACh (nmol/mg protein)
	Brain (U/mg protein)	Serum (U/L)	
Control group	571.1 ± 21.2	737.6 ± 28.9	$8.1 \times 10^{-2} \pm 0.11 \times 10^{-2}$
AD-induced group	767.0 ± 11.7^{a} (34.3 %)	906.6 ± 8.36^{a} (22.7 %)	$5.55 \times 10^{-2} \pm 0.11 \times 10^{-2a}$ (- 31.5 %)
AD + Rivastigmine group	602.5 ± 21.0^{b} (- 21.44 %)	758.2 ± 26.4^{b} (- 16.22 %)	$7.21 \times 10^{-2} \pm 0.10 \times 10^{-2\,b}$ (29.91 %)
AD + *R. graveolens* (750 mg/kg b.wt)	672.9 ± 14.3^{bc} (- 12.27 %)	835.4 ± 22.0^{bc} (- 7.85 %)	$6.92 \times 10^{-2} \pm 0.10 \times 10^{-2\,b}$ (24.7 %)
AD + *R. graveolens* (375 mg/kg b.wt)	621.4 ± 10.5^{b} (- 19.0 %)	782.2 ± 13.3^{b} (- 13.72 %)	$6.75 \times 10^{-2} \pm 0.14 \times 10^{-2bc}$ (21.62 %)
AD + *P. harmala* (375 mg/kg b.wt)	638.4 ± 14.6^{b} (- 16.76 %)	803.9 ± 18.4^{bc} (- 11.33 %)	$6.75 \times 10^{-2} \pm 0.14 \times 10^{-2bc}$ (21.62 %)
AD + *P. harmala* (187.5 mg/kg b.wt)	639.2 ± 15.0^{b} (- 16.66 %)	814.5 ± 16.1^{b} (- 10.16 %)	$6.3 \times 10^{-2} \pm 0.082 \times 0^{-2\,bc}$ (13.51 %)

Data were expressed as means ± standard error (SE) for 10 animals / group.
a: *P< 0.05 vs negative control.*
b: *P< 0.05 vs AD group.*
c: *P< 0.05 vs AD+Rivastigmine group.*
(%): percent of difference with respect to the corresponding control value.

The results of Table (2) illustrate effects of treatment of adult male rats with Rivastigmine and/or the selected medicinal plants total extract on brain and serum C Reactive Protein (CRP), total nuclear factor Kappa B_{65} (total NF Kappa B_{65}) levels and serum cyclooxygenase-2 (Cox-2) activity.

Our findings revealed that $AlCl_3$ administration produced significant elevation ($P< 0.05$) in brain and serum CRP (158.13 % and 71.01 %, respectively), total NF-κB_{65} 124.2 % and 58.05 %, respectively) and COX-2 activities (114.11 and 126.72 % respectively), levels when compared with the negative control group.

Data in the present study showed that Al administration induced significant elevation in brain and serum CRP levels, Table (2). These findings are in agreement with that of Ravaglia et al. [100]. C-reactive protein is a well-known serum protein which increases during inflammation and deposits in damaged tissues [101]. Moreover, increased serum concentration of high-sensitivity C-reactive protein (hsCRP) has been associated with poor memory [102], poor global cognitive performance [103], vascular dementia [100] and AD [104].

CRP can be locally produced in the brain and its expression is upregulated in AD affected brain areas [105]. This suggestion is in consistant with a prominent hypothesis forwarded to explain the pathogenesis of AD is the inflammatory hypothesis [106]. That suggested the inflammation observed might be induced by the pathologic features of AD, including senile plaques, neurofibrillary tangles, or components of degenerated neurons [107]. These pathologic changes are believed to stimulate glial cells to produce proinflammatory cytokines and inflammation reactive proteins such as CRP, these might then act *via* paracrine and/or autocrine pathways to stimulate glial cells to further produce additional Aβ (1-42), P-Tau, and proinflammatory molecules. Thus, a positively reinforcing cycle was established in which inflammatory mediators play a dual role by both stimulating glial cells and activating molecular pathways, leading to neurodegeneration [106].

The present study demonstrated that, aluminum administration induced significant elevation in brain and serum NF-κB_{65} levels, Table (2). This result was in consistent with that of Becaria et al. [17]. High NF-κB activity has been observed in AD brain [108]. The NF-κB pathway appears to be involved in the pathogenic mechanisms of AD [109]. Aluminium could increase the inflammatory processes in the brains of mice [110] as it could upregulate genes encode pro-inflammatory signaling elements, including NF-κB subunits and IL-1β precursor [16]. Also Al could promote the production of reactive oxygen species (ROS) in the brain and several studies have linked increased intraneuronal generation of ROS and NF-κB activiation [111].

