New insights in the horizon for the treatment of Alzheimer’s Disease: A proposal based on experimental study

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ABSTRACT

The current study aimed to evaluate the anti-inflammatory and anti-apoptotic efficacy of Salvia triloba and Piper nigrum total extract in modulation of neuroinflammation insults characteristic for Alzheimer’s disease in experimental rat model. Male Sprague Dawley rats were classified into five groups: (1), control group; (2), AD-induced group which was orally administered with aluminium chloride (AlCl₃) in a dose of 17 mg/kg b. wt. daily for one month; (3), AD-induced group which was treated orally with rivastigmine in a dose of 0.3 mg/kg b. wt. daily for three months; (4), AD-induced group divided into two subgroups each subgroup was treated orally with one of the selected medicinal plants extract in a dose of 750 mg/kg b. wt. for Salvia triloba L. and 187.5 mg/kg b. wt. for Piper nigrum daily for three months and (5), AD-induced group divided into two subgroups each subgroup was treated orally with one of the selected medicinal plants extract in a dose of 375 mg/kg b. wt. for Salvia triloba L. and 93.75 mg/kg b. wt. for Piper nigrum daily for three months. Brain and serum acetycholinesterase activity (AchE), Cyclooxygenase 2 (COX 2), Leukotriene B4 (LTB4) and B-cell lymphoma 2 (Bcl 2) levels were detected. Histological investigations of brain sections of all studied groups were also carried out. The results showed that administration of AlCl₃ resulted in significant elevation in brain and serum AchE, COX 2 and LTB4 levels accompanied with significant depletion in brain and serum Bcl 2 level. Histological investigations of the brain of rats administered AlCl₃ showed the appearance of amyloid plaques characterizing AD. While, treatment of rats with the selected medicinal plant total extract caused marked improvement in the measured biochemical parameters as well as in the histological features of the brain. In conclusion, Salvia triloba and Piper nigrum have a potent anti-inflammatory as well as anti-apoptotic effects against neuroinflammation insults characterizing Alzheimer’s disease.

Keywords: Alzheimer, inflammation, Salvia triloba, Piper nigrum, Rat

INTRODUCTION

Dementia is an organic brain disease characterized by a progressive decline in cognitive function depending on neurodegeneration, which affects elder population in their daily activities such as memory, speaking, and problem dissolving. The most well-known type of neurodegenerative dementia in elderly is Alzheimer’s disease (AD), which proceeds at stages from mild and moderate to severe and gradually destroys the brain [1]. AD is characterized by a progressive memory decline as well as serious cognitive disability due to the progressive dysfunction and death of nerve cells that are responsible for the storage and processing of information [2]. Incidence of AD increases with
age, doubling every 5-10 years [3]. Several mechanisms have been postulated to explain AD pathogenesis, Aβ toxicity, cholinergic dysfunction, tau protein hyperphosphorylation, oxidative damage, synaptic dysfunction and inflammation secondary to senile plaques [4] and formation of neurofibrillary tangles (NFTs) [5].

Risk Factors of AD include genetic factor: ε4 allele of the apolipoprotein E gene (ApoE) on chromosome 19q13 [6]. Environmental factors that have been associated with an increased risk of AD in one or more studies include exposure to metals such as aluminium [7]. Al^{3+} could permeate the BBB and accumulate in the brain [8], involve in the development of AD [9] and induce expression of inflammatory cytokines genes [10] such as TNF-α and IL-1β [11]. Inflammatory changes include activation of microglia and astrocytes, and infiltrating inflammatory cells in the cerebral inflammation with increased levels of proinflammatory cytokines [12].

Apoptosis has also been associated with the pathophysiology of AD. Al is thought to induce cell death via activation of apoptotic mechanisms mediated through either mitochondrial or endoplasmic reticulum stress processes [13]. Al induces neuronal apoptosis in vivo as well as in vitro by its effect on the functioning of both the endoplasmic reticulum and mitochondria [14].

The acetylcholinesterase inhibitor (AChEI) rivastigmine can significantly reduce agitated behavior in patients suffering from dementia [15]. Rivastigmine administration modulates the acetylcholine system [16]. Furthermore, Many herbal treatments have been tested and demonstrated beneficial effects in different AD related models as well as in clinical trials [17].

The East Mediterranean Sage plant (S. triloba L.), is an aromatic herb belongs to the Lamiaceae family [18]. It contains essential oil content such as monoterpenoids, 1, 8-cineole, linalool, α- and α, β-pinene [19], limonene, myrcene, β-caryophyllene, spathulenol, β- caryophyllene oxide, viridiflorol, δ-3-carene and α-bisabolol [20]. These essential oil constituents have been showed to posse hypoglycemic activity, cholinergic activity [21], antitumor activity [22], antifungal activity against several human pathogens [23] as well as cytotoxic activities [24]. Also, hypoglycemic, analgesic, antiinflammatory and antiulcer effects were evaluated [25]. Anti-oxidative properties, antibacterial, anti-inflammatory, fungistatic, virustatic, astringent, eupeptic, hypolipidemic anti-hydrating effects was demonstrated [26]. Salvia species (sage) were reported to be used for memory-enhancing purposes in European folk medicine [27]. Moreover, it has been revealed that extracts of Salvia species for anticholinesterase activity [28] which can use as a treatment for dementia [29].

