Research Article

AMELIORATION OF NEUROINFLAMMATION AND APOPTOSIS CHARACTERIZING ALZHEIMER'S DISEASE BY NATURAL PRODUCTS

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ABSTRACT

Objective: The current study was planned to investigate anti-inflammatory and anti-apoptotic effects of *Ruta graveolens* and *Pegenum harmala* total extract against neurodegeneration characterizing Alzheimer's disease (AD) in rat model.

Methods: Adult male *Sprague Dawley* rats were classified into five groups. Group (1) control group; group (2) AD group, was administered $AlCl_3$ daily for one month; group (3) AD group treated orally with rivastigmine daily for three months; group (4) AD group divided into two subgroups, the first was treated orally with *R. graveolens* (750 mg/kg b.wt.) and the second was treated with *R. graveolens* (375 mg/kg b.wt.) daily for three months and group (5), AD group divided into two subgroups the first was treated orally with *P. harmala* (375 mg/kg b.wt.) and the second was treated with *P. harmala* (187.5 mg/kg b.wt.) daily for three months. Brain and serum acetycholinesterase (AchE) activity, monocyte chemotactic protein-1 (MCP-1), leukotriene B_4 (LTB_4) and B-cell lymphoma 2 (Bcl-2) levels were detected. Brain histological investigation of all studied groups was carried out.

Results: It showed that $AlCl_3$ administration resulted in significant elevation in brain and serum AchE, MCP-1 and LTB_4 levels accompanied with significant depletion in brain and serum Bcl-2 level. Brain histological investigation of rats administered $AlCl_3$ showed appearance of Aβ plaques characterizing AD. Treatment of rats with the selected extracts caused marked improvement in the measured biochemical parameters as well as in the histological feature of the brain.

Conclusion: *R. graveolens* and *P. harmala* possess anti-inflammatory and anti-apoptotic effect against neuroinflammation characterizing AD.

Keywords: Alzheimer's disease; *Ruta graveolens*; *Pegenum harmala*; Neuroinflammation; Apoptosis; Rat.

INTRODUCTION

The most well-known type of neurodegenerative dementia in elderly is Alzheimer's disease (AD) which approximately 60% of patients with dementia [1]. AD is a disease that affects memory, thought, reasoning and language as well as serious cognitive disability and death of nerve cells. Memory is an organism's mental ability to store, retain and recall information. The hippocampus plays an important role in learning and memory [2]. Incidence of AD increases with age, doubling every 5-10 years [3]. Pathologically, AD is charaterized by senile pleques due to abnormal accumulation of extracellular amyloid β (Aβ) and the intracellular neurofibrillary tangles (NFTs) which are responsible for the neuronal loss, degeneration of cholinergic system [4], oxidative damage, synaptic dysfunction and inflammation [5].

The major risk factors for AD are advancing age, cardiovascular diseases, diabetes; obesity, cancer, low educational levels, head trauma and exposure to heavy metals such as aluminium (Al), cupper, iron and zinc [6]. Al has neurotoxic effect by many mechanisms; promoting formation and accumulation of senile plaques and neurofibirally tangles leading to progressive neuronal degeneration and death [7]. It has been demonstrated that Aβ (1-40) binds up to 4 Al atoms and that binding increased the β- sheet content of the peptide [8].

Cerebral inflammation as well as systemic immunological alterations has been reported in the pathogenesis of AD [9]. There is compelling evidence that in AD, the Aβ deposition is associated with a local inflammatory response, which is initiated by the activation of microglia and the recruitment of astrocytes. These cells secrete a number of cytokines and neurotoxic products that may contribute to neuronal degeneration and cell death [10]. Defective immune system may eventually be ineffective in defending cells from Aβ, leading to neurodegeneration characterizing AD. This might give an explanation why cellular changes precede by decades the clinical onset of AD [11].

Apoptosis has been also associated with the pathophysiology of AD. Aβ has been known to induce apoptosis both [12]. Stimuli for apoptosis in AD include increased oxidative stress, dysregulation of ion homeostasis, growth factor deprivation, accumulation of Aβ, metabolic impairment, reduced clearance of toxin, mitochondrial dysfunction, DNA damage and protein aggregation [13].

