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Effect of some medicinal plant extracts on the oxidative stress status in Alzheimer's disease induced in rats

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Abstract. – BACKGROUND/AIM, Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Increased oxidative stress has been shown to be a prominent and early feature in AD. Medicinal plants with antioxidant activities have been used traditionally in the treatment of several human diseases. The present study aims to investigate the effect of *Salvia triloba* and *Piper nigrum* plant extracts on the oxidative stress status in Alzheimer's disease induced in rats.

MATERIALS AND METHODS, 70 male rats were enrolled in this study and were classified into 7 groups (ten each). Group 1: control group, group 2: AD-induced rats by aluminum chloride, and served as positive control; group 3: AD group treated with Rivastigmine in a dose of 0.3 mg/kg b. wt. daily for three months; group 4 & 5: AD group treated with total extract of *Salvia triloba* in a dose of 750 or 375 mg/kg b. wt. respectively, daily for three months; group 6 & 7: AD group treated with total extract *Piper nigrum* in a dose of 187.5 or 93.75 mg/kg b. wt. respectively, daily for three months. After three months of treatment animals' sera and brain samples were collected. Malondialdehyde (MDA), nitric oxide (NO) and total antioxidant capacity (TAC) were determined in serum while superoxide dismutase (SOD) in erythrocyte. Brain samples were divided sagitally into two portions, the first portion was separated for determination of acetylcholine (Ach) and acetycholinesterase (AchE). The second portion was used for histopathological investigation.

RESULTS, The results indicated that extracts of *Salvia triloba* and *Piper nigrum* as well as Rivastigmine showed significant increase in brain Ach, serum TAC and SOD and significant decreases in brain AchE, MDA and NO in AD-induced rats. Moreover, histological investigation of brain sections showing nearly normal histological structure of hippocampus. Treatment with *Salvia triloba* in a dose of 750 mg/kg b. wt. was more powerful in protection from Alzheimer's disease than *Piper nigrum*, as indicated by both biochemical and histopathological findings.

CONCLUSION, This study revealed that the treatment of AD-induced rats with *Salvia triloba* and *Piper nigrum*, total plant extracts significantly reduced the oxidative stress status and ameliorates the neurodegeneration characteristic of Alzheimer's diseases in rats. Noteworthy, *Salvia triloba* extract showed more interest in improvement Alzheimer's disease in rats.

Key Words:

Alzheimer's disease, Oxidative stress, *Salvia triloba*, *Piper nigrum*, Extract.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily affects the elderly population and is considered to be responsible for the majority of dementia cases in people aged 65 or older. This disease is characterized by numerous symptoms such as memory and language impairment, cognitive dysfunction and behavioural disturbances (i.e., depression, agitation and psychosis), which become progressively more severe. Due to its debilitating nature, an enormous social and economic burden is placed on society[1].

Neuroimaging of the patient with AD or other dementias may reveal atrophy of the brain, such as enlarged ventricles and sulci and narrowed gyri, although these features are not always present[2]. Neuronal loss is the main neuropathologic feature underlying the symptoms of AD. Microscopically, AD is characterized by the presence of senile plaques and neurofibrillary tangles (NFTs). Plaques are extracellular deposits of filamentous β-amyloid, a protease cleavage product of amyloid precursor protein[3]. Several mechanisms have been postulated to explain AD pathogenesis, including a beta toxicity, cholinergic dysfunction, tau protein hyperphosphorylation, oxidative damage, synaptic dysfunction, and inflammation secondary to senile plaques[4].

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Increased oxidative stress has been shown to be a prominent and early feature of vulnerable neurons in AD. Exposure to oxidative stress induces the accumulation of intracellular reactive oxygen species (ROS), which in turn causes cell damage in the form of protein, lipid, and DNA oxidations. Elevated ROS levels are also associated with increased deposition of amyloid-β and formation of senile plaques, a hallmark of the AD brain. If enhanced ROS exceeds the basal level of cellular protective mechanisms, oxidative damage and cell death will result. Therefore, substances that can reduce oxidative stress are sought as potential drug candidates for treatment or preventive therapy of neurodegenerative diseases such as AD[5].

Medicinal plants have been traditionally used in the treatment of several human diseases and their pharmacological and therapeutic properties have been attributed to different chemical constituents isolated from their crude extracts. Of particular importance, chemical constituents with antioxidant activity can be found at high concentrations in plants and can be responsible for their preventive effects in various degenerative diseases, including cancer, neurological and cardiovascular diseases[6]. Thus, the antioxidant properties of plants have a full range of perspective applications in human healthcare[7].

Salvia is an important genus in the family Laminaceae[8]. The East Mediterranean Sage (*Salvia triloba*) is a native plant to the Mediterranean region, which has been used in traditional medicine by many Asian and Middle Eastern countries to treat several ailments. The plant has been reported in old Arabic literature to improve the mental power. The leaves of the plant are boiled as an herbal tea for the relief of headaches, stomachaches, abdominal pain and many other disorders. The aqueous and oil extracts of sage have been shown to possess antioxidant, anti-inflammatory, anticancer and antimicrobial activities[9]. Its high antioxidant activity could be attributed to on the synergetic effects of the high phenolic contents isolated from this herb such as hydroxybenzoic acid derivatives, caffeic acid derivatives (e.g., rosmarinic acid), ferulic acid as well as flavonoid derivatives; luteolin and quercetin[10].