Black pepper (Piper nigrum) is a flowering vine belongs to Piperaceae family [30]. Piper revealed the presence of a variety of natural products e.g. monoterpenes and sesquiterpene, flavone, dihydroflavone, dihydrochalcone and Omethylflavonoids (P) and, cinnamoyl amides and alkyl amides [31]. Favorinoids [32] and triterpenes [33], in part, have been demonstrated to exert anti-inflammatory activity [34]. Piperine, the active constituent of pepper possesses bioavailability enhancing activity with various structurally and therapeutically diverse drugs [35]. The other potential activities of piperine include hepatoprotective [36], anti-metastatic [37], antithyroid [38], immunomodulatory and antitumor activity [39]. Piperine could be useful for the control of CNS-related conditions, including mood disorders and moderate or mild depression states [40]. Moreover, it has been demonstrated that Piper nigrum L extracts showed potent inhibitory activity on acetylcholinesterase (AChE) [41].

The current study was aimed to evaluate the anti-inflammatory and anti-apoptotic efficacy of Salvia triloba ans Piper nigrum total methanolic extract in modulation of neuroinflammation insults characteristic for AD in experimental rat model.

**MATERIALS AND METHODS**

**Chemical**
Aluminium Chloride (Alcl₃) was purchased from Sigma Co. (New Jersey, NJ, USA). Its molecular weight was 133.34. Rivastigmine (Exelon 1.5 mg) was purchased from Novartis Co. (Germany).

**Medicinal plants**
Salvia triloba L. and Piper nigrum were purchased from local specialized market, Cairo, Egypt (Seeds, and the spices and medicinal plants Co., Cairo, Egypt).
Salvia triloba L. and Piper nigrum taxonomical features were kindly confirmed by Prof. M.N. El-Hadidi, Professor 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, with number 012.10.10 for Salvia triloba and 012.10.09 for Piper nigrum.

Plant extraction
Alcoholic extract of Salvia triloba and Piper nigrum was carried out according to Orhan and Aslan [42] and Rasheed et al. [43] respectively. The dried aerial parts of Salvia triloba (250 g) and seeds of Piper nigrum (100 g) 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 45 °C in a rotatory evaporator (Heidolph, Germany) till dryness. The yield of dry extract was 0.5 g% for Salvia triloba and 15 g% for Piper nigrum.

Experimental design
The present study was conducted on seventy adult male Sprague Dawley rats weighing from 150 to 200 g obtained from the Animal House Colony of the National Research Centre (NRC), Cairo, Egypt. The animals were maintained on standard laboratory diet and water ad libitum, housed in stainless steel cages in a temperature controlled (23 ± 1°C) 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 guidelines for animal experiments which were approved by the Ethical Committee of Medical Research, National Research Centre, Egypt. After an acclimatization period of one week, AD was induced in the experimental animals by using AlCl₃ orally in a dose of 17 mg/kg b. wt daily for one month [44]. The animals used in the current study were classified into 5 main groups: (1): Normal healthy animals served as untreated negative control group. (2): Animals inducted with AD served as untreated positive control 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 [45] as a reference drug for comparison daily for three months. (4): Animals inducted with AD and this group was divided into two subgroups each subgroup was treated with one of the selected medicinal plants extract in a dose of 750 mg/kg b. wt. (the most save dose which was obtained in the chronic study) for Salvia triloba L. and 187.5 mg/kg b. wt. for Piper nigrum daily for three months. (5): Animals inducted with AD and this group was divided into two subgroups each subgroup was treated with one of the selected medicinal plants extract in a dose of 375 mg/kg b. wt. (the half of most save dose which was obtained in the chronic study) for Salvia triloba L. and 93.75 mg/kg b. wt. for Piper nigrum daily for three months.

Samples collection
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 was according to the method Van Herck et al. [46]. 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 was frozen at -20 °C for the biochemical analyses.

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 and then weighed. One half of each brain was homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose (pH 7.4) [47]. The homogenate was centrifuged at 1800 xg for 10 min. at 4 °C and the supernatant (10%) was separated for the different biochemical analyses. Also, brain total protein concentration was measured to express the concentration of different brain parameters per mg protein [48]. The second half 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. [49] using kit purchased from Bio diagnostic Co., Egypt. Serum and brain acetylcholinesterase activity colorimetrically according to method of Den Blaawen et al. [50] using kit purchased from Quimica Clinica Aplicada S.A Co., Amposta, Spain. 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. Leukotriene B4 (LTB4) level in serum and brain was estimated by ELISA technique according to Maclof et al. [51] Method using kit purchased from Cayman Chemical Co., USA. Serum and brain B-cell lymphoma 2 (Bcl2) level was detected using ELISA technique according to the method of Barbareschi et al. [52] 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, deparaffinized and stained by hematoxylin and eosin (H&E) stain [53] for histopathological examination through the light microscope.

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 [54]. 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 effects of treatment of adult male rats with Rivastigmine and/or the selected medicinal plants total extract on brain and serum AchE activity in different experimental groups.

