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Research Article
Neuromodulatory Influences of Nutraceuticals on D-galactose Induced Hippocampal Neuronal Metabolic Dysfunction Model

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Abstract

Background and Objective: D-galactose induced neurotoxicity is widely known model for survey aging and related oxidative neurotoxicity and memory impairment. The present study was conducted to explore the possible role of Linseed Oil (LO) and Coenzyme Q10 (CoQ10) as compared with standard neuroprophylactic *Ginkgo biloba* extract to protect hippocampal cells against toxic effects induced by D-galactose (D-gal). **Materials and Methods:** The rats (50 males) were divided randomly into five groups, 10 rats for each, the 1st normal control, the 2nd served as aging treated with D-gal, the 3rd treated with *Ginkgo biloba* plus D-gal, the 4th group treated with linseed oil plus D-gal, the 5th group treated with Coenzyme Q10 plus D-gal. The experiment lasted for 6 weeks then behavioral tests were examined and then all animals were decapitated for biochemical, neurochemicals, histopathology and immunohistochemistry analysis. **Results:** Administration of D-gal for 6 weeks significantly impaired behavioral test, oxidative defense, decreased endogenous antioxidant, impaired neurotransmitters contents, increased acetylcholinesterase, inhibited neurogenesis, increased 8-hydroxy-2-deoxyguanosine, decreased ATP and increased apoptotic factors as compared to normal group. Six weeks of linseed oil and coenzyme Q10 treatments significantly improved neurobehavioral alterations, brain derived neurotrophic factor and apoptosis as compared to D-gal. **Conclusion:** The present study concluded that dietary intake rich with D-gal disrupt neuronal cell structure in accordance with aging which accumulate most neuronal aberration and treating with natural substances may be beneficial in normal aging troubles. In addition, CoQ10 showed best protective effect after 6 weeks of treatment compared with LO and GB which normalized most selected paramours.

Key words: D-galactose, linseed oil, coenzyme Q10, hippocampus, rats, neurobehavioral changes, brain injury, cognitive impairment

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aging is a progressive biological activity associated with multiple structural and biochemical alteration in biological system in addition to cognitive impairment[1]. Reactive Oxygen Species (ROS) induced DNA injury which is the main cause of aging[2,3]. There are multiple animal models for aging research such as; D-galactose (D-gal) model is convenient and can be resemble to natural aging researches. D-galactose metabolized normally in the body by D-galactokinase and galactose-1-phosphate uridyl transferase. However, chronic administration of D-gal can be converted into hydrogen peroxide leading to the formation of a superoxide anion[4]. Administration of D-gal shows aging symptoms such as; impairment of spatial learning, memory, decline in cognitive functions, neuroinflammation and increase apoptosis[5,6]. Hippocampus is responsible for learning, memory function and age-related neurodegeneration[7]. Therefore, dietary supplements and foods rich in natural antioxidants were shown to be beneficial in aging disorders[8].

Linseed "Flaxseed, *Linum usitatissimum*" has been the focus of increased interest in the field of diet and disease research due to its Omega-3 polyunsaturated fatty acids content. It is also the richest natural source of mammalian lignans that effectiveness is through an antioxidant effect[9].

Nutritionally, linseed oils rich with α-linolenic acid (ALA) which considered an essential fatty acid used as dietary supplements[10]. Thus, may benefit human body as antiarrhythmic and neuroprotective functions[11]. Up to date, no studies focused on the neuroprotective role of linseed oil against D-gal induced aging alterations and neurotoxicity.

Coenzyme Q (CoQ, ubiquinone) is a unique lipid-soluble antioxidant that is generated in animals[12]. It is an essential compound of the mitochondrial electron transport chain and is therefore essential for the production of ATP[13]. It is an important controller of lifespan in normal aging[14]. It is chemically similar to vitamin K in its structure, but it is not considered a vitamin because it is synthesized in the body. Furthermore, CoQ10 is a powerful antioxidant capable of recycling and restore other antioxidants[15] such as; vitamin E and vitamin C.