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae[11]. The plant have been used effectively for the treatment of AD. Piperine is a major plant alkaloid present in black pepper (*Piper nigrum*) and long pepper (Piper longum), which are among the most common spices consumed by a large number of people worldwide. This compound is known to possess several pharmacological actions, such as antimicrobial, antifungal, anti-inflammatory and antioxidant effects[12]. Piperine has been demonstrated in *in vitro* studies to protect against oxidative damage by inhibiting or quenching free radicals and ROS, lower lipid peroxidation *in vivo* and beneficially influence cellular thiol status, antioxidant molecules and antioxidant enzymes in a number of experimental situations of oxidative stress[13].

The aim of present work is to study the effect of total plant extracts of *Salvia triloba* and *Piper nigrum* on the oxidative stress status in Alzheimer's disease induced in rats.

Materials and Methods

Materials

Aluminium Chloride ($AlCl_3$), with M. wt. 133.34 was purchased from Sigma-Aldrich Co. (Germany). Rivastigmine of Exelon 1.5 mg, was purchased from Novartis Co. (Cairo, Egypt) While the aerial part of *Salvia triloba* L., and seeds of *Piper nigrum* were purchased from local market in Cairo, Egypt, and identified kindly by Prof. Ibrahim El-Garf, Faculty of Science, Cairo University.

Preparation of Medicinal Plant Extracts

Extraction was carried out according to Orhan and Aslan[14] and Rasheed et al[15]. The aerial part of *Salvia triloba* L., and seeds of *Piper nigrum* 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 Instruments GmbH & Co. KG, Schwabach, Germany).

Chronic Toxicity Study

Three dose levels of each plant extract (a highest-dose, which determined from the acute study and causing no death during the 2 weeks, an intermediate dose the half of the highest dose and a lowest-dose the half of the intermediate dose) were used in the chronic toxicity study as follows:

***Salvia triloba* (Sage):** The highest-dose level (3000 mg/kg b. wt./day), the intermediate-dose level (1500 mg/kg b. wt./day) and the lowest-dose level (750 mg/kg b. wt./day) caused no death cases for 3 months.

***Piper nigrum* (Black pepper):** The highest-dose level (750 mg/kg b. wt./day), the intermediate-dose level (375 mg/kg b. wt./day) and the lowest-dose level (187.5 mg/kg b. wt./day) caused no death cases for 3 months.

Induction of Alzheimer's Disease in Rates

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[16].

Experimental Design

The present study was conducted on 70 adult male Sprague Dawley rats weighing from 150 to 200 g 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 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 used were distributed into seven groups (10 rats each) as follows:

Group (1): Normal healthy animals served as untreated negative control group.
Group (2): AD-induced rats served as untreated positive control group.
Group (3): AD-induced rats treated daily for three months with Rivastigmine in a dose of 0.3 mg/kg b.wt.[17].
Group (4): AD-induced rats treated daily with *Salvia triloba* extract for three months, in a dose of 750 mg/kg b. wt. (the most save dose which was obtained in the chronic study).
Group (5): AD-induced rats treated daily with *Salvia triloba* extract for three months, in a dose of 375 mg/kg b. wt. (the half of most save dose which was obtained in the chronic study).
Group (6): AD-induced rats treated daily with *Piper nigrum* extract for three months, in a dose of 187.5 mg/kg b. wt. (the most save dose which was obtained in the chronic study).
Group (7): AD-induced rats treated daily with *Piper nigrum* extract for three months, in a dose of 93.75 mg/kg b. wt. (the half of most save dose which was obtained in the chronic study).

Brain Tissue Sampling and Preparation

At the end of the experimental period, (after three months), the animals were kept fasting for 12 hours. Blood samples were collected using the orbital sinus technique of Sandford[18]. Each blood sample was divided into two portions, the first small portion was taken on EDTA for superoxide dismutase assay, while the second large portion were 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 –20ºC for further biochemical analysis (malondialdehyde, nitric oxide, total antioxidant capacity).

After taking blood samples, 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. Then each brain was sagitally divided into two portions. The first portion 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[19]. The homogenate was centrifuged at 3000 rpm for 10 min at 4ºC. The supernatant (10%) was separated for biochemical analysis (acetylcholine and acetycholinesterase). The second portion of each brain was fixed in formaline buffer (10%) for histopathological investigation.

Biochemical Analyses

Brain acetylcholine (Ach) level was determined by the colorimetric method using choline/acetylcholine assay kit of Biovision incorported Co., Mountan View, CA, USA, according to the method of Oswald et al[20]. Also, brain acetycholinesterase (AchE) activity was done colorimetrically according to method of Den Blawen et al[21], using kits of Biostc Co. Italy. Moreover, brain total protein concentration was carried out to express the concentration of different brain parameters per mg protein, according to the method of Lowry et al[22], using kits of Biodiagnostic Co., Cairo, Egypt.