The Aβ1–42 peptides also activate astrocytes resulting in activation of NF-κB and production of induced nitric oxide synthase (iNOS) [112]. It was suggested that NF-κB and p38 kinase signaling pathways are involved in Aβ- induced responses in microglial and astroglial cells [113].

The results of the current study showed that, Al administration induced significant elevation in brain and serum COX-2 activity as compared to untreated negative control group, Table (2). These results in agreement with Hoozemans et al., [114] who showed elevation of neuronal COX- 2 protein level as well as COX activity in several areas of the AD brain and may correlate with levels of Aβ and plaque density [115]. Moreover, it has been reported that Al overload induced significant increase of COX-2 mRNA expression and protein level not only in cortical neurons but also in hippocampal neurons [116]. Several studies reported increased neuronal COX-2 immunoreactivity compared to control brain tissues [117]. COX-2 mRNA appears to be elevated in the frontal cortex in AD. A well controlled post mortem study indicated a higher variability of COX-2 mRNA in the brains of AD patients compared to age matched controls [118]. The transcription of COX-2 mRNA is induced by synaptic activity [119]. Accordingly, the increased synaptic activity associated with seizures markedly increases COX-2 expression [120]. Increased levels of COX-2 mRNA and protein staining in AD tissue [121]. COX-2 mRNA rises rapidly in response to inflammatory stimuli such as IL-1β suggesting that COX-2 is the isoform that mediates inflammation [122]. Immunocytochemical evidence shows that the increased levels of COX-2 content in the subsets of pyramidal layer neurons of the hippocampal formation correlates with neuronal atrophy [123] consistent with the previous evidence showing that, in the AD brain (and Down's syndrome), COX-2 protein content is preferentially elevated in neurons with neurofibrillary tangles (NFT) and in damaged axons [124].

The studies of COX in ischemia noted above also suggest that intraneuronal COX-2 levels may contribute to neuronal death by production of free radicals [125]. Moreover, free radicals have been reported to cause cell death through activation of JNK [126] and aberrant activation of JNK signaling pathway in neurons and glial cells has been reported to be neurotoxic and stimulate the production of pro-inflammatory cytokines, induction of iNOS, and COX-2 in microglial cells and even further activation of these cells [127].

It has been reported that expression of the COX-2 and cytosolic phospholipase A2 (cPLA2) are strongly activated

during AD, indicating the induction of proinflammatory gene pathways as a response to brain injury. Neurotoxic metals such as Al and zinc, both implicated in AD etiopathogenesis, and arachidonic acid, a major metabolite of brain cPLA2 activity, each polymerize hyperphosphorylated tau to form NFT-like bundles [128]. COX-2 is highly expressed in pyramidal neurons of AD cases [129]. Endothelial COX-2 induction is rapid and is linked to fever development, BBB changes and possibly regulation of blood flow [130]. It has also been reported that the extent of COX-2 expression correlates with the amount of Aβ and the degree of progression of AD pathogenesis [121]**.**

The expression levels of COX-1 and COX-2 change in the different stages of AD pathology. In an early stage, when low-fibrillar Aβ deposits are present and only very few neurofibrillary tangles are observed in the cortical areas, COX-2 is increased in neurons. The increased neuronal COX-2 expression parallels and colocalizes with the expression of cell cycle proteins. COX-1 is primarily expressed in microglia, which are associated with fibrillar Abeta deposits. This suggests that in AD brain COX-1 and COX-2 are involved in inflammatory and regenerating pathways respectively [114]. In AD, the expression of COX-2, the inducible isoform, increases in response to inflammatory agents in neurons and glial cells [131]. The apparent early up-regulation of COX-2 in hippocampal neurons of the AD brain [121]**.**

The free radical hypothesis of aging states that tissue damage from reactive oxygen species may underlie multisystem failure [132], and these mechanisms may also occur in the progression of AD. Free radical-mediated lipid peroxidation has been shown to activate cyclooxygenase (COX)-2 [133]. Furthermore, the two step oxygenase and peroxidase action of COX leading to the formation of a reactive oxygen species and prostaglandin H2 (PGH2) [125]. However, aggregated synthetic Aβ1-40 peptides have been shown to induce COX-2 expression in neuroblastoma cells, and Aβ1-40 has been shown to stimulate COX-2 oxygenase and peroxidase activity in a cell free system [134].

