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EVALUATION OF THE POSSIBLE ANTIOXIDANT EFFECTS OF *PEGANUM HARMALA* AND *GINKGO BILOBA* IN AMELIORATING ALZHEIMER'S DISEASE IN RAT MODEL

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ABSTRACT: Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder leading to dementia in the elderly inhabitants. Increasing evidence advises that oxidative stress that is normally associated with aging is an obvious and early feature of AD and plays a role in its pathogenesis. The present study was designed to evaluate the possible neuroprotective activity of *Ginkgo biloba* (Gb) and / or *Peganum harmala* (Ph) extract against AD in a rat model. AD was induced by chronic administration intraperitoneal injection of scopolamine (0.7 mg kg^{-1}, i.p.) to rats for a period of 7 days. The Gb (120 mg/kg b.wt.) and Ph (187.5 mg/kg b.wt.) were administrated to AD rat group orally daily for a period of 30 days. The results revealed that the levels of thiobarbituric acid reactive substances and Xanthine oxidase were significantly increased, even though the activities of superoxide dismutase and catalase, as well as the reduced glutathione, were significantly decreased in the brain homogenate of Alzheimer group. Additionally, brain acetylcholinesterase as well as alkaline phosphatase, acid phosphatase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activities were significantly increased. On the other hand, the administration of Gb, Ph and co-administration of those test herbs acquired potential therapeutic effects on improving the neurodegenerative disorder in rats through suppressing lipid peroxidation, augmenting endogenous antioxidant enzymes, and reducing acetylcholinesterase activity in the brain. It might be concluded that a mixture extract, by its antioxidant constituents, could modulate scopolamine-induced oxidative stress and enzyme activities in the brain.

INTRODUCTION: Alzheimer's disease is a pathological brain disease and the most frequent cause of dementia [1, 2]. Patients with AD suffer a gradual deterioration of memory and other cognitive functions, which eventually causes a complete incapacity and fatal within 3 to 9 years after diagnosis [3].

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Aging is the significant hazard factor of AD in the general population, it influences considerably on the everyday activities of older adults, being one of the key factors behind disability in older age. AD affects one person in eight over sixty-five and almost half over eighty five years of age, it is the 5th leading cause of death of the elder people [4]. There was clearly a predicted 46.8 million worldwide people developed dementia, and the incidence is expected to rise in the coming years, with 74.7 million cases approximated to occur in 2030 and 131.5 million in 2050 [5]. The etiology of AD is multifactorial, including genetic, environmental, and lifestyle.

Several other factors such as absence of acetyl-choline, the development of atherosclerotic lesions and inflammation of the brain - also play an important role in the progression of AD [6]. Albeit AD is most likely connected with various etiologies and pathophysiologic mechanisms, oxidative stress looks as a noteworthy area of the pathophysiologic process [7].

Oxidative stress (OS), a process increased in the brain with aging, is induced by an imbalance in the redox state, including the generation of abnormal reactive oxygen species (ROS) or the dysfunction of the antioxidant system [8]. These ROS assume a part in neurodegenerative diseases [9 - 12].

There has been an expanding enthusiasm for as far back as decades about intercessions that may enhance cognitive performance in older age or, in any event, defer the beginning of dementia. Because of the nonattendance of a cure against dementia and AD, the public health concern has concentrated more lately on banning of cognitive decline [13]. Lately, the role of food and nutrition in preventing or delaying chronic impairment in the elderly population has brought a great attention, because of their ability to influence biochemical and biological processes, bioactive nutrients are considered flexible factors capable of conserving a healthy brain status [14].

Ginkgo biloba (Gb) has long been considered to have therapeutic properties, and its extracts are presently the most investigated and an adopted herbal treatment for cognitive disorders and AD [15], and it is summarized as an antidementia medication in the Anatomical Treatment Chemical Classi-fication system. In addition, *in vitro* and *in vivo* studies have reported that Gb plays a key role in the maturation and development of neuro-fibrillary tangles [16]. From medical trials with Gb, it is often estimated that Gb delays progression of symptoms in Alzheimer's dementia by about 6 - 9 months [17]. Extract of Gb contains flavonols quercetin, kaempferol as well as terpenes that's giving this extract its unique polyvalent pharmacological action for use as a neuroprotective function [18]. It is well known due to the anti-inflammatory, anti-apoptotic and antioxidant properties which contribute its ability to scavenge free radicals [19].

Earlier investigation reported the essential prophylactic role of Gb in the prevention of several diseases associated with oxidative tissue damage [20, 21].

Peganum harmala (Ph) belongs to the family of Zygophyllaceae [22, 23]. An assortment of pharmacological and biological activities of Ph inclusive of antibacterial, antifungal and mono-amine oxidase (MAO) inhibition has been mentioned [24, 25]. This herb is wealthy in alkaloids; β- carbolines including harmalol, harmaline, norharmane, harmol, harmine and harmane [26]. The harmine alkaloids exhibit in *Peganum harmala* have been accounted for to sufficiently supportive to utilize in the management of neurodegenerative disorders of the type Alzheimer's diseases [27] through its acetylcholinesterase (AChE) inhibitory activity [28]. The aqueous extract of *Peganum harmala* should save your signs and symptoms and reduced oxidative stress markers in rats with Parkinson's disease caused by 6-hydroxydopamine [29].

The current study was planned to ascertain the possible neuroprotective activity of *Pegenum harmala* and *Ginkgo biloba* extract administered individually or in combination, in safeguarding the brain against the Alzheimer's disease in a rat model.

MATERIALS AND METHODS:

Experimental Animals: Male Wistar rats obtained from the Animal house of King Fahad clinical research Center, Jeddah, Kingdom of Saudi Arabia, weighing 220 - 250 g, have been used. The animals preserved under preferred situations of temperature and humidness and a 12-h mild dark cycle with access to food and water for one-week *ad libitum* prior to experimentation. Animals were cared for in accordance with the principles of the "Guide to the Care and Use of Experimental Animals" (Committee on the Care and Use of Laboratory Animals 1985) and by King Fahd Research Center, King Abdul-Aziz University.