Malondialdehyde (MDA) and nitric oxide (NO) levels were estimated in serum by the colorimetric methods described by Ohkawa et al[23] and Berkels et al[24] respectively, using kits of Biodiagnostic Co., Cairo, Egypt. Superoxide dismutase (SOD) level was estimated in erythrocyte by the method of Nishikimi et al[25], while total antioxidant capacity (TAC) level was measured in serum by the method of Koracevic et al[26] using kits of Biodiagnostic Co., Cairo, Egypt.

Histopathological Examination

The second portion of each brain was fixed in formaline buffer (10%) for 24 hours. 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 56ºC in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains [27] for histopathological examination using the light microscope.

Statistical Analysis

In the present study, all results were expressed as Mean + SE 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 (SPSS Inc, Chicago, IL, USA) followed by least significant difference (LSD) to compare significance between groups[28]. Difference was considered significant when p value was > 0.05.

Results

Effects of Treatment of Total Plant Extracts on Brain Acetylcholine (Ach) and Acetylcholinesterase (AchE) Activities on AD-induced Rats

The results in Table I showed the effects of treatment with the selected medicinal plant *Salvia triloba,* and *Piper nigrum* total extracts on brain cholinergic markers as represented by Ach and AchE activities in AD-induced rats. In comparison with control group, aluminum chloride administration in adult male rats (AD-induced group) induced significant reduction in brain Ach level (–31.48%) and significant elevation in brain AchE activity (34.3%). Treatment of AD-induced rats with Rivastigmine produced significant increase in brain Ach level (29.9%) and significant decrease in brain AchE activity (–21.44%) comparing with AD-induced group.

Treatment of AD-induced rats with *S. triloba* extracts exhibited significant increase in brain Ach level (12.4% for 750 mg/kg b.wt., and 22.88% for 375 mg/kg b.wt.), as well as treatment with *P. nigrum* produced significant increase in brain Ach levels (26.12% for 187.5 mg/kg b.wt., and 9.91% for 93.75 mg/kg b.wt.), comparing with AD-induced group (Table I). On the other hand, brain AchE levels exhibited significant decrease after treatment with each *S. triloba* in a dose of 750 mg/kg b.wt. (–16.23%) and/or in a dose of 375 mg/kg b.wt. (–10.73%), Also significant decreases were recorded after treatment with each *P. nigrum* in a dose of 187.5 mg/kg b.wt. (–10.8%) and/or 93.75 mg/kg b.wt. (–5.16%), comparing with AD-induced group (Table I).

Effects of Treatment of Total Plant Extract on Oxidative Stress Status (MDA & NO) in AD-induced Rats

The results in Table II showed the effects of treatment with the selected medicinal plant *Salvia triloba,* and *Piper nigrum* total extracts on oxidative stress biomarkers as represented by malondialdheyde (MDA) and nitric oxide (NO) activities in serum of AD-induced rats. In com-

Table I. Effects of treatment of medicinal plant extracts on brain acetylcholine (Ach) and aetylcholnesterase (AchE) activities in AD-induced rats.

	Ach (nmol/mg protein)	AchE (U/mg protein)
1. Control group	$8.10 \times 10^{-2} \pm 0.14$	571.1 ± 26.85
2. AD group	$5.55 \times 10^{-2} \pm 0.14^{a}$ (-31.48%+)	767.0 ± 14.9^{a} (34.3%+)
3. AD group treated with Rivastigmine	$7.21 \times 10^{-2} \pm 0.12^{b}$ (29.9%)	602.5 ± 26.64^{b} (-21.44%)
4. AD group treated with Salvia (750 mg/kg b.wt)	$6.24 \times 10^{-2} \pm 0.20^{bc}$ (12.4%)	642.5 ± 20.0^{b} (-16.23%)
5. AD group treated with Salvia (375 mg/kg b.wt)	$6.82 \times 10^{-2} \pm 0.15^{b}$ (22.88%)	684.7 ± 8.9^{bc} (-10.73%)
6. AD group treated with Piper (187.5 mg/kg b.wt)	$7.0 \times 10^{-2} \pm 0.31^{b}$ (26.12%)	684.1 ± 17.26^{bc} (-10.8%)
7. AD group treated with Piper (93.75 mg/kg b.wt)	$6.1 \times 10^{-2} \pm 0.18^{bc}$ (9.91%)	727.4 ± 13.37^{c} (-5.16%)

[a]Significance change from control group at $p < 0.05$. [b]Significance change from AD group at $p < 0.05$. [c]Significance change from Rivastigmine group at $p < 0.05$. (%+): Percent of difference from control group. (%): Percent of difference from AD group.

Table II. Effects of treatment of medicinal plant extracts on oxidative stress status (MDA & NO) in AD-induced rats.