The activation of NF-kB has previously been shown in neurons surrounding amyloid plaques in AD [135]. Jung et al. [136] reported that activation of NF-κB increased the expression of COX-2. Furthermore, a correlation between the presence of the transcription factor NF-kB in the cell nucleus and the level of COX-2 mRNA was found in brain tissues of AD patients and age matched controls, suggesting that NF-kB is involved in the induction of COX-2 in the human brain [137]. Interestingly, NF-kB is involved in the induction of COX-2 [138]**.**

Treatment of AD-induced rats with Rivastigmine or *P. nigrum* produced significant decrease (P< 0.05) in brain and serum CRP levels (-34.17 %, -31.9 % for Rivastigmine; -35.90, -31.38 for *R. graveolens* (750 mg/kg b.wt), -32.01, -29.70 for *R. graveolens* (375 mg/kg b.wt), -25.37, -20.28 for *P. harmala* (375 mg/kg b.wt) and -18.05, -17.44 for *P. harmala* (187.5 mg/kg b.wt)). In comparing with AD-induced group treated with Rivastigmine, the treatment with *P. harmala* (187.5 mg/kg b.wt) caused marked improvement in brain and serum CRP levels. The treatment with *P. harmala* (375 mg/kg b.wt) caused observable changes in serum CRP levels compared with AD-induced group treated with Rivastigmine but not as did the Rivastigmine.

The present data revealed that the treatment with Rivastigmine or the tested medicinal plants total extracts caused a significant decrease (P< 0.05) in brain and serum NF-κB_{65} level (-42.14 %, -22.70 % for Rivastigmine; -39.22, -22.08 for *R. graveolens* (750 mg/kg b.wt), -37.95, -17.85 for *R. graveolens* (375 mg/kg b.wt), -33.74, -21.28 for *P. harmala* (375 mg/kg b.wt) and -32.63, -18.63 for *P. harmala* (187.5 mg/kg b.wt)) when compared with the untreated AD-induced group. While, the treatment of AD-induced group with *P. harmala* (187.5 mg/kg b.wt) extract caused marked improvement in brain NF-κB_{65} level as compared to AD-induced group treated with Rivastigmine but, they could not reduce brain NF-κB_{65} level as did the Rivastigmine.

Treatment of AD-induced group with Rivastigmine or most of the selected medicinal plants total extract produced significant decrease (P< 0.05) in brain and serum COX-2 activities (-46.14, -36.79 % for Rivastigmine; -46.06, -26.40 % for *R. graveolens* (750 mg/kg b. wt.); -44.17, -22.69 % for *R. graveolens* (375 mg/kg b. wt.); -45.64, -34.47 % for *P. harmala* (375 mg/kg b. wt.) and -35.79, -30.83 % for *P. harmala* (187.5 mg/kg b. wt.)) as compared to AD-induced group. Meanwhile, in comparison with AD-induced group treated with Rivastigmine, the treatment with *R. graveolens* (375 mg/kg b.wt) caused marked change in brain and serum COX-2 activities.

Treatment of AD-induced rats with Rivastigmine significantly decreased brain and serum CRP levels, Table (2) and this could be explained by the anti-inflammatory activity of Rivastigmine. Rivastigmine can ameliorate neurological dysfunction and memory deficits in animals *via* its ability to downregulate the inflammatory activation of immune

cells through the increased level of ACh acting on nicotinic α7 receptors [139]. The anti-inflammatory effects of Rivastigmine rely on the cholinergic immune system [140]. It has been reported that peripheral administration of acetylcholinesterase inhibitor, Rivastigmine, in mice significantly attenuates the production of IL-1β in the hippocampus and blood, concomitantly with the reduction in acetylcholinesterase activity. It has been demonstrated that IL-1β and IL-6 strongly induce the expression of CRP in the brain tissue [141]. These findings demonstrated that cholinergic enhancement produces central and peripheral anti-inflammatory effects [142] with consequent reduction in CRP production.

Treatment of AD-induced rats with *R. graveolens* produced significant decrease in brain and serum CRP level as compared to Al-intoxicated positive control group, Table (2). Ratheesh et al. [143] have been revealed that of CRP level was found to be decreased significantly in methanolic extract of *ruta* administrated rats, may due to Polyphenolic, Alkaloid and coumarin constituents of *R. graveolens* which are a potent antiinflammatory components [144].