Medicinal plants are considered very important in primary health care system. Medicinal plants are widely used as alternative therapeutic tool for the prevention or treatment of many diseases in different parts of the world [14]. *Ruta graveolens* L. (rue) is a native of the Mediterranean region but cultivated throughout Europe and many Asian countries and it belongs to the family Rutaceae [15]. It contains more than 120 compounds of different classes of natural products such as acridone alkaloids, coumarins, flavonoids and furoquinolines [16]. This plant has different established effects like anti-inflammatory [15], antioxidant [17]. Traditionally, *R. graveolens* L. is used for the treatment of rheumatism, arthritis and other inflammatory conditions [17]. Moreover, *R. graveolens* contains essential oil rich with active compounds reported to have acetylcholinesterase (AChE) inhibitory activity [18].

Pegenum harmala (Peganum) is a small genus belonging to the family Zygophylaceae and mainly distributed in the Mediterranean region [19]. A variety of pharmacological and biological activities of *P. harmala* such as antibacterial, antifungal and monoamine oxidase (MAO) inhibition has been reported [20]. This plant is rich in alkaloids; β- carbolines including harmalol, harmaline, norharmane, harmol, harmine and harmane [19]. A series of β -carbolines and β - carbolinium salts characterized by their *in vitro* acetylcholinesterase (AChE) and butyrlcholinesterase (BChE) inhibitory activity [21].

The current study was aimed at investigating the efficiency of *R. graveolens* and *P. harmala* total extract in management of neuroinflammatory insults and neuronal apoptosis characterising Alzheimer's disease in experimental animal model.

MATERIALS AND METHODS

Chemical and drug

- **Aluminium Chloride (AlCl₃)** 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.

Plants extraction

Extraction of R. graveolens and P. harmala plant was carried out according to Kuzovkina et al. [19] and Berrougui et al. [22]**,** respectively. The dried aerial parts of R. graveolens and seeds of P. harmala were macerated in 500 ml of 70% methanol, left at room temperature for three days, and then filtered. The residue was repeatedly extracted with fresh methanol (70 %). The combined filtrates were evaporated under reduced pressure at 45 ˚C in a rotatory evaporator (Heidolph, Germany) till dryness.

Animals

The present study was conducted on seventy adult male Sprague Dawley rats weighing from 150 to 200 g, 4 months old 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*, housed in polypropylene 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.

Experimental set-up

After an acclimation period of one week, the animals were classified into 5 main groups: (1): Normal healthy animals served as negative control group. (2): AD-induced group, in which, the animals were received $AlCl_3$ orally in a dose of 17 mg/kg b. wt daily for one month [23], and rats were scarified after 3 months, at the end of the experiment. (3): AD-induced group treated orally with the conventional therapy for AD (rivastigmine) in a dose of 0.3 mg/kg b.wt (after stopping $AlCl_3$ administration (1 month, induction of AD)) [24] daily for three months as a reference drug for comparison. (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. (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.

At the end of the experiment, blood samples were collected after 12 hours fasting using the orbital sinus technique, under light anaesthesia by diethyl ether, according to the method Van Herck et al. [25]. Each blood sample was left to clot in clean dry test tubes, and then centrifuged at 1800 xg for 10 min. at 4 ˚C to obtain serum. The clear serum samples were frozen at -20 ºC for biochemical analysis.

After blood collection, the rats were killed by decapitation and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried, weighed and then divided mid-sagitally into two halves. One half of each brain was homogenized immediately to give 10% (w/v) homogenate in ice-cold medium

containing 50 mM Tris-Hcl (pH 7.4) and 300 mM sucrose [26]. The homogenate was centrifuged at 1800 xg for 10 min at 4 ºC and the supernatant (10%) was separated for the different biochemical analysis. The second half of each brain was fixed in formalin buffer (10%) for histological investigation.