In comparison with the negative control group, AlCl₃ administration produced significant elevation (P< 0.05) in brain and serum AchE activities (34.3 % and 22.7 % respectively)

Treatment of AD-induced rats with rivastigmine or with either one of the selected medicinal plants extract resulted in significant decrease (P< 0.05) in brain and serum AchE activities (-21.44 %, -16.22 % for rivastigmine; -16.3 %, -13.1 % respectively for S. triloba at a dose 750 mg/kg b. wt. and by -10.73 %, -7.32 % for S. triloba (375 mg/kg b. wt.); -10.81 %, -8.7 % for P. nigrum (187.5 mg/kg b. wt.)). Meanwhile, treatment of AD-induced rats with P. nigrum extract in a dose of 93.75 mg/kg b. wt showed insignificant decrease (P> 0.05) in brain and serum AchE activities (-5.16 %, -4.73 %) as compared with the untreated AD-induced group. The treatment with S. triloba (375 mg/kg b. wt.) or P. nigrum (187.5 or 93.75 mg/kg b. wt.) extract could not reduce brain and serum AchE activities as did the Rivastigmine.

Table (1): Effect of treatment of AD-induced rats with the selected medicinal plant total extracts on brain and serum acetylcholinesterase (AchE) activities and brain acetylcholine (Ach) level.

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>AchE</th>
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<tbody>
<tr>
<td></td>
<td>Brain (U/mg protein)</td>
</tr>
<tr>
<td>control group</td>
<td>571.1 ± 21.2</td>
</tr>
<tr>
<td>AD-induced group</td>
<td>767.0 ± 11.7 (∗) (34.3 %)</td>
</tr>
<tr>
<td>AD + Rivastigmine group</td>
<td>602.5 ± 21.0 (∗) (-21.44 %)</td>
</tr>
<tr>
<td>AD + S. triloba (750 mg/kg b.wt)</td>
<td>642.3 ± 15.7 (∗) (-16.3 %)蔬菜</td>
</tr>
<tr>
<td>AD + S. triloba (375 mg/kg b.wt)</td>
<td>684.7 ± 7.0 (∗) (-10.73 %)</td>
</tr>
<tr>
<td>AD + P. nigrum (187.5 mg/kg b.wt)</td>
<td>684.1 ± 13.6 (∗) (-10.81 %)</td>
</tr>
<tr>
<td>AD + P. nigrum (93.75 mg/kg b.wt)</td>
<td>727.4 ± 10.5 (∗) (-5.16 %)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± standard error (SE) for 10 animals / group.

a: Significance change at P < 0.05 in comparison with control group.

b: Significance change at P < 0.05 in comparison with AD-induced group.

c: Significance change at P < 0.05 in comparison with AD+Rivastigmine group.

(%) : percent of difference with respect to the corresponding control value.

The results of the current study showed that, Al administration induced significant elevation (P< 0.05) in brain and
serum COX-2 activities (114.11 and 126.72 % respectively), and LTB$_4$ (187.84 % and 44.76 % respectively) levels when compared with the negative control group (Table 2).

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; -36.61, -25.24 % for S. triloba (750 mg/kg b. wt.); -44.86, -31.12 % for P. nigrum (187.5 mg/kg b. wt.)) and insignifcant decrease (P> 0.05) in serum COX-2 activity as compared to AD-induced group. Treatment of AD-induced group with S. triloba (375 mg/kg b.wt) produced significant decrease (P< 0.05) in brain COX-2 activity and insignificant decrease (P> 0.05) in serum COX-2 activity as compared to AD-induced group. Meanwhile, in comparison with AD-induced group treated with Rivastigmine, the treatment with S. triloba, S. triloba (375 mg/kg b.wt) and P. nigrum (93.75 mg/kg b. wt) caused marked change in brain and serum COX-2 activities. Treatment with P. nigrum (93.75 mg/kg b.wt) caused observable change in brain COX-2 activity as compared to AD-induced group treated with Rivastigmine but not 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 LTB$_4$ levels (-46.95 %, -19.04 % for Rivastigmine; -30.82, -15.36 % for S. triloba (750 mg/kg b. wt.); -35.52, -15.17 % for P. nigrum (187.5 mg/kg b. wt) and -34.27, -14.02 % for P. nigrum (93.75 mg/kg b. wt.)) as compared to the untreated AD-induced group. Treatment of AD-induced group treated with S. triloba (375 mg/kg b.wt) produced significant decrease (P< 0.05) only in serum LTB$_4$ level (-23.63 %) as compared to the untreated AD-induced group. Treatment with S. triloba (750 mg/kg b.wt) extract caused significant change in brain LTB$_4$ level as compared to AD-induced group treated with Rivastigmine but not as did the Rivastigmine.

Table (2): Effect of treatment with Rivastigmine and the selected medicinal plants total methanolic extracts on brain and serum COX-2 activities and LTB$_4$ levels in AD-induced rats