	MDA (nmol/mg protein)	NO (µ/mg protein)
1. Control group	6.2 ± 0.4	4.70 ± 0.07
2. AD group	10.23 ± 0.703[a] (65.0%+)	10.52 ± 0.30[a] (123.83%+)
3. AD group treated with Rivastigmine	8.28 ± 0.52[b] (-19.1%)	5.61 ± 0.091[b] (-46.67%)
4. AD group treated with Salvia (750 mg/kg b.wt)	8.22 ± 0.3[b] (-19.65%)	6.71 ± 0.166[bc] (-36.21%)
5. AD group treated with Salvia (375 mg/kg b.wt)	8.91 ± 0.49[b] (-12.9%)	7.16 ± 0.078[bc] (-21.94%)
6. AD group treated with Piper (187.5 mg/kg b.wt)	10.06 ± 0.30[c] (-1.66%)	7.03 ± 0.18[bc] (-33.17%)
7. AD group treated with Piper (93.75 mg/kg b.wt)	9.84 ± 0.22[c] (-3.81%)	7.11 ± 0.05[bc] (-32.41%)

[a]Significance change from control group at $p < 0.05$. [b]Significance change from AD group at $p < 0.05$. [c]Significance change from Rivastigmine group at $p < 0.05$. (%+): Percent of difference from control group. (%): Percent of difference from AD group.

parison with control group, aluminum chloride administration in adult male rats (AD-induced group) induced significant elevation in each of serum MDA (65.0%) and NO levels (123.83%) as compared with control group. In contrast the treatment of AD-induced rats with rivastigmine produced significant decrease in serum MDA level (–19.1%) and significant decrease in serum NO level (–46.67%), comparing with AD-induced group.

Treatment of AD-induced rats with *S. triloba* extracts exhibited significant decrease in serum MDA level (–19.65% for 750 mg/kg b.wt., and –12.9% for 375 mg/kg b.wt.), while treatment with *P. nigrum* exhibited non-significant decrease in serum MDA levels (–1.66% for 187.5 mg/kg b.wt., and –3.81% for 93.75 mg/kg b.wt.), comparing with AD-induced group. Serum NO levels exhibited significant decreases after treatment with each *S. triloba* in a dose of 750 mg /kg b.wt. (-36.21%) and/or in a dose of 375 mg/kg b.wt. (–21.94%), Also significant decreases were recorded after treatment with each *P. nigrum* in a dose of 187.5 mg/kg b.wt. (–33.17%) and/or 93.75 mg/kg b.wt. (–32.41%), comparing with AD-induced group (Table II).

Effects of Treatment of Total Plant Extracts on Antioxidant Status (TAC & SOD) in AD-induced rats

Table III revealed the effects of treatment with the selected medicinal plant total extracts on antioxidant status as represented by measurement of serum total antioxidant capacity (TAC) and superoxide dismutase activities (SOD) in AD-induced rats. In comparison with control group, aluminum administration in adult male rats (AD-induced group) induced significant depletion in serum TAC (–32.66%) and SOD activity (–31.78%). In contrast the treatment of AD-induced group rats with rivastigmine produced significant increase in brain TAC (35.41%) and significant increase in serum SOD activities (38.22%), comparing with AD-induced group.

Treatment of AD-induced rats with *S. triloba* extracts exhibited significant increase in serum TAC (33.39% for 750 mg/kg b.wt., and 26.67% for 375 mg/kg b.wt.), as well as treatment with *P. ni-*

Table III. Effects of treatment of medicinal plant extracts on antioxidant status (TAC & SOD) in AD-induced rats.

	TAC (mmol/mg protein)	SOD (U/mg protein)
1. Control group	18.37 ± 0.65	2.80 ± 0.064
2. AD group	12.37 ± 0.94[a] (-32.66%+)	1.91 ± 0.0313[a] (-31.78%+)
3. AD group treated with Rivastigmine	16.75 ± 0.59[b] (35.41%)	2.64 ± 0.10[b] (38.22%)
4. AD group treated with Salvia (750 mg/kg b.wt)	16.50 ± 0.73[b] (33.39%)	2.24 ± 0.103[bc] (17.27%)
5. AD group treated with Salvia (375 mg/kg b.wt)	15.67 ± 0.72[b] (26.67%)	1.99 ± 0.047[c] (4.20%)
6. AD group treated with Piper (187.5 mg/kg b.wt)	16.20 ± 0.72[b] (30.96%)	2.20 ± 0.06[bc] (15.18%)
7. AD group treated with Piper (93.75 mg/kg b.wt)	16.69 ± 0.75[b] (34.92%)	2.08 ± 0.029[bc] (8.90%)

[a]Significance change from control group at $p < 0.05$. [b]Significance change from AD group at $p < 0.05$. [c]Significance change from Rivastigmine group at $p < 0.05$. (%+): Percent of difference from control group. (%): Percent of difference from AD group.

grum produced significant increase in serum TAC (30.96% for 187.5 mg/kg b.wt., and 34.92% for 93.75 mg/kg b.wt.), comparison with AD-induced group. On the other hand, serum SOD levels exhibited significant increase after treatment with each *S. triloba* in a dose of 750 mg /kg b.wt. (17.27%) and/or in a dose of 375 mg/kg b.wt. (4.20%), Also significant increases were recorded after treatment with each *P. nigrum* in a dose of 187.5 mg/kg b.wt. (15.18%) and/or 93.75 mg/kg b.wt. (8.90%), comparing with AD-induced group (Table III).