Biochemical assays

Quantitative estimation of total protein content in the brain was carried out according to the method of Lowry et al. [27] to express the concentration of different brain parameter per mg protein [28]. Brain and serum acetycholinesterase activity was determined colorimetrically according to the method of Den Blawen et al. [29] using kit purchased from Quimica Clinica Aplicada S.A Co., Amposta, Spain**.** Brain and serum monocyte chemoattractant protein-1 (MCP-1) level was detected according to Ikawa et al. [30] method using ELISA kit purchased from Invitrogen Co., Camarillo, USA. Leukotriene B_4 (LTB_4) level in brain and serum was estimated by ELISA technique according to Maclof et al. [31] method using kit purchased from Cayman Chemical Co., USA. Brain and serum B-cell lymphoma 2 **(**Bcl2) level was detected using ELISA technique according to the method described by Barbareschi et al. [32] using kit purchased from Bender MedSystems GmbH,Vienna, Austria**.**

Histopathological examination

After twenty four hours of brain tissue fixation, washing was done in tap water, then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 ˚C in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μ thick by slidge microtome. The obtained tissue sections were collected on glass slides, deparffinized and stained by hematoxylin and eosin (H&E) stain [33] for histopathological examination through the light microscope Nikon, Japan, with objectives 5x.

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 [34]. Difference was considered significant when P value was $<$ 0.05. Percentage difference representing the percent of variation with respect to corresponding control group was also calculated using the following formula.

$$\% \text{ difference} = \frac{\text{treated value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS

The results in Table (1) showed the effect of treatment with rivastigmine and the selected medicinal plants extract on cholinergic markers represented by brain and serum AchE activity 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 activity (34.3 % and 22.7 % respectively). However, treatment of AD-induced group with rivastigmine or with either one of the selected medicinal plants extract resulted in significant inhibition (P< 0.05) in brain and serum AchE activity (-21.44 %, - 16.36 % 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 with the untreated AD-induced group. Also, the results in Table (1) showed that the treatment with *R. graveolens* (750 mg/kg b.wt) extract caused significant increase (P< 0.05) in brain and serum AchE activity as well as the treatment with *P. harmala* (375 mg/kg b.wt) extract caused significant increase (P< 0.05) in serum AchE activity in comparison with AD-induced group treated with rivastigmine.

Table 1: Effect of treatment with rivastigmine and the selected medicinal plant total methanolic extracts on brain and serum acetylcholnesterase (AchE) activity in AD-induced rats

	AchE	
	Brain U/mg protein	Serum U/L
Negative control group	571.1 ± 21.2	737.6 ± 28.9
AD-induced group	767 ± 11.7[a] (34.3 %)	906.6 ± 8.36[a] (22.7 %)
AD + Rivastigmine group	602.5 ± 21.0[b] (- 21.44 %)	758.2 ± 26.4[b] (- 16.36 %)
AD + *R. graveolens* (750 mg/kg b.wt)	672.9 ± 14.3[bc] (- 12.27 %)	835.4 ± 22.0[bc] (- 7.85 %)
AD + *R. graveolens* (375 mg/kg b.wt)	621.4 ± 10.5[b] (- 19.0 %)	782.2 ± 13.3[b] (- 13.72 %)
AD + *P. harmala* (375 mg/kg b.wt)	638.4 ± 14.6[b] (- 16.76 %)	803.9 ± 18.4[bc] (- 11.33 %)
AD + *P. harmala* (187.5 mg/kg b.wt)	639.2 ± 15.0[b] (- 16.66 %)	814.5 ± 16.1[b] (- 10.16 %)

Data are 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 data in Table (2) illustrated the influenece of treatment with rivastigmine and the selected medicinal plants extract on brain and serum MCP-1 and LTB$_4$ levels in AD-induced rats. Our findings in the current study revealed that AlCl$_3$ administration produced significant elevation (P< 0.05) in brain and serum MCP-1 (141.06 % and 45.35 % respectively) and LTB$_4$ (187.84 % and 44.76 % respectively) levels when compared with the negative control group. While, the treatment of AD-induced group with rivastigmine or the selected medicinal plant extracts showed significant depletion (P< 0.05) in brain and serum MCP-1 levels (-53.37 %, -27.5 % for rivastigmine; -31.72 %, -16.05 % for *R. graveolens* (750 mg/kg b. wt); -44.45 %, -25.83 % for *R. graveolens* (375 mg/kg b. wt); -42.65 %, -22.16 % for *P. harmala* (375 mg/kg b. wt) and -41.65 %, -16.81 % for *P. harmala* (187.5 mg/kg b. wt)) as compared to the untreated AD-induced group. Meanwhile, in comparison with AD-induced group treated with rivastigmine, the treatment with *R. graveolens* (750 mg/kg b.wt) or *P. harmala* (187.5 mg/kg b.wt) extract caused significant increase (P< 0.05) in brain and serum MCP-1 level.