Induction of Experimental Alzheimer's Disease: Scopolamine hydrochloride was purchased from Sigma -Aldrich, St, Louis, MO, USA. Scopolamine was solved with a 0.9% saline solution and being injected in a volume of (0.7 mg / kg^{-1}, i.p.) of body weight to rats for a period of 7 days.

Plant Material:

***Ginkgo biloba*:** *Ginkgo biloba* capsules were used in this study, each capsule containing (260) mg of ginkgo leaves manufactured by EIMC United Pharmaceuticals in Egypt with M.O.H Reg. no. 589/2012. Each capsule was solved in 3 ml of distilled water. The drug was given daily (120 mg/kg body weight) by oral gavage syringe for 30 days.

Peganum harmala: Dry plant seeds of *Peganum harmala* were obtained from the herbal market in Cairo, Egypt. The crude extract was obtained according to the method Al-Izzy [30].

Preparation of Extract: Seeds of Ph were purified washed and dried under fresh air, then ground in an electrical grinder to get fine powder of the seeds. Then, 100 gm of the ground seeds was infused in 500 ml of distilled water for 24 hours at room temperature. Agitation of the infusion by using a magnetic stirrer had been done alternatively. Then the infusion was filtered by filter paper (Wattman no.1) and the deposit was discarded. The extract was administrated to rats at doses (187.5 mg/kg b.wt.) daily by oral gavage syringe for 30 days.

The Experimental Design: 50 male Wistar rats were randomly assigned to five groups of ten animals each as follows:

Group1: (C) Normal, healthy rats served as an untreated negative control group.

Group 2: (AD) rats received Scopolamine hydrochloride (0.7 mg kg^{-1}, i.p.) for 7 days to induce AD and served as an untreated positive control group [31].

Group 3: (AD + Gb) rats received Scopolamine (0.7 mg kg^{-1}, i.p.) and treated orally with *Ginkgo biloba* (120 mg/kg b.wt.) daily for 30 days [17].

Group 4: (AD + Ph) rats received Scopolamine (0.7 mg kg^{-1}, i.p.) and treated orally with *Peganum harmala* (187.5 mg/kg b.wt.) daily for 30 days [25].

Group 5: (AD + Ph + Gb) rats received Scopolamine (0.7 mg kg^{-1}, i.p.) and treated orally with mixture of *Peganum harmala* (187.5 mg/kg b.wt.) and *Ginkgo biloba* (120 mg/kg b.wt.) daily for 30 days.

Methods:

Measurement of Body Weight (Biological Evolution): The rats in all groups were monitoring the change in body weight by measuring their weight before the start of treatment and every week during the experimental period.

Brain Tissue Sampling and Preparation: At the end of the experimental duration, the rats were sacrificed by using decapitation after an overnight fast of 12 hours. The whole brain of each rat was rapidly removed, washed with ice-cold saline, blotted dry and weighed. Then, the brain become perfused with a PBS (phosphate buffered saline) solution, pH 7.4. Containing 0.16 mg/ml heparin to remove any red blood cells and clots. Then homogenize the tissue in 5 - 10 ml cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.4. 1 mM EDTA and 1 mL/L Triton X-100) per gram tissue. The homogenate was centrifuged at 4,000 rpm for 15 minutes at 4 °C and the resultant supernatant was used for different determinations.

Biochemical Analysis: Brain acetyl cholinesterase (AChE), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), acid phosphatase (AP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities as well as, lipid peroxidation assay (TBARS), Xanthine oxidase (XO), reduced glutathione estimation (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated according to the protocol mentioned in the assay kits supplied by the biodiagnostic Chemical Company (Egypt).

Statistical Evaluation: All values had been expressed as mean ± standard deviation ($\bar{X} \pm SD$) obtained from the experiments (n = 10). Statistical analysis became performed with one-way analysis of variance (ANOVA) test and independent pattern t-test using the Mega Stat Excel (version 10.3, Butler University).

RESULTS: As shown in **Table 1**, the body weights (BWTs) and brain weight of the rats in different treatment groups were matched to the mean value within the normal group. It was observed a significant decrease in the final body weight of the Alzheimer group compared with normal group. However, no significant decreased had been found in brain weights as compared with

a normal group. Treating of AD group with either Gb and /or Ph exhibits significant improvement of the body weight in comparison with untreated AD group.

TABLE 1: INITIAL, FINAL BODY WEIGHT (g) AND BRAIN WEIGHT, IN CONTROL AND AD RATS TREATED WITH *GINKGO BILOBA*, *PEGANUM HARMALA* OR THEIR MIXTURE AFTER 4 WEEKS OF TREATMENT

Experimental groups	Initial body weight (g)	Final body weight (g)	Brain weight (g)
Normal	235.000 ± 7.517	277.600 ± 26.264	1.800 ± 0.065
AD	237.400 ± 6.148	249.200 ± 22.643**	1.620 ± 0.369
AD + Gb	239.000 ± 5.000	267.500 ± 17.111	1.763 ± 0.092
AD + Ph	239.600 ± 3.507	262.200 ± 24.576	1.843 ± 0.092
AD + Gb-Ph	240.600 ± 4.159	274.400 ± 23.776	1.836 ± 0.098

Each value represents the mean of 10 rats ± SD. There is significantly different from control values at 0.01^{**}

Regarding antioxidants, the data indicated that, AD induced a significant reduction in brain SOD and CAT activities as well as GSH content in comparison to a control group **Table 2**. However, treated AD group with the aqueous extract of Gb or and Ph for 30 days relieved the effects of scopolamine and resulted in a significant increase in the antioxidant activity of these enzymes when compared to AD untreated rats.