Treatment of AD-induced rats with *P. harmala* produced significant decrease in brain and serum CRP levels as compared to Al-intoxicated positive control group, Table (2). As it has been suggested that CRP may be closely linked to TNF-a production [145]. Thus *P. harmala* inhibit CRP production, due to harmine which attenuates inflammatory gene expression (TNFα, IL-1β, iNOS) and macrophage accumulation in adipose tissue [146]. Importantly, 9-methyl-b-carboline (9-me-BC) also reduced the expression of inflammatory modulators such as chemokine (C-X-C motif) ligand 9 (Cxcl9), tumor necrosis factor (TNF), Fas ligand and interferon regulatory factor 1 [147]**.**

Rivastigmine treatment in AD-induced rats caused a significant decrease in brain and serum NF-κB_{65} levels, Table (2). Rivastigmine can ameliorate neurological dysfunction and memory deficits in animals, *via* its ability to down-regulate the inflammatory activation of immune cells through an increased level of ACh acting on nicotinic α7 receptors [139]. This property of AChEIs depends on the activation of the α7 nicotinic acetylcholine receptors (nAChR) on T-cells. Accordingly, the α7 nAChR was identified as an anti-inflammatory target in macrophages [148] as the activation of these receptors reduced pro-inflammatory cytokine production and NF-κB -dependent transcription [149]. These observations were supported by the evidence showing a role for acetylcholine in suppression of cytokine release through a 'cholinergic anti-inflammatory pathway. Additionally, it has been reported that AChEIs directly inhibit the release of cytokines from microglia and monocytes [150].

Treatment of AD-induced rats with *R. graveolens* produced significant decrease in brain and serum total NF Kappa B_{65} levels as compared to Al-intoxicated positive control group, Table (2). Coumarins isolated from *R. graveolens* such as 5, 7-dihydroxy-4- methylcoumarin and 7, 8-Dihydroxy-4-methylcoumarin were known to inhibit the activation of NF-κB [151]. These compounds have been reported to inhibit the pro-inflammatory mediators like NO and IL-1β through suppression of NF-κB activation. Moreover, the crude extract of *R. graveolens* L. plant, represses activation of p65/NF-κB by LPS in macrophage cells by inhibiting the activation of IkBα and effectively suppress nuclear translocation of NF-κB [152].

Treatment of AD-induced rats with *P. harmala* produced significant decrease in brain and serum total NF Kappa B_{65} levels as compared to Al-intoxicated positive control group, Table (2). This may due to Antiinflammatory activity *P. harmala* [55]**.** Harmine significantly inhibited the translocation/activation of NF- κB subunits such as p65, p50, and c-Rel. It also has been found that harmine inhibited the nuclear translocation of AP-1 factors as c-fos, ATF-2, and CREB [153].

Treatment of AD-induced rats with Rivastigmine produced significant decrease in brain and serum COX-2 activity as compared to AD-induced group, Table (2). This could be attributed to its anti-inflammatory properties [140].

Treatment of AD-induced rats with *R. graveolens* produced significant decrease in brain and serum COX-2 activity as compared to AD-induced rats, Table (2). It has been demonstrated that, supplementation with methanolic extract of R. graveolens (MER) decreases the activity of COX as well as inhibitory effect on COX-2 gene expression in monocyte, which suggested that MER, protect against the inflammation [144]. Moreover, Ratheesh et al. [143] showed that treatment with MER showed significant decrease in COX and LOX (5-LOX is the key enzyme involved in the synthesis of leukotrienes from arachidonic acid) activity on rats.

Shen et al. [154] quercetin is the hydrolyzed product of rutin (flavonoid constituents of rue) showed a significant decrease in the prostaglandin level associated with a decrease in COX-2 protein expression *in vitro* in LPS-treated murine macrophages, by different mechanism, Inhibition of both *inos* and *COX-2* genes expression by the present extract points towards the possible involvement of nuclear factor kappa B (NF-kappa B), a common activator of *inos* and *COX-2* promoters. NF-kappa B activation appears to involve a redox-sensitive step [155] and thus, the phenolic and flavonoid compounds of this plant may have inhibitory effect at this level also by their natural antioxidant property. Thus, *R. graveolens* L. unravels a novel molecular mechanism of combating the pro-inflammatory challenge by the endotoxin in murine macrophage cells and demands further research to establish its anti-inflammatory therapeutic potential [44].