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 LTB$_4$ levels (-46.95 %, -19.04 % for rivastigmine; -36.02 %, -16.93 % for *R. graveolens* (375 mg/kg b. wt); -34.29 %, -11.83 % for *P. harmala* (375 mg/kg b. wt) and -33.80 %, -8.47 % for *P. harmala* (187.5 mg/kg b. wt)) as compared to the untreated AD-induced group. Treatment of AD-induced group treated with *R. graveolens* extract (750 mg/kg b. wt) produced significant decrease (P< 0.05) only in brain LTB$_4$ level (-23.63 %) as compared to the untreated AD-induced group. Meanwhile, in comparison with AD-induced group treated with rivastigmine, the treatment with *R. graveolens* (750 mg/kg b.wt) extract caused significant increase (P< 0.05) in brain and serum LTB$_4$ level as well as the treatment with *P. harmala* (187.5 mg/kg b.wt) extract caused significant increase (P< 0.05) only in serum LTB$_4$ level.

Table 2: Effect of treatment with rivastigmine and the selected medicinal plant total methanolic extracts on brain and serum MCP-1 and LTB$_4$ levels in AD-induced rats.

	MCP-1		LTB$_4$	
	Brain pg/mg protein	Serum pg/ml	Brain pg/mg protein	Serum pg/ml
negative control group	1.8 ± 0.10	67.53±1.47	0.22 ± 0.011	24.01± 0.58
AD-induced group	4.3 ± 0.36[a] (141.06%)	98.15± 6.70[a] (45.35 %)	0.64 ± 0.046[a] (187.84%)	34.76±0.64[a] (44.76 %)
AD + Rivastigmine group	2.01±0.14[b] (-53.37 %)	71.17±2.22[b] (-27.5 %)	0.34 ± 0.038[b] (-46.95 %)	28.14 ±0.96[b] (-19.04 %)
AD + *R. graveolens* (750 mg/kg b.wt)	2.94 ± 0.11[bc] (-31.72 %)	82.40 ± 2.03[bc] (-16.05 %)	0.49±0.033[bc] (-23.63 %)	32.57 ±0.73[c] (-6.30 %)
AD + *R. graveolens* (375 mg/kg b.wt)	2.39 ± 0.15[b] (-44.45 %)	72.80± 2.43[b] (-25.83 %)	0.41 ± 0.048[b] (-36.02 %)	28.87± 0.77[b] (-16.93 %)
AD + *P. harmala* (375 mg/kg b.wt)	2.47 ± 0.063[b] (-42.65 %)	76.4 ± 2.43[b] (-22.16 %)	0.42 ± 0.058[b] (-34.29 %)	30.65± 0.89[b] (-11.83 %)
AD + *P. harmala* (187.5 mg/kg b.wt)	2.51 ±0.19[bc] (-41.65 %)	81.65 ± 1.19[bc] (-16.81%)	0.423±0.061[b] (-33.80 %)	31.82±1.92[bc] (-8.47 %)