TABLE 2: THE EFFECT OF THE AQUEOUS EXTRACT OF GB OR / AND PH ON BRAIN SOD, CAT ENZYME ACTIVITY AND GSH LEVELS IN SCOPOLAMINE INDUCED AD RATS

Groups	GSH (M mol/g)	CAT (µ/g)	SOD (µ/gm)
Normal	0.553 ± 0.088	0.404 ± 0.046	330.688 ± 80.646
AD	0.380 ± 0.080 ***	0.168 ± 0.046 ***	148.241 ± 24.370 ***
AD + Ph	0.483 ± 0.071	0.300 ± 0.050 $^{*\,\#\#}$	297.780 ± 72.380 $^{\#\#\#}$
AD + Gb	0.446 ± 0.034 $^{*\,\#}$	0.320 ± 0.151$^{\#\#}$	284.891 ± 83.966 $^{\#\#}$
AD + Ph-Gb	0.505 ± 0.146 $^{\#}$	0.371 ± 0.093 $^{\#\#\#}$	301.185 ± 84.354 $^{\#\#\#}$

Data were expressed as $\bar{X}$ ± SD. There is significantly different from control values at P <0.05*, 0.01**, 0.001***. There is significantly different from the AD value at P <0.05$^{\#,}$ 0.01$^{\#\#,}$ 0.001$^{\#\#\#}$

The data presented in **Table 3**, showed that the XO and MDA levels were significantly increased in (P-value = 0.000) in AD group when compared to the normal group. Treatment of AD group with Gb or /and Ph ameliorated the levels of XO and MDA by reducing them significantly relative to the untreated AD group.

TABLE 3: THE EFFECT OF THE AQUEOUS EXTRACT OF GB OR / AND PH ON THE OXIDATIVE STRESS IN THE BRAIN OF THE EXPERIMENTAL GROUPS

Groups	XO (µ/ml)	MDA (N mol/g)
Normal	25.172 ± 4.348	15.003 ± 1.728
AD	37.282 ± 7.457$^{***\wedge\wedge\wedge}$	24.167 ± 5.021 ***
AD+ Ph	29.672 ± 2.052 $^{\wedge\wedge\,\#\#}$	18.603 ± 4.773 $^{\#\#}$
AD +Gb	28.433 ± 4.002$^{\wedge\,\#\#\#}$	18.360 ± 1.075$^{\#\#}$
AD+ Ph-Gb	23.785 ± 2.241 $^{\#\#\#}$	17.755 ± 4.544 $^{\#\#\#}$

Data were expressed as $\bar{X}$ ± SD. There is significantly different from control values at P <0.05*, 0.01**, 0.001***, There is significantly different from the AD value at P <0.05$^{\#}$, 0.01$^{\#\#}$, 0.001$^{\#\#\#}$, There is a significant difference from AD + Gb-Ph value at P<0.05$^{\wedge}$, 0.01$^{\wedge\wedge}$, 0.001$^{\wedge\wedge\wedge}$

It is clear from the results that injection of rats with scopolamine caused a significant elevation (P-value = 0.000.0.000, 0.002) in the activity of AChE, LDH and AP respectively compared to that of the control group. The level of these enzymes was significantly suppressed by the administration of rats with Gb or /and Ph compared to the untreated AD group **Table 4**. As regards to AST and ALT enzyme, the data demonstrated in **Table 5** showed there were highly significant increases (P - value = 0.000) in their activities in AD group when compared to the normal group. In other hand, the activity of ALP enzyme in AD group was slightly significantly elevated relative to the normal group. The results of the current study showed a significant attenuation of the Gb or / and Ph, effects on AST and ALT activity in the AD groups by decreasing it significantly when compared to the untreated AD group. Whilst, AD groups treated with either Gb nor Ph showed non-significant change in ALP activity when compared to the untreated AD group.

TABLE 4: THE EFFECT OF AQUEOUS EXTRACT OF GB OR / AND PH ON BRAIN ACETYLCHOLINESTERASE, LACTATE DEHYDROGENASE AND ACID PHOSPHATASE ACTIVITIES IN SCOPOLAMINE-INDUCED AD RATS

Groups	AChE µ/mg	LDH µ/L	AP µ/L
Normal	411.904 ± 48.831	13.395 ± 2.411	6.163 ± 1.284
AD	539.901 ± 45.411***	40.532 ± 9.524 ***	10.416 ± 2.496 **
AD + Ph	473.990 ± 52.407$^{*\#}$	24.922 ± 7.050 $^{** \#\#\#}$	7.298 ± 2.180 $^{\#}$
AD + Gb	463.056 ± 46.840 $^{\#\#}$	23.058 ± 5.904 $^{** \#\#\#}$	7.210 ± 3.524 $^{\#}$
AD + Ph-Gb	447.331 ± 60.483$^{\#\#}$	19.910 ± 4.986 $^{* \#\#\#}$	7.139 ± 1.891 $^{\#}$

Data were expressed as $\bar{X}$ ± SD. There is significantly different from control values at P <0.05*, 0.01**, 0.001***, There is significantly different from the AD value at P <0.05$^{\#}$, 0.01$^{\#\#}$, 0.001$^{\#\#\#}$

TABLE 5: THE EFFECT OF THE AQUEOUS EXTRACT OF GB OR / AND pH ON THE BRAIN ENZYME ACTIVITIES IN SCOPOLAMINE-INDUCED AD RATS

Groups	AST (µ/L)	ALT (µ/L)	ALP (µ/L)
Normal	24.589 ± 4.341	12.101 ± 1.653	343.575 ± 66.741
AD	39.053 ± 7.454 ***	25.815 ± 4.966***	560.459 ± 254.092*
AD + Ph	22.322 ± 2.232 $^{\#\#\#}$	16.947 ± 2.076$^{**\#\#\#}$	436.262 ± 67.228
AD + Gb	25.124 ± 3.265 $^{\#\#\#}$	17.672 ± 2.260$^{**\#\#\#}$	408.561 ± 150.492
AD + Ph-Gb	24.124 ± 1.863 $^{\#\#\#}$	14.778 ± 2.700 $^{\#\#\#}$	396. 614 ± 160.867

Data were expressed as $\bar{X}$ ± SD. There is significantly different from control values at P <0.05*, 0.01**, 0.001***, There is significantly different from the AD value at P <0.05$^{\#}$, 0.01$^{\#\#}$, 0.001$^{\#\#\#}$

DISCUSSION: There is growing evidence that AD became a major medical, economic, and social problem that is deteriorating as the increasing number of elderly people. The increasing in brain oxidative stress during brain aging could be reversed by antioxidants. Currently, available drug therapies for Alzheimer's disease and other diseases that because dementia consists primarily of acetylcholinesterase inhibitors and some neuroprotective agents [32]. These drugs cause side effects, thus an alternative bioactive compound from plants with little or no side effects should be used to replace these drugs. Therefore, the present research was undertaken to check out the effects of Gb and Ph, (both individual and combinational) in the scopolamine-treated rat model of Alzheimer.