Histopathological Investigation of Brain Section in Different Studied Groups

Microscopic examination of brain section of control rat showed normal morphological structure of the hippocampus (Figure 1A). On the other hand, microscopic investigation for brain section of aluminium intoxicated rat (AD-induced group) demonstrated various sizes of amyloid plaques in the hippocampus (Figure 1B).

Histological investigation of brain section of AD-induced rats treated with rivastigmine and/or *Salvia triloba* in a dose of 750 mg /kg b.w revealed more or less normal histological structure of the hippocampus and all amyloid plaques that are formed under the influence of $AlCl_3$ administration disappeared (Figures 2A and B), as well as the treatment with *Piper nigrum* in a dose of 187.5 mg/kg b.wt. showed normal histological structure of the hippocampus, but with few disturbance in hippocampus cells arrangement (Figure 2C).

Moreover, the treatment with *Salvia triloba* in a dose of 375 mg /kg b.wt. or *P. nigram* in a dose with 93.75 mg/kg b.wt. showing normal histological structure of hippocampus with dislocation of some hippocampus cells in AD-induced rats treatment with *Salvia triloba*, and with few disturbance in hippocampus cells arrangement associated with dislocation of some cells in AD-induced rats treatment with *P. nigrum* (Figures 3A and B).

Disscussion

The number of patients suffering from Alzheimer's disease (AD) all over the world is rising continually and becomes one of the biggest challenges for most societies throughout the world[29]. According to the free radical theory, aging can be considered as a progressive, inevitable process partially related to the accumulation of oxidative damage into biomolecules (nucleic acids, lipids, proteins or carbohydrates) due to an imbalance between pro-oxidants and antioxidants in favor of the former[30]. A large body of evidence implicates oxidative damage in AD pathogenesis[31]. It is believed that oxidative damage to critical molecules occurs early in the pathogenesis of AD and precedes pronounced neuropathological alterations[32].

Aluminum has been implicated as most important risk factor in aging related changes[34] and particularly in neurodegenerative disease[33]. In the present study the aluminum intoxicated rats (AD group) showed significant reduction in Ach, while significant elevation in AchE activities were reported in brain of rats, as well as the microscopic investigation for brain section revealed

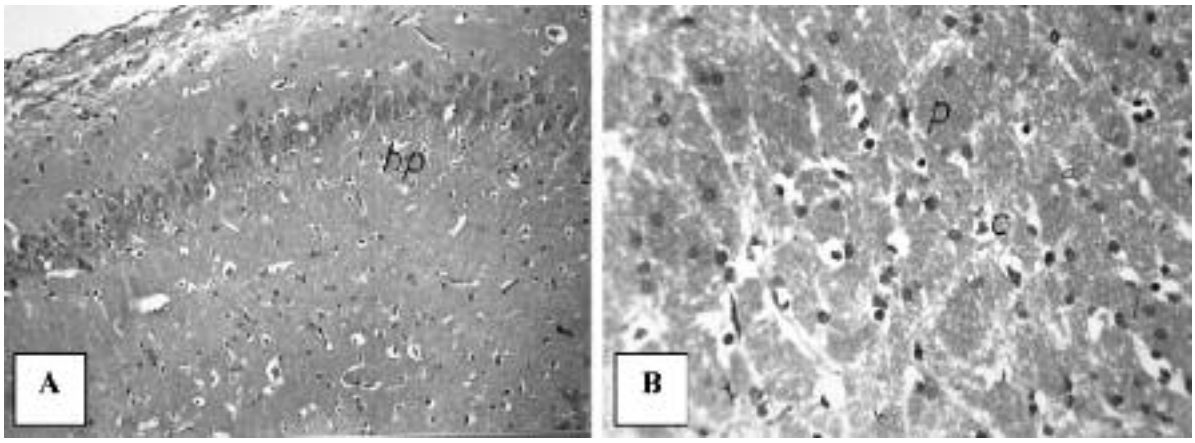

Figure 1. *A*, Micrograph of brain section of control rat (group 1) showing normal histological structure of the hippocampus (hp). *B*, Micrograph of brain section of AD-induced rats (group 2) showing encephalomelacia (*c*) with plaques formation (p) in hippocampus (H&E*64).