Data are 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 in Table (3) demonstrated the effect of treatment of AD-induced rats with rivastigmine and the selected medicinal plants extract on brain and serum Bcl-2 levels. The current results revealed that $AlCl_3$ administration produced significant reduction (P< 0.05) in brain and serum Bcl-2 levels (-43.25 % and -42.98 % respectively) when compared with the negative control group. However, the treatment of AD-induced group with rivastigmine or most of the selected medicinal plants extract exhibited significant elevation (P< 0.05) in brain and serum Bcl-2 levels (63.59 %, 58.10 % for rivastigmine; 62.91 %, 51.52 % for *R. graveolens* (375 mg/kg b. wt); 48.68 %, 26.17 % for *P. harmala* (375 mg/kg b.wt))

as compared to the untreated AD-induced group. Treatment of AD-induced rats with *R. graveolens* (750 mg/kg b. wt.) or *P. harmala* (187.5 mg/kg b.wt) produced significant elevation (P< 0.05) only in brain Bcl-2 level (33.85 %, 47.64 % respectively) as compared to the untreated AD-induced group. In comparison with AD-induced group treated with rivastigmine, the treatment of AD-induced rats with *R. graveolens* (750 mg/kg b.wt) extract caused significant decrease (P< 0.05) in brain and serum Bcl-2 levels as well as the treatment with *P. harmala* (375 or 187.5 mg/kg b.wt) extract caused significant decrease (P< 0.05)but only in serum Bcl-2 level.

Table 3: Effect of treatment with rivastigmine and the selected medicinal plants total methanolic extract on brain and serum Bcl-2 levels in AD-induced rats.

| | Bcl-2 | |
	Brain ng/mg protein	Serum ng/ml
negative control group	5.50 ± 0.38	3.46 ± 0.10
AD-induced group	3.12 ± 0.22[a]	1.97±0.027[a]
	(-43.25 %)	(-42.98 %)
AD + Rivastigmine treated group	5.10 ± 0.39[b]	3.12 ± 0.11[b]
	(63.59 %)	(58.10 %)
AD + *R. graveolens* (750 mg/kg b.wt)	4.18 ± 0.11[bc]	2.12 ± 0.09[c]
	(33.85 %)	(7.54 %)
AD + *R. graveolens* (375 mg/kg b.wt)	5.08 ± 0.21[b]	2.99 ± 0.15[b]
	(62.91 %)	(51.52 %)
AD + *P. harmala* (375 mg/kg b.wt) treated rats	4.64 ± 0.27[b]	2.49 ± 0.114[bc]
	(48.68 %)	(26.17 %)
AD + *P. harmala* (187.5 mg/kg b.wt)	4.61 ± 0.30[b]	2.14 ± 0.053[c]
	(47.64 %)	(8.45 %)

Data are 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.

Histological investigation

Microscopic examination of brain sections of negative control group (Figs. 1, 2, 3 and 4) showed no histopsthological alteration and normal histological structure of the meninges, hippocampus, medulla oblongata, cerebral cortex and cerebellum.
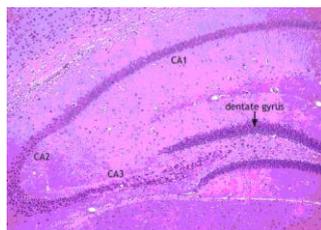
Micrographs of brain section of AD-induced group showed sever congestion in the blood vessels with oedema in the meninges (Fig. 5). The cerebrum showed neuronal degeneration with oedema and gliosis (Figs. 6 and 7), associated with focal gliosis in the cerebrum (Fig.8). Also, the histological examination of brain section of AD-induced rat showed encephalomalacia and plaques formation in the hippocampus (Figs. 9 and 10),

Micrograph of brain section of AD-induced rats treated with rivastigmine showing no histopathological alteration in the
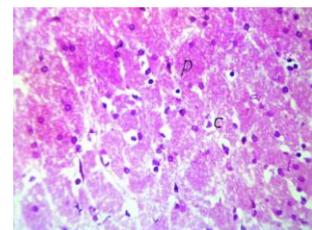
hippocampus (Fig. 11).