The present results demonstrate that there was a decrease in SOD, CAT activities and GSH level with the concomitant increase in MDA and XO levels in the brain of AD group as compared to the normal group. This finding is in accordance with the results of [31, 33]. Various specialized clinical research has reported strong evidence that oxidative stress is included in the pathogenesis of Alzheimer's disease. The susceptibility of brain to oxidative stress other than many tissues is caused by the neuronal membranes that comprise a very excessive percentage of long chain polyunsaturated fatty acids. Consequently, lipids consider as a one of the targets of oxidative change by way of free radicals in neurodegenerative disorders [34]. The elevated oxidative stress in brains can be by inducing the activation of a cascade of redox-sensitive cell signal pathways [35]. This likely displays the increase in lipid peroxidation due to either an increase in the production of free oxidative radicals [36 - 38] or a decrease in the antioxidant defense mechanisms such as α-tocopherol [39] as a result directing the role of free radicals in the progression of Alzheimer's disease.

The current results demonstrated that XO activity was significantly increased in AD group when compared with the normal group. These results are in coincide with [40]. They increased in brain XO activity may be contributing to the increase in intracellular calcium, which activates proteases that catalyze conversion of xanthine dehydrogenase to xanthine oxidase, which in turn catabolizes purine bases to form O^{2-}. Therefore, diminished energy metabolism can increase intraneuronal calcium, which leads to excitotoxicity, and these converging mechanisms are able to be producing ROS. Each of these mechanisms are thought to occur in neurodegenerative diseases, mainly AD [41]. The depletion of brain GSH in Alzheimer rat model has been reported previously by [42, 44].

GSH is the most plenteous endogenous antioxidant in the brain, which found mainly in the reduced form within the cells. Therefore, it has been shown to react with free radicals and prohibit generation of hydroxyl free radicals [45, 47]. The decreased level of GSH in scopolamine- treated animals indicates

that there is an increased generation of free radicals and reduced activity of glutathione system in fighting oxidative stress [45].

Catalase performs a major role in detoxifying superoxide anions, which furthermore damages the cell walls and macromolecules [48]. The decreased in the catalase activity in scopolamine - induced AD group is accompaniments with other studies [31, 49, 50]. Marisco [51] and Ishola [52] deduced that Scopolamine has been found to increase oxidative stress and impaired the anti-oxidative defense system.

The decreased activity of SOD may be due to the toxic effect of Scopolamine by promoting oxidative stress in the brain and this may affect brain functioning and growth. Moreover, this decrease may be as a result of imbalances between oxidants and antioxidant level in support of the oxidants [49, 53]. This result is steady with the study of Otitoju [55].

The data obtained from the present study mentioned that the administration of a normal water extract of Gb and / or Ph to scopolamine stimulate AD rats caused a significant decrease in the level of TBARS, XO and elevated the SOD and CAT enzyme activities and GSH contents when compared with the untreated AD group.

The antioxidative effects of Ph were studied by Rezaei [29], who concluded that the aqueous extract of *Peganum harmala* could prevent signs and decreased oxidative stress markers in rats with Parkinson's disease brought on by 6-hydroxydopamine. Hamden [55] decided that, Ph contains effective constitutes which can also have the ability of scavenging free radicals and modulate the expression of genes encoding antioxidant enzymes inclusive of estrogens, growth factors and Vitamin E supplement. Moreover, the alkaloid extract of seeds of Ph has a prospect enough at the control of neurodegenerative disorders of the type Alzheimer's diseases [27]. Farzin and Mansouri [56] indicated that β- carbolines (harmane, norharmane and harmine) induce an antidepressant-like impact, while, harmaline and harmane are capable of decrease voltage-gated calcium channel currents at concentrations which can be can be to be sufficient for neuroprotective effects *in vivo*.

Concerning the possible ameliorative role of *Ginkgo biloba*, the present results revealed that the free radical scavenging and antioxidant activity of Gb is confirmed via restoration of XO and MDA levels along with a significant increase of CAT and SOD activities and GSH level in the brain as compared with the values recorded in normal rats. These results agree with other previous findings [57, 58].This probably due to biologically active ingredients which are rich in Gb [59, 60]. Further, Ginkgo leaves extract is identified to prevent the creation of Aβ from a β-amyloid precursor protein (APP), a critical method within the pathogenesis of Alzheimer's disease [61]. Alternatively, the Gb inhibits ROS accumulation induced by Aβ (especially flavonol quercitin) and also reduces neuron apoptosis, in which apoptosis is considered to be one of the major causes for neurodegenerative diseases. [62] suggested that the neuroprotective effects of Gb can be due to their antioxidant properties, antiapoptotic effects, and improvement of energy metabolism.

The present study confirmed that scopolamine caused AD produced a significant increase in AChE activity in contrast with the control group. This finding is in accordance to the results of [63 - 65]. The preserving of the acetylcholine level within the brain is important in the proper healthcare of AD patients. One particular method in that is blocking the activity of AChE, the enzyme-degrading acetylcholine [60].