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>Brain (ng/mg protein)</th>
<th>Serum (ng/mL)</th>
<th>Brain (pg/mg protein)</th>
<th>Serum (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>7.82±0.42 (114.11 %)</td>
<td>8.16±0.16</td>
<td>0.22 ± 0.011</td>
<td>24.01±0.58</td>
</tr>
<tr>
<td>AD-induced group</td>
<td>16.74±0.79 (93.75 mg/kg b.wt)</td>
<td>15.51±0.63 (126.72 %)</td>
<td>0.64 ± 0.046 (187.84%)</td>
<td>34.76±0.64 (44.76 %)</td>
</tr>
<tr>
<td>AD + Rivastigmine group</td>
<td>9.02±0.63 (187.5 mg/kg b.wt)</td>
<td>11.70±1.18 (93.75 mg/kg b.wt)</td>
<td>0.34 ± 0.038 (187.84%)</td>
<td>28.14±0.96 (187.84%)</td>
</tr>
<tr>
<td>AD + S. triloba (750 mg/kg b.wt)</td>
<td>10.61±1.02 (375 mg/kg b.wt)</td>
<td>13.84±0.35 (126.72 %)</td>
<td>0.44±0.028 (187.84%)</td>
<td>29.42±0.91 (187.84%)</td>
</tr>
<tr>
<td>AD + S. triloba (375 mg/kg b.wt)</td>
<td>10.78±0.20 (187.5 mg/kg b.wt)</td>
<td>16.70±0.35 (126.72 %)</td>
<td>0.57 ± 0.065 (187.84%)</td>
<td>31.35±0.95 (187.84%)</td>
</tr>
<tr>
<td>AD + P. nigrum (187.5 mg/kg b.wt)</td>
<td>9.23±0.29 (375 mg/kg b.wt)</td>
<td>12.75±1.03 (126.72 %)</td>
<td>0.41 ± 0.03 (187.84%)</td>
<td>29.40±1.19 (187.84%)</td>
</tr>
<tr>
<td>AD + P. nigrum (93.75 mg/kg b.wt)</td>
<td>13.32±0.35 (750 mg/kg b.wt)</td>
<td>13.99±0.97 (126.72 %)</td>
<td>0.42 ± 0.045 (187.84%)</td>
<td>29.89±1.22 (187.84%)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± standard error (SE) for 10 animals / group.

a: Significance change at P < 0.05 in comparison with control group.
b: Significance change at P < 0.05 in comparison with AD-induced group.
c: Significance change at P < 0.05 in comparison with AD + Rivastigmine group.

The results in Table (3) demonstrated the effect of treatment of AD-induced rats with Rivastigmine and the selected medicinal plants total extract on brain and serum B cell lymphoma 2 (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 total extracts exhibited significant elevation (P< 0.05) in brain and serum Bcl-2 levels (63.59 %, 58.10 % for Rivastigmine; 49.09, 47.28 % for S. triloba (750 mg/kg b. wt.); 48.25, 18.17 % for S. triloba (375 mg/kg b.wt) and 57.37, 36.71 % for P. nigrum (187.5 mg/kg b.wt.)) as compared to the untreated AD-induced group. In comparison with AD-induced group treated with Rivastigmine, the treatment with S. triloba (375 mg/kg b.wt), P. nigrum (187.5 mg/kg b.wt) or P. nigrum (93.75 mg/kg b.wt) extract caused significant change but only in serum Bcl-2 level.
Treatment with *P. nigrum* (93.75 mg/kg b.wt) extract caused significant change but only in brain Bcl-2 level in comparison with AD-induced group treated with Rivastigmine. But the treatment with the extracts could not change brain and serum Bcl-2 levels as did the Rivastigmine.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bcl-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brain ng/mg protein</td>
</tr>
<tr>
<td>control group (n=10)</td>
<td>5.50 ± 0.38</td>
</tr>
<tr>
<td>AD-induced group</td>
<td>3.12 ± 0.22</td>
</tr>
<tr>
<td>AD + Rivastigmine group</td>
<td>5.10 ± 0.39</td>
</tr>
<tr>
<td>AD + <em>S. triloba</em> (750 mg/kg b.wt)</td>
<td>4.65±0.059</td>
</tr>
<tr>
<td>AD + + <em>S. triloba</em> (375 mg/kg b.wt)</td>
<td>4.63±0.23</td>
</tr>
<tr>
<td>AD + <em>P. nigrum</em> (187.5 mg/kg b.wt)</td>
<td>4.91±0.18</td>
</tr>
<tr>
<td>AD + <em>P. nigrum</em> (93.75 mg/kg b.wt)</td>
<td>3.80±0.11</td>
</tr>
</tbody>
</table>

Data are expressed as means ± standard error (SE) for 10 animals/group.

a: Significance change at P < 0.05 in comparison with control group.
b: Significance change at P < 0.05 in comparison with AD-induced group.
c: Significance change at P < 0.05 in comparison with AD+Rivastigmine group.

Microscopic examination of brain sections of negative control group (Figs. 1, 2, 3 and 4) showed no histopathological alteration and normal histological structure of the meninges, hippocampus, medulla oblongata, cerebral cortex and cerebellum.

Micrographs of brain section of AD-induced group showed severe 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 ependymal malacia 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 Al-intoxicated rats and treated with *S. triloba* (750 mg/kg b.wt.) showing severe congestion in the blood vessels with pericellular and perivascular oedema were detected in the cerebrum (Fig. 12), cerebral ependymal malacia with plaques formation were observed in the striatum (Fig. 13 and 14) and intact normal histological structure was observed in hippocampus (Fig 15 and 16). While, micrograph of brain section of Al-intoxicated rats and treated with *S. triloba* (375 mg/kg b.wt) showing congestion in the blood vessels with perivascular oedema were detected in the cerebrum (Fig. 17).