Micrograph of brain section of AD-induced rats treated with *R. graveolens* (750 mg/kg b. wt.) showed congestion with perivascular oedema in the hippocampus (Fig. 12), and focal gliosis in the cerebrum (Fig. 13). While, micrograph of brain section of AD-induced rats treated with *R. graveolens* (375 mg/kg b. wt.) showed no histopathological alteration in the hippocampus (Fig. 14).
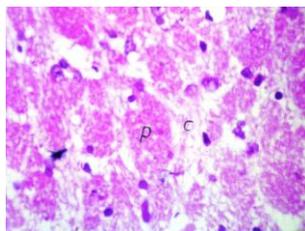
Micrograph of brain section of AD-induced rats treated with *P. harmala* (375 mg/kg b.wt.), showed perivascular oedema and vacuolization in the striatum of the cerebrum (Fig. 15) but normal histological structure of the hippocampus (Fig 16). Micrograph of brain section of AD-induced rats treated with *P. harmala* (187.5 mg/kg b.wt.) showed focal gliosis in the cerebrum (Fig. 17), while the hippocampus was intact (Fig. 18).
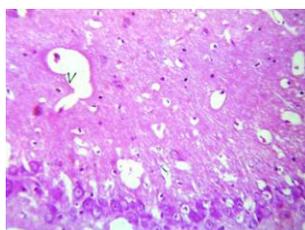

(a): Micrograph of brain section of negative control rat showing normal histological structure of the hippocampus "H&E 40"
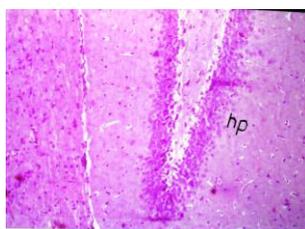

(b): Micrograph of brain section of AD-induced rat showing encephalomelacia (c) with plaques formation (p) in the hippocampus "H&E 64"
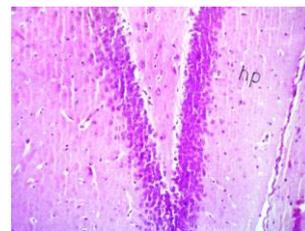
(c): Micrograph of brain section of AD-induced rat showing the magnification of Fig. 2 to identify the encephalomelacia (c) with plaques formation (p) in hippocampus "H&E 80"

(d): Micrograph of brain section of AD-induced rat treated with rivastigmine showing intact normal histological structure of hippocampus "H&E 64"
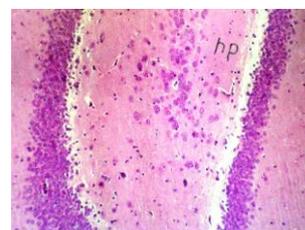

(e): Micrograph of brain section of AD-induced rats treated with *R. graveolens* (750 mg/kg b. wt.) showing congestion (v) with perivascular oedema in hippocampus "H&E 64"

(f): Micrograph of brain section of AD-induced rats treated with *R. graveolens* (375 mg/kg b. wt) showing intact histological structure of hippocampus (hp) "H&E 40 "
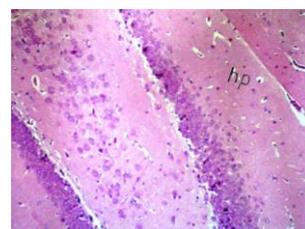

(g): Micrograph of brain section of AD-induced rats treated with *P. harmala* (375 mg/kg b. wt.) showing normal histological structure in hippocampus "H&E 40"

(h): Micrograph of brain section of AD-induced rats treated with *P. harmala* (187.5 mg/kg b. wt.) showing intact histological structure of hippocampus (hp) "H&E 40"

DISCUSSION

The present findings revealed that AlCl₃ administration induced significant elevation in brain and serum AchE activity. These results are in agreement with those of Kumar et al. [35]. The activity of AchE has been shown to be elevated after Al administration and this could be attributed to allosteric interaction between Al and the peripheral anionic site of enzyme molecule to modify the secondary structure and eventually its activity [36].

Treatment of AD-induced rats with rivastigmine produced significant decline in brain and serum AchE activity. These results are in agreement with those of Liang and Tang [37]. Rivastigmine is a novel AchE inhibitor that displays specific activity for central AchE over peripheral AchE [38] by interacting with the esteratic site in ChE molecules [39].

Treatment of AD-induced rats with *R. graveolens* resulted in significant inhibtion in brain and serum AchE activity. Antiacetylcholinesterase activity of *R. graveolens* has been reported [40] which may be contributed to its constituent (terpenes, alkaloids, flavonoids, coumarins, furanocoumarins, triacylglycerines, and glycosides) that exhibite inhibitory influence on AchE activity [41].