On the other side, AChE activity in AD rats treated with Ph and Gb, (both individual and combinational) was inhibited. It absolutely was documented that Gb promote the ability of learning and memory by increasing Ach level and inhibiting AChE activity [67, 68]. Regard the Ph, previous reviews have noted that Ph indicates a potential therapeutic effect on AD, because of the cholinesterase inhibitory activities of harmine, harmaline, harmalol, harmol, and vasicine presented in plant [69 - 71]. Further, Singh [72] explained that the deoxypeganine from harmala seeds has activity similarly to reversibly behaving cholinesterase inhibitors. It suppresses acetyl-cholinesterase and monoamine oxidase, thereby protecting against the degradation of acetylcholine and dopamine, which is therefore beneficial in treating Alzheimer's dementia.

One of the major findings of the present study was the elevation of the activity of brain LDH in Alzheimer's group. These results are relative to the findings of [73 - 75]. LDH serves as an essential metabolic enzyme in brain cells and it is released into the bloodstream from injuries brain cells. Thus, LDH level in serum or brain cell is a reliable index to assess cerebral ischemic injury [76]. Elevated extracellular LDH is a biomarker of oxidative stress and disrupted cell sincerity throughout the lipid peroxidation and oxidative stress.

Tao [74] reported that increased level production of LDH cause Aβ accumulation is probably involved in the pathophysiology of AD. Administration of Gb and Ph to AD groups caused a pronounced reduction in the elevated activity of LDH as compared with Alzheimer's group. Much of those decrease could be due to the antioxidant characteristic in their constituents as flavonoids and terpenoids in Ginko and harming alkaloids from the seeds of the, which protect the cellular membrane probity from scopolamine-induced oxidative damage and restore the antioxidant system, therefore, enhance brain function.

As shown in a present study, scopolamine increased the brain AP activity in the AD group as compared with control rat group. These findings are in agreement with some studies reported by [77, 78]. Change in the activity of AP causes Lysosomal storage disorders [79] and neurodegenerative diseases [80]. Moreover, dysfunction lysosome system results in the formation of Tau insoluble aggregates in lysosomes of AD [81]. A significant reduction in brain AP activity was recorded in AD group treated with Gb and /or Ph in the present study as compared to the untreated AD group. The effect of Gb in reducing AP agrees with Tan [82]. In addition, Mohamed and El-Moneim [83], observed an improvement in AP activity in rats administrated of $AlCl_3$ after treatment with Gb.

In the present study, a significant increase in brain AST, ALT, and ALP activities were observed in the AD untreated group as compared to control rats. These results are in accordance with the findings of Sumathi [84] who noticed an increased in the activities of ALP, ACP, ALT and AST in all the brain parts of aluminium induced neurotoxicity group when compared with control rats. AST and ALT are important enzymes of brain; their activities are related to the maintenance of amino acid homeostasis and might be an indicator of mitochondrial injury [85]. Brain is enriched in amino compounds, it is possible that the promote transamination activity within the brain tissue served to neutralize the biochemical and biological responses that happen due to the amino compounds in response to the scopolamine effects [86]. Treated AD^+ group with the water extract of Gb and /or Phameliorate the enzyme activities which were brought back close to normal compared to the untreated AD^+ group.

This result indicates that Gb extract may play important roles in the improvement of the brain of the Alzheimer's disease through augmentation of bloodstream flow and inhibition of platelet activating factor, it may also protect the cell membrane against injury induced by free radicals and has protective effects against brain ischemia / reperfusion injury [87]. Moreover, the protective impact of Ph extract may be related to the richness of the plant in substances of phenolic characteristics which may decrease the free - radical lipid peroxidation level leading to the stabilizing of membrane structures [55].

CONCLUSION: In conclusion, the present study revealed that *Peganum harmala* and *Ginkgo biloba* extracts possibly have a protective role against AD by inhibiting oxidative stress, increase the antioxidant enzymes and improve the alteration of enzyme activities in the brain. Therefore, the receipt of these herbs may encourage older people to improve general health condition.

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REFERENCES:

1. Barker DJ, Eriksson JG, Forsén T and Osmond C: Fetal origins of adult disease: strength of effects and biological basis. International journal of epidemiology 2002; 31(6): 1235-1239.
2. Wilson WJ: A Text Book of the truly disadvantaged: The inner city, the underclass, and public policy. University of Chicago Press, Second Edition 2012.