Micrograph of brain section of Al-intoxicated rats and treated with *P. nigrum* (187.5 mg/kg b.wt.) showed focal gliosis (Fig. 18), neuronal degeneration with ependymal malacia and plaques formation (Fig. 19), congestion with perivascular oedema (Fig. 20) in the cerebrum and normal histological structure in hippocampus (Fig 21). Moreover, micrograph of brain section of Al-intoxicated rats and treated with *P. nigrum* (93.75 mg/kg b.wt.) showed focal gliosis in the cerebrum (Fig. 22), while the hippocampus was intact (Fig. 23).
Fig (1): Micrograph of brain section of negative control rats showing normal histological structure of the meninges (m) and cerebral cortex “H&E *40”

Fig (2): Micrograph of brain section of negative control rats showing normal histological structure of the hippocampus “H&E *40”

Fig (3): Micrograph of brain section of negative control rats showing normal histological structure of the medulla oblongata (mo) “H&E*40”

Fig (4): Micrograph of brain section of negative control rats showing normal histological structure of the cerebellum (cr) “H&E*40”

Fig (5): Micrograph of brain section of AD-induced group showing severe congestion with oedema (v) in meninges “H&E*80”

Fig (6): Micrograph of brain section of AD-induced group showing neuronal degeneration (arrow) with oedema in between in cerebrum “H&E*64”
Fig (7): Micrograph of brain section of AD-induced group showing the magnification of (Fig 6) to identify the neuronal degeneration (arrow) and oedema (o) with gliosis (g) in between in cerebrum "H&E*80"

Fig (8): Micrograph of brain section of AD-induced group showing focal gliosis in cerebrum "H&E*80"

Fig (9): Micrograph of brain section of AD-induced group showing encephalomalacia (c) with plaques formation (p) in hippocampus "H&E*64"

Fig (10): Micrograph of brain section of AD-induced group showing the magnification of (fig 21) to identify the encephalomalacia (c) with plaques formation (p) in hippocampus "H&E*80"

Fig (11): Micrograph of brain section of AD-induced group treated with Rivastigmine showing intact normal histological structure of hippocampus "H&E*64"
Fig (12): Micrograph of brain section of AD-induced group treated with *S. triloba* (750 mg/kg b. wt.) showing severe congestion (v) with pericellular and perivascular oedema in cerebrum "H&E*64"

Fig (13): Micrograph of brain section of AD-induced group treated with *S. triloba* (750 mg/kg b. wt.) showing cerebral encephalomelacia (c) with plaques formation (p) in striatum "H&E*64"

Fig (14): Micrograph of brain section of AD-induced group treated with *S. triloba* (750 mg/kg b. wt.) showing the magnification of (fig 13) to identify the encephalomelacia (c) with plaques formation (p) in striatum "H&E*80"

Fig (15): Micrograph of brain section of AD-induced group treated with *S. triloba* (750 mg/kg b. wt.) showing normal histological structure in hippocampus "H&E*40"

Fig (16): Micrograph of brain section of AD-induced group treated with *S. triloba* (375 mg/kg b. wt.) showing normal intact histological structure of hippocampus (hp) "H&E*40"

Fig (17): Micrograph of brain section of AD-induced group treated with *S. triloba* (375 mg/kg b. wt.) showing Congestion with perivascular oedema in the cerebrum "H&E*64"
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. [55] and Zhang et al. [56] The study of Zubenko
and Hanin [57] showed that Al enhanced the activity of AchE in vivo and in vitro 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 [58].

The second proposed mechanism for Al-induced enhancement of AchE activity in the brain depends on the ability of Al to promote the accumulation of insoluble Aβ (1–42) protein [59]. Aβ (1–42) induced elevation of AchE activity is mediated through the direct inhibitory action of Aβ on nicotinic acetylcholine receptors (nAchR) [60]. The accumulation of Al in the brain and the decline of nAchRs functions contribute to AD and other forms of dementia [61].

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 [62]. Rivastigmine is a novel acetylcholinesterase (AChE) inhibitor that displays specific activity for central AchE over peripheral AchE [63]. It is licensed in the UK for the treatment of AD and memory dysfunction [64]. 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 [65]. Rivastigmine appears to inhibit cholinesterases (ChEs) in plaques and tangles with the same potency as those in neurons and axons [66]. Rivastigmine exerts its inhibitory effects on brain AchE activity by interacting with the esteratic site in ChE molecules [67].

Treatment of AD-induced rats with S. triloba extract resulted in significant decrease in brain and serum AchE activities in the studied groups. Acetylcholinesterase inhibitory activity of S. triloba has been previously reported by Loizzo et al. [68] and Orhan and Aslan [42]. Salvia species essential oil or their metabolites have been found to reach the brain (crossing the gastrointestinal and blood–brain barriers) and exert an effect on cognition by inhibiting AchE in different brain areas, consistent with evidence of inhibition of the rat brain enzyme in vivo [27] and rat brain AchE in vivo [69]. This activity of S. triloba could be attributed to its active constituents terpenes [70]. Also, the essential oil of S. triloba contains cyclic monoterpenes 1, 8-cineole and α-pinene [71], which possess AchE inhibitory activity [72]. Flavonoids and other phenolic compounds are also present in S. triloba [73] and these compounds are known to possess cholinesterase inhibitory action. Salvia essential oils and its individual monoterpenoid constituents have been found to inhibit cholinesterases as well as nicotinic and muscarinic activities of cholinergic system that are involved in the memory retention process [27].