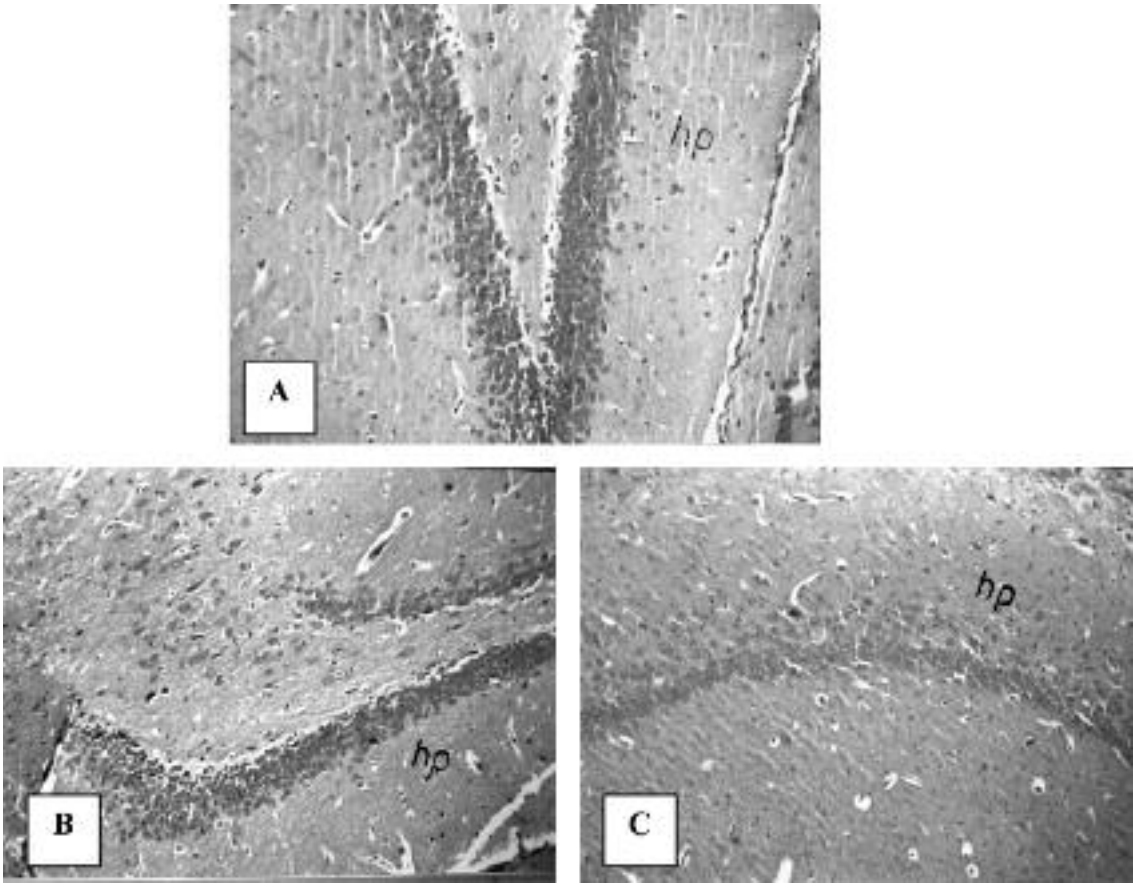

Figure 2. *A*, Micrograph of brain section of AD-induced rats treated with rivastigmine (group 3) showing normal morphological structure of the hippocampus (hp). *B*, Micrograph of brain section of AD-induced rats treated with Salvia triloba (750 mg/kg b. wt.) plant extract (group 4) showing normal morphological structure of the hippocampus. C) Micrograph of brain section of AD-induced rats treated with Piper nigrum (187.5 mg/kg b. wt.) plant extract (group 5) showing normal morphological structure of the hippocampus with few disturbance in hippocampus cells arrangement (H&E*40).

the presence of amyloid plaques in the hippocampus of rats. The mechanism of aluminum induced neurodegeneration is not clearly known. However, it has been reported that aluminum potentiates the activity of ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions to cause oxidative damage leading to neurodegeneration[35]. Moreover, aluminum promotes the formation of amyloid-β plaque[32] and aggregation of tau protein in Alzheimer disease[36].

The present study showed that oxidative stress was found in Alzheimer group of rats which indicated by statistically significant elevation in the mean serum levels of MDA and NO and significant decrease in the activities of SOD and TAC. These results are coincided with Gustaw-Rothenberg et al[37]. They stated that in both AD and vascular dementia (VaD) groups, the level of oxidative stress parameter (MDA) was higher compared with controls. Other studies support our findings and describe an increased level of the peripheral MDA in AD[32,38,39].

Also, Dickstein et al[40] stated that growing evidence appears to implicate oxidative stress as the common factor rendering the brain vulnerable to environmental insults, and it has been shown to play an important role in the pathogenesis of AD. They reported that accumulated oxidative stress affects nitric oxide (NO) function to relax endothelial vasculature, increases vascular endothelial permeability, and further reduces CBF. These are thought to occur because of the reduced bioavailability of NO and the increase in free radicals. At the same time, contradictory reports regarding the peripheral level of MDA in AD were reported by Galbusera et al[41]. These inconsistencies could be due to the fact that thiobarbituric acid-reactive substances (TBARS) are nonspecific