Treatment of AD-induced rats with *P. harmala* caused significant depletion in brain and serum AchE activity. Acetylcholinesterase inhibitor activity of *P. harmala* [21] is probably contributed to its alkaloids [42], coumarins [43] and β-carbolines which have anti-acetylcholinesterase and anti-butyrylcholinesterase activity [21]. Recent report has been shown that the extract of *P. harmala* inhibits the breakdown of brain acetylcholine *via* decreasing the activity of AchE [44].

The current study revealed that Al administration induced significant elevation in brain and serum MCP-1 levels. Al promots the accumualtion of insoluble Aβ (1-42) protein [45]. Aβ is able to induce MCP-1 expression in the astrocytes with consequent increase in its production [11].

Rivastigmine treatment in AD-induced rats produced significant depletion in brain and serum MCP-1 levels. Rivastigmine has been found to ameliorate neurological dysfunction and memory deficits in animals *via* its ability to down-regulate the inflammatory activation of immune cells [46].

Treatment of AD-induced rats with *R. graveolens* produced significant decrease in brain and serum MCP-1 levels. Anti-inflammatory activity of *R. graveonalens* [15] could be attributed to the coumarins and coumarinic derivatives which significantly inhibits IL-6 and TNF production induced by lipopolysaccharide that stimulates alveolar macrophages and reduces MCP-1 release [47]. It has been demonstrated that neutrophil elastase stimulated the release of MCP-1 from peritoneal macrophages [48] and neutrophil elastase inhibitor such as sivelestat could reduce the release of MCP-1 [49]. Bissonnette et al. [47] demonstrated the potency of coumarinic derivatives as anti-elastases *in vitro*. Thereby, these active constituents in *R. graveonalens* could deplete MCP-1 level both in brain and in serum of the treated rats as shown in the present study.

Treatment of AD-induced rats with *P. harmala* caused significant reduction in brain and serum MCP-1 levels. The antiinflammatory activity of *P. harmala* [50] could be attributed the alkaloid content [51] of *P. harmala* extract.

The results of the current study showed that Al administration

induced significant elevation in brain and serum LTB$_4$ levels. Aβ has been found to induce proinflammatory response in microglia which evidenced by increased LTB$_4$ release [52]. The changes in cytochrome P450 4Fs (CYP4F) levels, due to Al intoxication triggers inflammation, inversely correlate with the levels of LTB$_4$ in the brain following injury [53] as these have been shown to catalyze the omega-hydroxylation of endogenous eicosanoids such as LTB$_4$ [54].

Treatment of AD-induced rats with rivastigmine produced significant decrease in brain and serum LTB$_4$ level as compared to AD-induced group. This could be attributed to its anti-inflammatory properties [45].

Treatment of AD-induced rats with *R. graveolens* resulted in significant decrease in brain and serum LTB$_4$ levels. Ratheesh et al. [55] demonstrated that the treatment with methanolic extract of *R. graveonales* (MER) leads to significant decrease in lipooxygenase enzyme activity (the key enzyme involved in the synthesis of leukotrienes from arachidonic acid) in rats. Matern et al. [56] attributed this effect of *R. graveonales* to its content (coumarins) which possess anti-inflammatory properties.

Treatment of AD-induced rats with *P. harmala* revealed significant depletion in brain and serum LTB$_4$ levels. Alkaloid compounds found in the extract of *P. harmala* have been shown to have anti-inflammatory activity which seems to be mediated by inhibition of lipooxygenase and/or cyclo-oxygenase activity or by inhibiting the release of cytokines such as TNF-α, IL-1β and IL-8 resident peritoneal macrophages and mast cells [57].

The results of the current study showed that Al administration induced significant reduction in brain and serum anti-apoptotic marker (Bcl-2) levels. In accordance with our results, Jin et al. [58] demonstrated that Al could influence the activities of learning and memory and also reduce the expression of Bcl-2 level.

Al induces neuronal apoptosis through exerting stress on both the endoplasmic reticulum and mitochondria, with a response that leads to cross talk between the endoplasmic reticulum and mitochondria, leading to activation of apoptosis, down regulation of the anti-apoptotic protein Bcl-2 [59].