The current study revealed that P. nigrum treatment in AD-induced rats produced a significant decrease in brain and serum acetylcholinesterase (AchE) activity in the studied groups. P. nigrum extract has been shown to inhibit the activity of AchE significantly [41]. Recently Chonpathompikunlert et al. [74] demonstrated that piperine, the major constituent of piper is responsible for the attenuation of memory impairment, the inhibition of AchE activity and the amelioration of neurodegeneration of the brain. These authors stated that the cognitive enhancing effect of Piperine might occur partly via the cytoprotective effect and the inhibition of AchE in hippocampus region. Moreover, terpenoids, glycosides and coumarins from plants belonging to family Piperaceae that have been reported to possess AchE inhibitory potential [75]. By the same way, P. nigrum extract could elevate brain Ach level and improve cholinergic transmission in the brain.

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. [76] 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 [77]. 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 [78]. Several studies reported increased neuronal COX-2 immunoreactivity compared to control brain tissues [79]. 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 [80]. The transcription of COX-2 mRNA is induced by synaptic activity [81]. Accordingly, the increased synaptic activity associated with seizures markedly increases COX-2 expression [82]. Increased levels of COX-2 mRNA and protein staining in AD tissue [83]. COX-2 mRNA rises rapidly in response to inflammatory stimuli such as IL-1β suggesting that COX-2 is the isoform that mediates inflammation [84]. 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 [85] 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 [86].

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 [87]. Moreover, free radicals have been reported to cause cell death through activation of JNK [88] 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 [89].

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 [90]. COX-2 is highly expressed in pyramidal neurons of AD cases [91]. Endothelial COX-2 induction is rapid and is linked to fever development, BBB changes and possibly regulation of blood flow [92]. 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 [83].

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 [76]. In AD, the expression of COX-2, the inducible isoform, increases in response to inflammatory agents in neurons and glial cells [93]. The apparent early up-regulation of COX-2 in hippocampal neurons of the AD brain [83].

The activation of NF-κB has previously been shown in neurons surrounding amyloid plaques in AD [94]. Jung et al. [95] reported that activation of NF-κB increased the expression of COX-2. Furthermore, a correlation between the presence of the transcription factor NF-κB 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-κB is involved in the induction of COX-2 in the human brain [96]. Interestingly, NF-κB is involved in the induction of COX-2 [97].

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 [98].

Treatment of AD-induced rats S. triloba produced significant decrease in brain and serum COX-2 activity as compared to Al-intoxicated positive control group, Table (2). The essential oils of Salvia species from different locations showed inhibition of the COX-2 enzyme [99]. Moreover, Salvianolic acid B (Sal-B), isolated from Salvia miltiorrhiza Bge, can effectively suppress COX-2 expression in a variety of cancer cell lines [100]. Cryptotanshinone is one of the major constituents of tanshinones (tanshinone I and tanshinone IIA), isolated from Salvia miltiorrhiza Bunge that exerts anti-inflammatory effects in vivo and in vitro by selectively inhibiting COX-2 activity [101]. The anti-inflammatory properties are thought to be based on the inhibition of lipoxygenases and cyclooxygenases and the interference of rosmarinic acid with the complement cascade [102].

Treatment of AD-induced rats with P. nigrum produced significant decrease in brain and serum COX-2 activity as compared to AD-induced group, Table (2). Extracts and single constituents of P. nigrum possess inhibitory properties that are specific to cyclooxygenase-1, an important enzyme in the formation of prostaglandins, which in turn are important mediators of the development of inflammatory diseases [103].

Piper extracts and piperine possess inhibitory activities on prostaglandin and leukotrienes inhibitory effect and thus exhibit anti-inflammatory activity. Constituents of piper species exhibit inhibitory activity on prostaglandin and leukotriene biosynthesis in vitro [104]. It was suggested that the aqueous extract of Piper sarmentosum (AEPS) possessed arachidonate COX inhibitor property [34].

The actual mechanisms of these anti-inflammatory activity due to bioactive compound, the report of Dawson and Snyder [105] revealed that the in vivo anti-inflammatory activity of flavonoids and flavones derivatives occur through modulation of pro-inflammatory gene expression such as inducible NO synthase and cyclooxygenase-2.
was corroborated that anti-inflammatory activity of allylpyrocatechol (APC) isolated from *piper* induced a decrease in COX-2 which translated into a dramatic decrease in prostaglandins (PGE2). This inhibition may contribute to inhibition of IkB phosphorylation, as observed previously that would inhibit the generation of NF-κB dependent cytokines, expression of iNOS and COX-2, thereby reducing inflammation [106].

The results of the current study showed that Al administration induced significant elevation in brain and serum LTB4 levels, Table (2). Aβ has been found to induce proinflammatory response in microglia which evidenced by increased LTB4 release [107].