Treatment of AD-induced rats with *P. harmala* produced significant decrease in brain and serum COX-2 activity as compared to AD-induced rats, Table (2). Harmine treatment significantly down-regulated the expression of VEGF, iNOS and COX-2 transcript levels by B16F-10 melanoma cells. Harmine treatment down-regulated the level of expression of VEGF, iNOS and COX-2 mRNA expression [156].

Table (2): Effects of treatment with the selected medicinal plants total extract on brain and serum CRP and Total NF-Kappa β_{65} levels on AD-induced rats

Parameter / Group	CRP		Total NF-Kappa B_{65}		Cox-2	
	Brain mg/mg protein	Serum mg/L	Brain pg/mg protein	Serum pg/ml	Brain ng/mg protein	Serum ng/ml
- ve control	3.06 ± 0.21	$0.34\pm0.7\times10^{-2}$	8.3 ± 0.22	1766.5 ± 16.1	7.82±0.42	8.16±0.16
+ ve control	7.9 ± 0.60^{a} (158.13 %)	$0.58\pm2.4\times10^{-2a}$ (71.01 %)	18.6 ± 0.83^{a} (124.2 %)	2333.2 ± 58.1^{a} (58.05 %)	16.74 ± 0.79^{a} (114.11 %)	18.51 ± 0.63^{a} (126.72 %)
$AlCl_3$ + Rivastigmine	5.2 ± 0.31^{b} (-34.17 %)	$0.39\pm1.42\times10^{-2b}$ (-31.9 %)	10.77 ± 0.30^{b} (-42.14 %)	1803.5 ± 6.98^{b} (-22.70 %)	9.02 ± 0.63^{b} (-46.14 %)	11.70 ± 1.18^{b} (-36.79 %)
$AlCl_3$ + *R. graveolens* (750 mg/kg b.wt)	5.06 ± 0.25^{b} (-35.90 %)	$0.40\pm 2.8\times10^{-2b}$ (-31.38 %)	11.31 ± 0.19^{b} (-39.22 %)	1817.9 ± 114.3^{b} (-22.08 %)	9.03 ± 1.04^{b} (-46.06 %)	13.62 ± 1.11^{b} (-26.40 %)
$AlCl_3$ + *R. graveolens* (375 mg/kg b.wt)	5.37 ± 0.26^{b} (-32.01 %)	$0.41\pm1.75\times10^{-2b}$ (-29.70 %)	11.55 ± 0.63^{b} (-37.95 %)	1916.5 ± 213.2^{b} (-17.85 %)	9.34 ± 0.69^{b} (-44.17 %)	14.31 ± 0.42^{bc} (-22.69 %)
$AlCl_3$ + *P. harmala* (375 mg/kg b.wt)	5.89 ± 0.17^{b} (-25.37 %)	$0.46\pm3.0\times10^{-2bc}$ (-20.28 %)	12.33 ± 0.54^{b} (-33.74 %)	1836.6 ± 77.4^{b} (-21.28 %)	9.10 ± 0.78^{b} (-45.64 %)	12.13 ± 1.16^{b} (-34.47 %)
$AlCl_3$ + *P. harmala* (187.5 mg/kg b.wt)	6.47 ± 0.22^{bc} (-18.05 %)	$0.48\pm0.95\times10^{-2bc}$ (-17.44 %)	12.54 ± 0.83^{bc} (-32.63 %)	1898.4 ± 53.0^{b} (-18.63 %)	10.75 ± 0.34^{b} (-35.79 %)	12.80 ± 0.27^{b} (-30.83 %)

Data were expressed as means ± standard error (SE) for 10 animals / group.
***a:** P< 0.05 vs negative control.*
***b:** P< 0.05 vs AD group.*
***c:** P< 0.05 vs AD+Rivastigmine group.*
(%): percent of difference with respect to the corresponding control value.

CONCLUSION

The current study revealed that treatment of AD-induced rats with *R. graveolens* or *P. harmala* methanolic extracts, significantly ameliorates the cholinergic dysfunction, inflammation and apoptosis induced neurodegeneration characteristic of AD. These effects could be attributed to powerful antiinflammatory activity, the anticholinesterase effects, antioxidant capacity. Noteworthy, *R. graveolens* extracts revealed more pronounced modulatory effect on most of the measured biochemical parameters as well as histopathological feature of the brain. These results represented good therapeutic approaches for intervension against progressive neurological damage associated with AD with special reference to the inflammatory insults.

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