3. Querfurth HW and La Ferla FM: "Alzheimer's disease," The New England Journal of Medicine 2010; 362(4): 329-344.
4. Biradar SM and Joshi H: The Influence of Ethanolic Extract of Seeds of *Peganum harmala* Linn. On Behavioral and Biochemical Studies in Cognitive Deficit Mice. International Journal of Pharmaceutical and Phytopharmacological Research 2017; 4(1): 25-33.
5. Alzheimer's disease International, The Global Impact of Dementia. An analysis of prevalence, incidence, costs and trends. Alzheimer's Disease International, London 2015
6. Zhou Y, Wang J and Zhang L: Basic theory of fractional differential equations. World Scientific, Second Education 1998.
7. Huang WJ, Zhang X and Chen WW: Role of oxidative stress in Alzheimer's disease (Review). Biomedical reports 2016; 4 (5): 519-522.
8. Andreyev AY, Kushnareva YE and Starkov AA: Mitochondrial metabolism of reactive oxygen species. Biochemistry (Moscow) 2005; 70(2): 200-214.
9. Skulachev VP, Anisimov VN, Antonenko YN, Bakeeva LE, Chernyak BV, Erichev VP, Filenko OF, Kalinina NI, Kapelko VI, Kolosova NG and Kopnin BP: An attempt to prevent senescence: a mitochondrial approach. Biochimica et Biophysica Acta (BBA)-Bioenergetics 2009; 1787(5): 437-461.
10. Jomova K, Vondrakova D, Lawson M and Valko M: Metals, oxidative stress and neurodegenerative disorders. Molecular and cellular biochemistry 2010; 345(1-2): 91-104.
11. Kim GH, Kim, JE, Rhie, SJ and Yoon S: The role of oxidative stress in neurodegenerative diseases. Experimental neurobiology 2015; 24 (4): 325-340.
12. Niedzielska E, Smaga I, Gawlik M, Moniczewski A, Stankowicz P, Pera J and Filip M: Oxidative stress in neurodegenerative diseases. Molecular neurobiology 2016; 53(6): 4094-4125.
13. Mecocci P, Tinarelli C, Schulz RJ and Polidori MC: Nutraceuticals in cognitive impairment and Alzheimer's disease. Frontiers in pharmacology 2014; 5.
14. Abate G, Marziano M, Rungratanawanich W, Memo M and Uberti D: Nutrition and Ageing: Focusing on Alzheimer's disease. Oxidative medicine and cellular longevity 2017.
15. Weinmann S, Roll S, Schwarzbach C, Vauth C and Willich SN: Effects of *Ginkgo biloba* in dementia: systematic review and meta-analysis. BMC Geriatrics 2010; 10(1): 14.
16. Yarza R, Vela S, Solas M and Ramirez MJ: c-Jun N-terminal kinase (JNK) signaling as a therapeutic target for Alzheimer's disease. Frontiers in pharmacology 2016; 6: 321.
17. Kasper S and Schubert H: "*Ginkgo biloba* extract EGb 761 in the treatment of dementia: evidence of efficacy and tolerability." 2009; 494-506.
18. El Tabaa MM, Sokkar SS, Ramadan ES, El Salam IZ, Zaid A: Neuroprotective role of *Ginkgo biloba* against cognitive deficits associated with Bisphenol A exposure: An animal model study. Neurochemistry International 2017.
19. Naik SR, Pilgaonkar VW and Panda VS: Neuro-pharmacological evaluation of *Ginkgo biloba* phytosomes in rodents. Phytotherapy research 2006; 20(10): 901-5.
20. Okuyan B, Izzettin FV, Bingöl-Ozakpinar Ö, Turan P, Ozdemir ZN, Sancar M, Cirakli Z, Clark PM and Ercan F: The effects of *Ginkgo biloba* on nephrotoxicity induced by cisplatin-based chemotherapy protocols in rats. IUFS Journal of Biology 2012; 71(2): 103.
21. Khafaga AF and Bayad AE: *Ginkgo biloba* Extract Attenuates Hematological Disorders, Oxidative Stress and Nephrotoxicity Induced by Single or Repeated Injection Cycles of Cisplatin in rats: Physiological and Pathological Studies. Asian Journal of Animal Sciences 2016; 10: 235-246.
22. Qazan WS: The effect of low levels of dietary *Peganum harmala* L. and *Ballota undulata* or their mixture on chicks. J Anim Vet Adv 2009; 8: 1535-8.
23. Kumar P, Sharma R, Ray S, Mehariya S, Patel SK, Lee JK and Kalia VC: Dark fermentative bioconversion of glycerol to hydrogen by *Bacillus thuringiensis*. Bioresource Technology 2015; 182: 383-388.
24. Nenaah G: Antibacterial and antifungal activities of (beta)-carboline alkaloids of *Peganum harmala* (L.) seeds and their combination effects. Fitoterapia 2010; 81(7): 779-782.
25. Salem AM, Sabry GM, Ahmed HH, Hussein AA and Kotob SE: Amelioration of neuroinflammation and apoptosis characterizing Alzheimer's disease by natural products. Int J Pharm Pharm Sci. 2013; 5(2S): 87-94.
26. Abdel-Fattah AF, Matsumoto K, Murakami Y, Gammaz HA and Mohamed MF and Watanabe H: Central serotonin level-dependent changes in body temperature following administration of tryptophan to pargyline-and harmaline-pretreated rats. General Pharmacology: The Vascular System 1997; 28(3): 405-9.
27. Biradar SM, Joshi H and Tarak KC: The Cerebroprotective effect of isolated harmine alkaloids extracted of seeds of *Peganum harmala* L. on sodium nitrite-induced hypoxia and ethanol-induced neurodegeneration in young mice. Pakistan Journal of Biological Sciences 2013; 16(23): 1687.
28. He D, Wu H, Wei Y, Liu W, Huang F, Shi H, Zhang B, Wu X and Wang C: Effects of harmine, an acetyl-cholinesterase inhibitor, on spatial learning and memory of APP/PS1 transgenic mice and scopolamine-induced memory impairment mice. European journal of pharmacology 2015; 768: 96-107.
29. Rezaei M, Nasri S, Roughani M, Niknami Z and Ziai SA: *Peganum harmala* L. Extract Reduces Oxidative Stress and Improves Symptoms in 6-Hydroxydopamine-Induced Parkinson's disease in Rats. Iranian journal of pharmaceutical research: IJPR 2016; 15(1): 275.
30. Al-Izzy MY: Antimicrobial effects of aqueous and alcoholic extract of *Peganum harmala* L. seeds on two types of salivary isolated microorganisms in Al-Ramadi city. Journal of King Abdulaziz University: Medical Sciences 2010; 17(4): 3-17.
31. Kaur R, Parveen S, Mehan S, Khanna D and Kalra S: The Neuroprotective effect of ellagic acid against chronically scopolamine induced Alzheimer's type memory and cognitive dysfunctions: possible behavioural and biochemical evidences. Int. J. Preven. Med. Res. 2015; 1: 45-64.
32. Olivares DK, Deshpande V, Shi YK, Lahiri DH, Greig NT, Rogers J and Huang X: N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. Current Alzheimer Research 2012; 9(6): 746-758.
33. Sridharamurthy NB, Ashok B and Yogan R: Evaluation of Antioxidant and Acetyl Cholinesterase inhibitory activity of *Peltophorum pterocarpum* in Scopolamine treated Rats. International journal of drug development and research 2012; 4(3).