Cytochrome P450 4Fs (CYP4Fs) are constitute a subgroup of the cytochrome P450 superfamily and are involved in cellular protection and metabolism of numerous molecules, including drugs, toxins and eicosanoids which widely distributed in rat brain with each isoform having a unique distribution pattern throughout different brain regions. Brain injury due to Al intoxication triggers inflammation and elicits changes in mRNA expression of CYP4Fs in the frontal and occipital lobes and hippocampus [108]. CYP450 enzymes are involved in the resolution of inflammation and their expression levels and/or catalytic activities are changed by the pathophysiological processes [109]. The changes in CYP4F levels inversely correlate with the levels of leukotriene B4 (LTB4) in the brain following injury [108] as these have been shown to catalyze the omega-hydroxylation of endogenous eicosanoids such as LTB4 [110]. It has been demonstrated that brain injury immediately leads to inflammation involving increased concentrations of leukotrienes and prostaglandins [111] due to decreased CYP4F levels at the brain injury [112].

Treatment of AD-induced rats with Rivastigmine produced significant decrease in brain and serum LTB4 level as compared to AD-induced group, Table (2). This could be attributed to its anti-inflammatory properties [98].

Treatment of AD-induced rats with *S. triloba* produced significant decrease in brain and serum LTB4 level as compared to AD-induced rats, Table (2). The *in vitro* anti-inflammatory activity of essential oils and solvent extracts of *Salvia* species was evaluated using the 5-lipoxygenase assay. Essential oils of *Salvia runcinata* and *Salvia stenophylla* could be inhibited the 5-lipoxygenase enzyme as well [113]. Peana et al. [114] showed that linalool and its ester linalyl acetate exhibited anti-inflammatory activity.

*S. lavandulaefolia* ethanolic extracts and monoterpenoids present in the essential oil, a-pinene and geraniol, have demonstrated significant inhibition of eicosanoid leukotriene B4 (LTB4) Synthesis via inhibition of 5-lipoxygenase, enzyme produced eicosanoid leukotriene B4 (LTB4) [71].

Treatment of AD-induced rats with *P. nigrum* produced significant decrease in brain and serum LTB4 level as compared to AD-induced rats, Table (2). *Piper* extracts and piperine possess inhibitory activities on prostaglandin and leukotrienes inhibitory effect and thus exhibit anti-inflammatory activity. Constituents of piper species exhibit inhibitory activity on prostaglandin and leukotriene biosynthesis *in vitro* [104]. It has been revealed the presence of various physiologically active chemical constituents on *piper* including unsaturated amides, flavonoids, lignans, aristolactams, long and short chain esters, terpenes, steroids, prophenylphenols, and alkaloids [115]. Interestingly, these compounds exhibited cyclooxygenase-1 (COX-1) and 5-lipoxygenase (5-LO) inhibitory activity [34].

Species of *Piper* act as *in vitro* inhibitors not only of cyclooxygenase-1 but also of 5-lipoxygenase, which is responsible for the formation of pro-inflammatory leukotrienes that play an important role in leukocyte migration [116].

The results of the current study showed that Al administration induced significant reduction in brain and serum anti-apoptotic marker (Bcl-2) levels, Table (3). In accordance with our results, Jin et al. [117] demonstrated that Al could influence the activities of learning and memory and also reduce the expression of Bcl-2 level. Many studies have generated a strong body of evidence indicating the potential role of Al in inducing apoptosis and neurodegeneration in the brain *in vivo* [118] as well as *in vitro* [119]. In mitochondrial fraction derived from brain, the levels of Bcl-2 are decreased following Al administration in young adult rabbits [118]. Also it has been observed a dramatic down regulation of Bcl-2 expression following treatment of neurons with Al *in vitro* [120].

Indeed, Al has been shown to induce cell death with similar pathological and biochemical changes observed in AD [121]. Some of the important biochemical events attributed to cell death associated with AD are decreased levels of Bcl-2, and increased levels of Bax [122]. In fact, 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, increasing the level of the pro-apoptotic Bcl-2 associated x protein (Bax), activation of caspase-3 and release of cytochrome c [13].

From another point of view, Al has been known to induce oxidative stress on the neuronal cells and increase p53 expression by activating P38 mitogen-activated protein kinases (p38 MAPK) to initiate apoptotic cascade [123]. This increase in p53 protein was accompanied by a marked inhibition of Bcl-2 expression and an increase in Bax expression [120].

It has been reported that Aβ could activate p53 by direct interaction with p53 promoter [124]. This would lead to Bax and caspase-6 activation with subsequent reduction of Bcl-2 and execution of the cell death pathway [120].

Treatment of AD-induced rats with Rivastigmine produced significant elevation in brain and serum Bcl-2 levels, Table (3). The expression level of Bcl-2 increased with ACHEIs treatment [125]. The blockade of voltage-activated K currents by Rivastigmine may lead to the suppression of apoptosis and substantial increase in cell survival [126]. Treatment of AD-induced rats with S. triloba produced significant elevation in brain and serum Bcl-2 level as compared to AD-induced rats, Table (3). Antiapoptotic activity of S. triloba L. [127] has been documented. The simple phenolic PCA is one of the major benzoic acid derivatives from Salvia miltiorrhiza [128], was significantly effective to suppress the down-regulation of Bcl-2. It was suggested that the water-soluble extracts of Salvia miltiorrhiza could inhibit apoptosis by regulating the expression of Bax and Bcl-2 [129]. It has been demonstrated that induction of apoptotic cell death by MPP+ was associated with loss of mitochondrial membrane potential, the formation of ROS, GSH depletion and activation of caspase-3. In contrast, treatment of PC12 cells with PCA significantly prevented the above-mentioned mitochondrial dysfunction. Moreover, MPP+-induced apoptotic cell death was associated with the down-regulation of Bcl-2 and PCA was also significantly effective to suppress the down-regulation of Bcl-2 [129]. Moreover, it was suggested that the water-soluble extracts of Salvia miltiorrhiza could inhibit biliary obstruction-induced hepatocyte apoptosis by regulating the expression of Bax and Bcl-2 [127]. Salvianolic acid B (Sal B) extracted from Salvia Species [130] has been demonstrated to possess anti-apoptotic effect [131].