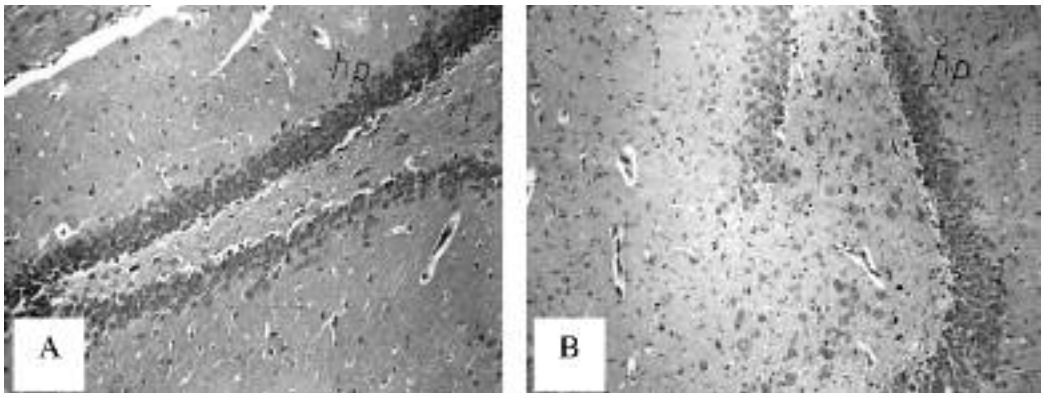

Figure 3. *A*, Micrograph of brain section of AD-induced rats and treated with S. triloba (375 mg/kg b. wt), showing normal intact histological structure of hippocampus (hp). Notice the dislocation of some hippocampus cells (arrow) (H&E*40). *B*, Micrograph of brain section of AD-induced rats treated with P. nigrum (93.75 mg/kg b. wt.) showing intact histological structure of hippocampus (hp). Notice with few disturbance in hippocampus cells arrangement (arrowhead) associated with dislocation of some cells (arrow) (H&E*40).

markers of membrane lipid peroxidation that can lead sometimes to artifactual values[42].

The present findings could support the hypothesis that decreased SOD and TAC activity could lead to an accumulation of H_2O_2. This could increase the stimulation of lipid peroxidation and protein oxidation, resulting in cellular damage[42]. Increased production of oxygen and nitrogen reactive species in mild cognitive impairment (MCI) and AD leads to a rapid consumption of plasma antioxidants. So, the antioxidant systems failed to protect the organism against the oxidative damage with subsequent development of the pathological alterations that characterize the neurodegenerative disorder[32,43].

Acetylcholine esterase inhibitors are the only agents approved by the Food and Drug Administration (FDA) for the treatment of AD. All other agents prescribed for the treatment of AD are used on an off-label basis. Current research into new drugs is focused on agents that will prevent, slow down and/or halt the progress of the disease process. Hence, the potential for developing medicinal herb-derived and food plant-derived prophylactic agents directed at age related disorders especially neurological and psychiatric disorders including memory dysfunction has increased the importance. In the present investigation we tried to use two different plants *Salvia triloba* extract and *Piper nigrum* extract as well as rivastigmine (as a reference drug) to know their effect on the status of oxidant/antioxidant system in cases of AD.

Rivastigmine was used as standardized drug where it is the only proven pharmacological therapy for the symptomatic treatment of AD[44]. Treatment of AD group of rats with rivastigmine exhibited an improvement in oxidative stress status as represented by a significant increase in brain Ach level and significant decrease in brain AchE activities than AD-induced rats. Moreover, rivastigmine produced significant decrease in serum MDA and NO levels, while significant increases in serum TAC and erythrocyte SOD levels were reported in comparison with AD group. These results were parallel with the histopathological finding in brain where, the amyloid plaques that are formed under the influence of $AlCl_3$ administration has been disappeared in comparison with AD group.

The efficacy of rivastigmine in the treatment of dementia has also been studied in patients with moderate to severe AD living in long-term care facilities. Rivastigmine treatment improves cognition, activities of daily living, and global function[45]. Rivastigmine binds to the AChE molecule in a pseudo-irreversible fashion; the acetyl moiety of AChE is dissociated rapidly, but the carbamyl moiety remains for some time longer. Rivastigmine is metabolized by the synapse rather than by hepatic cytochrome enzymes[46]. Andin's study[47], provides the first evidence that the glutamatergic system is modulated following AChE inhibition by rivastigmine; a finding which is likely to be of importance for the clinical effects.

Rivastigmine might act through the glutameric mechanism, decrease the oxidative stress and restore the antioxidant defense[48,49]. Besides selective acetylcholinesterase inhibitors also protect against

the Aβ-induced oxidative stress[50]. Rivastigmine protects behavioral changes, restores antioxidant defense enzyme in brain and improves mitochondrial enzyme level induced neurotocixity[51].

Salvia triloba and *Piper nigrum* plants are commonly used worldwide in household. In addition, they are used as important ingredients for various medicinal purposes in traditional medicine; both plants have been recommended by old Arabic literature for treatment of AD. The main aim of the present work is to assess the efficacy of *Salvia triloba* and *Piper nigrum* plant extracts on oxidative stress status in AD-induced in rats. The present study showed that treatment of AD-induced rats with *Salvia triloba, and Piper nigrum,* total extracts produced significant increase in brain Ach and significant decrease in brain AchE levels. Moreover, significant decreases in levels of oxidant markers (MDA and NO) and significant increases in levels of antioxidants (SOD and TAC) were reported in comparison with AD group. However, the the high dose 750 mg/kg b. wt. for *Salvia triloba* and 187.5 mg/kg b. wt. for *Piper nigrum* is more powerful than the low treatment with low dose for each plant, as well as treatment with *Salvia triloba* is more interest than *Piper nigrum* in improvement the AD diseases in rats as evidenced by the biochemical markers.