34. Kim GH, Kim JE, Rhie SJ and Yoon S: The role of oxidative stress in neurodegenerative diseases. Experimental neurobiology 2015; 24(4): 325-40.
35. Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C and Collin F: Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biol. 2018; 14: 450-464.
36. Mihalas BP, Iuliis GN, Redgrove KA, McLaughlin EA and Nixon B: The lipid peroxidation product 4-hydroxynonenal contributes to oxidative stress-mediated deterioration of the ageing oocyte. Scientific reports 2017; 7(1): 6247.
37. Phanindhra B, Raju AB, Vikas G, Anusha R and Deepika D: Effect of *Nyctanthes arbor tristis* leaf extract against scopolamine-induced cognitive impairment in rats. Herba Polonica 2014; 60(4): 34-49.
38. Hejazian SH, Karimi S, Hosseini M, Mousavi SM and Soukhtanloo M: Protection against brain tissues oxidative damage as a possible mechanism for improving effects of low doses of estradiol on scopolamine-induced learning and memory impairments in ovariectomized rats. Advanced biomedical research 2016; 5.
39. Zamana LV: Fluorine hydrogeological anomalies in Transbaikalia. Geokhimiya 1992; 2: 228-237
40. Sushma NJ, Sivaiah U, Suraj NJ and Rao KJ: Aluminium acetate induced oxidative stress in brain of albino mice. J. Pharmacol. Toxicol 2006; 1: 579-584.
41. Beal MF: Aging, energy, and oxidative stress in neurodegenerative diseases. Annals of neurology 1995; 38(3): 357-366.
42. Atakisi O, Erdogan HM, Atakisi E, Citil M, Kanici A, Merhan O and Uzun M: Effects of reduced glutathione on nitric oxide level, total antioxidant and oxidant capacity and adenosine deaminase activity. Eur Rev Med Pharmacol Sci. 2010; 14(1): 19-23.
43. Huang WJ, Zhang X and Chen WW: Role of oxidative stress in Alzheimer's disease (Review). Biomedical reports 2016; 4(5): 519-522.
44. Bains JS and Shaw CA: Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Research Reviews 1997; 25(3): 335-358.
45. Aprioku JS: Pharmacology of free radicals and the impact of reactive oxygen species on the testis. Journal of reproduction and infertility 2013; 14(4): 158.
46. Kurutas EB: The importance of antioxidants, which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal 2016; 15(1): 71.
47. Ganguly R, Hazra R, Ray K and Guha: Effect of *Moringa oleifera* in experimental model of Alzheimer's disease: Role of antioxidants. Annals of Neurosciences 2010; 12 (3): 33-36.
48. Sofic E, Salkovic-Petrisic M, Tahirovic I, Sapcanin A, Mandel S, Youdim M and Riederer P:"Brain catalase in the streptozotocin-rat model of sporadic Alzheimer's disease treated with the iron chelator–monoamine oxidase inhibitor, M30." Journal of Neural Transmission 2015; 122(4): 559-564.
49. Marisco PC, Carvalho FB, Rosa MM, Girardi BA, Gutierres JM, Jaques JA, Salla AP, Pimentel VC, Schetinger MRC, Leal DB and Mello CF: Piracetam prevents scopolamine-induced memory impairment and decrease of NTPDase, 5′-nucleotidase and adenosine deaminase activities. Neurochemical research 2013; 38(8): 1704-1714.
50. Ishola IO, Adamson FM and Adeyemi OO: Ameliorative effect of kolaviron, a biflavonoid complex from *Garcinia kola* seeds against scopolamine-induced memory impairment in rats: role of antioxidant defense system. Metabolic brain disease 2017; 32(1): 235-245.
51. Padurariu M, Ciobica A, Lefter R, Lacramioara Serban I, Stefanescu C and Chirita R: The oxidative stress hypothesis in Alzheimer's disease. Psychiatria Danubina 2013; 25(4): 0-409.
52. Otitoju O, Onwurah IN, Otitoju GT and Ugwu CE: Oxidative stress and superoxide dismutase activity in brain of rats fed with diet containing permethrin. Biokemistri, 2008; 20(2).
53. Hamden K, Silandre D, Delalande C, ElFeki A and Carreau S: Protective effects of estrogens and caloric restriction during aging on various rat testis parameters. Asian journal of andrology 2008; 10(6): 837-845.
54. Farzin D and Mansouri N: Antidepressant-like effect of harmane and the other β-carbolines in the mouse forced swim test. European Neuropsychopharmacology 2006; 16(5): 324-328.
55. Mohamed NE and El-Moneim AE: *Ginkgo biloba* extract alleviates oxidative stress and some neurotransmitters changes induced by aluminum chloride in rats. Nutrition 2017; 35: 93-9.
56. Belviranl M and Okudan N: The effects of *Ginkgo biloba* extract on cognitive functions in aged female rats: the role of oxidative stress and brain-derived neurotrophic factor. Behavioural brain research 2015; 278: 453-61.
57. Stefanovits-Bányai E, Szentmihályi K, Hegedűs A, Koczka N, Váli L, Taba G and Blázovics A: Metal ion and antioxidant alterations in leaves between different sexes of Ginkgo biloba L. Life sciences 2006; 78(10): 1049-1056.
58. Kobus-Cisowska J, Flaczyk E, Rudzińska M and Kmiecik D: Antioxidant properties of extracts of *Ginkgo biloba* leaves in meatballs. Meat Science 2014; 97(2): 174-180.
59. Singh G: Pharmaceutical Benefits of *Ginkgo biloba* (Tree of Life): A Review. Journal of Biomedical and Pharmaceutical Research 2013; 2(01).
60. Guo M, Suo Y, Gao Q, Du H, Zeng W, Wang Y, Hu X and Jiang X: The protective mechanism of Ginkgolides and Ginkgo flavonoids on the TNF-α induced apoptosis of rat hippocampal neurons and its mechanisms *in vitro*. Heliyon 2015; 1(1): e00020.
61. Kwon SH, Lee HK, Kim, JA, Hong SI, Kim HC, Jo TH, Park YI, Lee CK, Kim YB, Lee SY and Jang CG: Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. European journal of pharmacology 2010; 649(1): 210-217.
62. Stein C, Hopfeld J, Lau H and Klein J: Effects of *Ginkgo biloba* extract EGb 761, donepezil and their combination on central cholinergic function in aged rats. Journal of Pharmacy and Pharmaceutical Sciences 2015; 18(4): 634-646.
63. Ishola IO, Adamson FM and Adeyemi OO: Ameliorative effect of kolaviron, a biflavonoid complex from *Garcinia kola* seeds against scopolamine-induced memory impairment in rats: role of antioxidant defense system. Metabolic brain disease 2017; 32(1): 235-245.
64. Ellis JM: Cholinesterase inhibitors in the treatment of dementia. The Journal of the American Osteopathic Association 2005; 105(3): 145-158.
65. Kehr J, Yoshitake S, Ijiri S, Koch E, Nöldner M and Yoshitake T: *Ginkgo biloba* leaf extract (EGb 761®) and its specific acylated flavonol constituents increase dopamine and acetylcholine levels in the rat medial prefrontal cortex: possible implications for the cognitive

enhancing properties of EGb 761®. International psychogeriatrics 2012; 24(S1): S25-S34.