Treatment of AD-induced rats with P. nigrum produced significant elevation in brain and serum Bcl-2 as compared to AD-induced rats, Table (3). Anti-apoptotic potential of Piperine has been demonstrated [132]. The antiapoptotic effect of P. nigrum may be due to piperine was demonstrated to upregulate the expression of Bcl-2 [133].

Pathak and Khandelwal [134] have demonstrated oxidative stress followed by apoptosis leading to altered immune function and cell proliferative mitogenic response in murine thymocytes. Piperine supplementation effectively restored immune function and proliferation notably by curbing oxidative stress damage to murine thymocytes [132]. Vijayakumar et al. [135] showed that the antioxidant efficacy of black pepper and piperine in rats with oxidative stress which could be attributed to its free radical scavenging property [136] which may explain the antiapoptotic effects of piper. Moreover, changes in reactive oxygen species and GSH by cadmium may be associated with alterations in the expression of several antioxidant and apoptotic genes and interaction of piperine through redox sensitive pathways is quite plausible, since Li et al. [137] observed that piperine could upregulate the mRNA level of brain-derived neurotrophic factor in hippocampus of chronic mild stressed mice.

Photomicrograph of brain section of AD-induced rats showed the presence sever congestion in the blood vessels with oedema in the meninges. The cerebrum showed neuronal degeneration associated with focal gliosis. The hippocampus showed encephalomalacia and plaques formation. In accordance of our results Abd El-Rahman [138] demonstrated that Al administration causes the formation of neuritic plaques that appeared with dark center, neuronal damage and degeneration in the cerebral cortex and hippocampus. Moreover, histologic examination carried by Bihaqia et al. [139] showed increased neuronal loss, ghost cells haemorrhage and vacuolated cytoplasm after Al exposure in rats.

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. [139] who revealed that Rivastigmine reversed histopathological alterations caused by Al. Also the study of Bihaqia et al. [139] showed normal histological appearance of the brain cells treated with Rivastigmine tartrate. Bailey and Lahiri [16] observed
enhancement of neuronal morphology after treatment with Rivastigmine suggesting that Rivastigmine is considered as novel neuroprotective and/or neurorestorative agent for the brain. These properties of Rivastigmine comes from the inhibition of the hydrolytic enzyme, acetylcholinesterase (AChE), and thereby increasing the available pool of the neurotransmitter acetylcholine (ACh), which attenuates plaques and tangles formation [66].

Photomicrograph of brain section of AD-induced rats and treated with *S. triloba* (750 mg/kg b. wt.) showing severe congestion in the blood vessels with pericellular and perivascular oedema were detected in the cerebrum, cerebral encephalomalacia with plaques formation were observed in the striatum and intact normal histological structure was observed in hippocampus. While, micrograph of brain section of Al-intoxicated rats and treated with *S. triloba* (375 mg/kg b. wt.) showing congestion in the blood vessels with perivascular oedema were detected in the cerebrum. Tissue sections from Tg and CTS-M mice which were stained with an anti-Aβ antibody 6E10 showed that treatment Tg (APP/PS1) mice with cryptotanshinone (CTS), active component of *salvia* has been shown to reduce Aβ deposition in cortex and hippocampus [140]. The reduced Aβ peptide levels could be due to less available APP substrate for β-secretase cleavage caused by CTS-enhanced α-secretase activity [141].

Photomicrograph of brain section of AD-induced rats and treated with *P. nigrum* (187.5 mg/kg b. wt.) showed focal gliosis, neuronal degeneration with encephalomalacia and plaques formation, congestion with perivascular oedema in the cerebrum and normal histological structure in hippocampus. Moreover, micrograph of brain section of Al-intoxicated rats and treated with *P. nigrum* (93.75 mg/kg b. wt.) showed focal gliosis in the cerebrum, while the hippocampus was intact. This may be due to the presence of phenolic compounds detected such as hydroxybenzoic acid derivatives, caffeic acid derivatives (e.g., rosmarinic acid), ferulic acid as well as flavonoid derivatives and piperine which they have a potent antioxidant activity [142]. Chonpathompikunlert et al. [74] showed that piperine used in their study significantly improved memory impairment and neurodegeneration in hippocampus of rats.

**CONCLUSION**

In conclusion, the current study revealed that treatment of AD-induced rats with *S. triloba* or *P. nigrum* methanolic extract, significantly ameliorates the cholinergic dysfunction and inflammation-induced neurodegeneration characteristic of Alzheimer's disease. These effects could be attributed to powerful the anticholinesterase effects, anti-inflammatory activity and anti-apoptotic effects of these extract. These results represented good therapeutic approaches for intervention against progressive neurological damage associated with Alzheimer's disease with special reference to the inflammatory insults.

**REFERENCES**