Salvia triloba and *Piper nigrum* treatments inhibit acetyl cholinesterase, retarding the catabolism of acetylcholine and, therefore, resulting in increased synaptic availability of the neurotransmitter, have been shown to improve memory function in young and aged healthy human cohorts and are currently the only widely used treatment for Alzheimer's disease. Moreover, by suppressing the AchE enzyme, these extracts have been found to inhibit the breakdown of acetylcholine, a chemical messenger in the brain[51]. Some activities of *Salvia triloba*, particularly its reputation as being good for the memory, may be relevant to AD treatment was documented by Howes et al[53]. Moreover, other clinical studies have shown that *Salvia triloba* as well as the related plant Spanish sage, is effective in the treatment of mild to moderate AD[52,54]. However, despite these promising clinical observations, the precise mechanism for this herb remains not clearly understood.

Also, it is well known that aqueous extract of *Salvia triloba* (sage) has been shown to possess antioxidant activities due to presence of many phenolic compounds such as hydroxybenzoic acid derivatives, caffeic acid derivatives (e.g., rosmarinic acid), ferulic acid as well as flavonoid derivatives; luteolin and quercetin[10]. Other previous studies have shown that rosmarinic acid reduced iron-dependent anthracycline induced lipid peroxidation of rat cardiomyocytes[55], inhibited the hemolysis of rat erythrocytes induced by hydrogen peroxide[56], and attenuated ROS production induced by toxins in human hepatoma cell line[57]. On the other hand Iuvone et al[58] reported that rosmarinic acid could reduce Aβ42-induced ROS formation and lipid peroxidation, thus suggesting that this natural compound is a novel and effective neuroprotective agent against oxidative damage induced by Aβ. Because antioxidants are known to attenuate Aβ-induced oxidative injury[59], it is likely that the antioxidant properties of rosmarinic acid could contribute to its beneficial effect.

In the present study, the modulation occurred for the oxidant/antioxidant profile markers in AD rats treated by *S. triloba* support the antioxidant properties of the Egyptian plant *Salvia triloba* and hence its usefulness in the management of AD. Similar modulation in the oxidant/antioxidant profile markers could be easily noted for *Piper nigrum* group in our study. These results are in accordance with that of Singh et al[60] who concluded that *P. nigrum* could be considered as a potential source of natural antioxidant. Also our findings are coincided with Chonpathompikunlert et al[29] findings. Their data showed that piperine significantly improved memory impairment and neurodegeneration in hippocampus of rats. They stated that the possible underlying mechanisms might be partly associated with the decrease lipid peroxidation and acetylcholinesterase enzyme, in addition piperine supplementation in the diet reduces the risk of oxidative damage by augmenting antioxidant enzymes[12]. Our results correlate with Mittal and Gupta[61], who stated that piperine demonstrates direct antioxidant effects against various free radicals. They concluded that further researches about the precise underlying mechanism are still required.

Piper has been reported to have antioxidant activity may be due to piperine which has been reported that piperine protect against oxidative damage by inhibiting or quenching free radicals and ROS and by augmenting antioxidant enzymes[12]. Also, it has been evidenced that piperine lower lipid peroxidation and beneficially influence cellular thiol status, antioxidant molecules and antioxidant enzymes in a number of experimental situations of oxidative stress[13].

Treatment of AD-induced rats with rivastigmine, *Salvia triloba* plant extract or *Piper ni-*

grum plant extract revealed more or less normal structure of the hippocampus, all amyloid plaques that were formed under the effect of Al-Cl_3 administration are disappeared under the influence of these extracts that 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[10,12]. Moreover, the histopathological investigation revealed that treatment with *Salvia triloba* and/or *Piper nigrum* with 750 or 187.5 mg/kg. rat b. wt. respectively, are more powerful than the treatment with low dose for each plant extract, as well as *Salvia triloba* is more effective in ameliorate the toxicological changes induced in AD group of rats, and these findings are parallel with the biochemical results obtained in the present report.

In conclusion, this investigation revealed that the treatment of AD-induced rats with rivastigmine (reference drug), *Salvia triloba* and *Piper nigrum,* total extracts significantly reduced the oxidative stress status and ameliorates the neurodegeneration characteristic of Alzheimer's diseases in rats. Moreover, rivastigmine followed by *Salvia triloba* are more powerful than *Piper nigrum*, as well as the high dose of *Salvia triloba* (750 mg.kg b. wt.), showed more interest in improvement Alzheimer's disease as indicated by both biochemical and histopathological investigations. The effect of *Salvia triloba* and *Piper nigrum* were achieved through the powerful anticholinesterase activity and antioxidant capacity of these plants. These results represented good therapeutic approaches for intervention against progressive neurological damage associated with Alzheimer's disease with special reference to the oxidative insults.

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