66. Abd-Elhady RM, Elsheikh AM and Khalifa AE: Anti-amnestic properties of *Ginkgo biloba* extract on impaired memory function induced by aluminum in rats. International Journal of Developmental Neuroscience 2013; 31(7): 598-607.
67. Frost D, Meechoovet B, Wang T, Gately S, Giorgetti M, Shcherbakova I and Dunckley T: β-carboline compounds, including harmine, inhibit DYRK1A and tau phosphorylation at multiple Alzheimer's disease-related sites. PLoS One 2011; 6(5): e19264.
68. Yang Y, Cheng X, Liu W, Chou G, Wang Z and Wang C: Potent AChE and BChE inhibitors isolated from seeds of *Peganum harmala* Linn. by a bioassay-guided fractionation. Journal of ethnopharmacology 2015; 168: 279-286.
69. Liu W, Yang Y, Cheng X, Gong C, Li S, He D, Yang L, Wang Z and Wang C: Rapid and sensitive detection of the inhibiting activities of acetyl-and butyryl-cholinesterases inhibitors by UPLC–ESI-MS/MS. Journal of pharmaceutical and biomedical analysis 2014; 94: 215-220.
70. Tursunkhodjaeva F and Sokhibova N: Neuroprotective and antinociceptive activity of two new derivatives of Deoxypeganine Alkaloid 2015; 3: 56-60.
71. Harris RA: Cerebral lactate metabolism and memory: Implications for Alzheimer's disease 2017
72. Li T, Xie J, Wang Y, Wang S, Wu S, Wang Q and Ding H: "Protective effects of aloe-emodin on scopolamine-induced memory impairment in mice and H_2O_2-induced cytotoxicity in $PC1_2$ cells." Bioorganic and medicinal chemistry letters 2014; 24(23): 5385-5389.
73. Xu P, Wang K, Lu C, Dong L, Gao L, Yan M, Aibai S and Liu X: Protective effect of lavender oil on scopolamine induced cognitive deficits in mice and H_2O_2 induced cytotoxicity in PC12 cells. Journal of Ethnopharmacology 2016; 193: 408-415.
74. Wu J, Li R, Li W, Ren M, Thangthaeng N, Sumien N, Liu R, Yang S, Simpkins JW, Forster MJ and Yan LJ: Administration of 5-methoxyindole-2-carboxylic acid that potentially targets mitochondrial dihydrolipoamide dehydrogenase confers cerebral preconditioning against ischemic stroke injury. Free Radical Biology and Medicine 2017; 113: 244-54.
75. Braithwaite SP, Stock JB, Lombroso PJ and Nairn AC: Protein phosphatases and Alzheimer's disease. Progress in molecular biology and translational science 2012; 106: 343.
76. Jung IH, Lee HE, Park SJ, Ahn YJ, Kwon G, Woo H, Lee SY, Kim JS, Jo YW, Jang DS, Kang SS and Ryu JH: Ameliorating effect of spinosin, a C-glycoside flavonoid, on scopolamine-induced memory impairment in mice. Pharmacology, Biochemistry and Behavior 2014; 120: 88-94.
77. Coffey EE: A role for lysosomal pH dysfunction in Alzheimer's disease and strategies for its restoration. University of Pennsylvania 2015.
78. Zhang L, Sheng R and Qin Z: The lysosome and neurodegenerative diseases. Acta biochimica et biophysica Sinica 2009; 41(6): 437-445.
79. Mohamed NV, Plouffe V, Rémillard-Labrosse G, Planel E and Leclerc N: Starvation and inhibition of lysosomal function increased tau secretion by primary cortical neurons. Scientific reports 2014; 4.
80. Tan MS, Yu JT, Tan CC, Wang HF, Meng XF, Wang C, Jiang T, Zhu XC and Tan L: Efficacy and adverse effects of *Ginkgo biloba* for cognitive impairment and dementia. A systematic review and meta-analysis. Journal of Alzheimer's Disease 2015; 43(2): 589-603.
81. Mohamed NES and El-Moneim AEA: *Ginkgo biloba* extract alleviates oxidative stress and some neurotransmitter changes induced by aluminum chloride in rats. Nutrition 2017; 35: 93-99.
82. Sumathi TH, Shobana C, Mahalakshmi VA, Sureka R, Subathra MA, Vishali AR and Rekha K: Oxidative stress in brains of male rats intoxicated with aluminium and neuromodulating effect of *Celastrus paniculatus* alcoholic seed extract. Asian J Pharm Clin Res, 2013; 6(3): 80-90.
83. Cohen S: Phosphatases, In: Handbook of Neurochemistry. 3rd edn, (Ed. Lajtha A) Plenum Press, New York 1970: 87-131.
84. Muthuviveqqganandavel V, Muthuraman P, Muthu S and Srikumar K: Individual and combined biochemical and histological effect of Cypermethrin and Carbendazim in male albino rats. Journal of Applied Pharmaceutical Science 2011; 1(9): 121-129.
85. Zhang WR, Hayashi T, Kitagawa H, Sasaki C, Sakai K, Warita H, Wang JM, Shiro Y, Uchida M and Abe K: Protective effect of ginkgo extract on rat brain with transient middle cerebral artery occlusion. Neurological research 2000; 22(5): 517-532.

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