Treatment of AD-induced rats with rivastigmine produced significant elevation in brain and serum Bcl-2 levels. The expression level of Bcl-2 increased with ACHEIs treatment [60]. The blockade of voltage-activated K currents by rivastigmine may lead to the suppression of apoptosis and substantial increase in cell survival [61].

Treatment of AD-induced rats with *R. graveolens* and *P. harmala* produced significant elevation in brain and serum Bcl-2 levels. Coumarin and umbelliferone compounds in *R. graveonales* were found to have a mechanism of action similar to non steroidal anti-iflammatory drug (NSAID) in a carrageenan induced inflammation [62]. NSAIDs have anti-apoptotic effects as well as protective effects in many neurodegenerative diseases such as AD through the inhibition of apoptosis in chondrocytes [63].

P. harmala contains active constitutes that have the capacity of scavenging free radicals and to modulate the expression of genes encoding antioxidant enzymes such as estrogens, growth factors and vitamin E [64]. Nilsen and Brinton [65] demonstrated that estrogen increases antiapoptotic proteins, Bcl-2 and Bcl-xl, which prevent the activation of the permeability transition pore and thereby protect against the mitochondrial Ca^{2+}accumulation. Also, estrogen markedly upregulates Bcl-2 expression in primary cultured hippocampal neurons [66].

Photomicrograph of brain section of AD-induced rats showed the presence sever congestion in the blood vessels with oedema in the memninges. The cerebrum showed neuronal degeneration associated with focal gliosis. The hippocampus showed encephalomalacia and plaques formation. In accordance of our results Abd El-Rahman [67] demonstrated that Al administration causes the formation of neuratic plaques that appeared with dark center, neuronal damage and degeneration in the cerebral cortex and hippocampus.

Photomicrograph of brain section of AD-induced rats treated with rivastigmine showed no histopathological alteration in the hippocampus. These results are in agreement with the results of Bihaqia et al. [68] who showed normal histological appearance of the brain cells treated with rivastigmine tartrate and revealed that rivastigmine reversed histopathological alterations caused by Al.

Photomicrograph of brain section of AD-induced rats treated with *R. graveolens* (750 mg/kg b. wt.) showed some congestion with perivascular oedema, and focal gliosis in the cerebrum. Also, there was encephalomalacia with plaques formation in the hippocampus. While, photomicrograph of brain section of AD-induced rats treated with *R. graveolens* (375 mg/kg b. wt.) showed no histopathological alteration in the hippocampus. The neuroprotective effect of *R. graveonales* extract could be attributed to the flavonoids and coumarin contents of this plant. Flavonoids of *R. graveolens* could protect neuronal cells from oxidative stress [69] and coumarin derivatives could inhibit Aβ-induced cytotoxicity and reactive oxygen species production in brain cells [70].

Photomicrograph of brain section of AD-induced rats treated with *P. harmala* (375 mg/kg b. wt.) showed congestion in the blood vessels with pericellular, intercellular and perivascular oedema in the hippocampus, associated with perivascular oedema and vacuolization in the striatum but normal histological structure in hippocampus. While, photomicrograph of brain section of AD-induced rats treated with *P. harmala* (187.5 mg/kg b. wt.) showed focal gliosis in the cerebrum, while the hippocampus showed normal structure. Saponins the most active constitute in *P. harmal,* have a neuroprotective capacity to antagonize Aβ-induced cytotoxicity in the nerve cells [71].

CONCLUSION

In conclusion, the current study revealed that treatment of AD-induced rats with *R. graveolens* or *P. harmala* methanolic extracts, significantly ameliorates the cholinergic dysfunction and inflammation-induced neurodegeneration characterising Alzheimer's disease. These effects were achieved through the powerful anti-inflammatory activity and anti-apoptotic effects of these extract. Noteworthy, *R. graveolens* extracts revealed more pronounced modulatory effect on most of the measured biochemical parameters as well as histological feature of the brain than *P. harmala* extract. The selected medicinal plant extracts may represent good therapeutic approaches for intervension of the progressive neurological damage associated with Alzheimer's disease with special reference to the inflammatory and apoptotic insults.

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