The present research was conducted to study dietary intake of natural substances that could be beneficial in normal aging troubles and to explore the potential role of linseed oil and coenzyme Q10 as compared with standard neuroprophylactic *Ginkgo biloba* extract to protect hippocampal cells against toxic effects induced by D-galactose.

MATERIALS AND METHODS

This study was conducted in September, 2017 in Animal house of National Organization for Drug Control and Research, Giza, Egypt.

Animals: The adult male Sprague-dawley rats weighing 280-300 g were used in the present study. The animals were obtained from animal house of NODCAR and caged in 5 separate cages with natural ventilation and illumination at about 20±2°C and 14 h light period and 65% humidity, animal feed standard basal diet (*ad libitum*) and free access of water. Animals were allowed one week for adaptation before treatments. Animal handling, tissue collection were follow the instructions of the guidelines of the Research Ethical Committee of the NODCAR.

Chemicals: D-galactose was purchased from Loba Chemie, India and used in dose of D-gal (120 mg kg^{-1}) daily, sub-cutaneously (sc), for 6 successive weeks[7]. Standardized EGb 761 (Huisong, China) was used in dose of 150 mg kg^{-1} b.wt., according to Blecharz-Klin *et al.*[16]. Linseed oil (Isis Company for Food and Industries, Egypt) was used (LO, 1.5 g kg^{-1}; p.o, for 6 weeks), according to Rajesha *et al.*[17]. Coenzyme Q10 (Arab Company for Pharmaceutical and Medicinal Plant; MEPACO, Egypt) was used (CoQ10, 10 mg kg^{-1}; p.o, for 6 weeks), according to Emam *et al.*[18].

Experimental design: The protocols of following methodologies were adopted according to the guidelines of the Institutional Animal Ethics Committee of NODCAR. All the experimental procedures were carried out in accordance with international guide lines for the care and use of laboratory animals and with accordance with standard guidelines[19]. The rats were subjected to 50 male adult Sprague-dawley were divided randomly into five groups, 10 rats for each, the 1st served as normal control the 2nd served as control aging treated with D-galactose (D-gal, 120 mg kg^{-1}, subcutaneously) the 3rd treated with *Ginkgo biloba* (GB, 150 mg kg^{-1}, p.o.) plus D-gal, the 4th group treated with Linseed Oil (LO, 1.5 g kg^{-1}, p.o) plus D-gal, the 5th group treated with coenzyme Q10 (10 mg kg^{-1}, p.o.) plus D-gal. The experiment lasted for 6 weeks then behavioral tests were examined and then all animals were decapitated for biochemical, neurochemicals and histopathology and immunohistochemistry analysis.

Behavioral assessments

Measurement of spatial memory and learning by Morris Water Maze (MWM): Morris water maze[20] analyze its behavior via 2 parameters 1st is escape latency, which is the time it

takes to find the platform, the 2nd measured during probe trials: the escape platform is removed and the mice or rats are allowed to search for it for a fixed time (often 60 sec). Variables were measured time and path length in quadrants, time near platform and platform crossings.

Grid test (Catalepsy test): The grid test was measured according to Rahman *et al.*[21] and used as index of catalepsy or sensorimotor deficit.

Assessment of locomotor and exploratory activity by the open field test: The open-field test was measure rearing and ambulation according to Looser[22].

Tissue sampling: Tissue sampling were collected after 24 h of the end experiment and the rats were decapitated and both hippocampi were isolated one was kept in 10% formalin for histopathological examination while the other at -80°C for estimating the other biochemical parameters. For the determination of neurotransmitters, a 10% (w/v) homogenate was prepared in a 75% methanol for HPLC. Each homogenate was centrifuged at 10006×g (4°C) for 10 min. The resultant supernatant was divided into 2 halves, the first was dried using vacuum (70 millipore) at RT and its residues were derivatized for the determination of brain amino acids (HVA, GLU, GABA), whereas the second half was used for monoamines determination (5-HT, DA, NE). In another subset, hippocampi were homogenized in 10% (w/v) phosphate buffer (pH 7.6) for the assay of the other biochemical parameters[23].

Determination of malondialdehyde (MDA), reduced glutathione (GSH), Nitric Oxide (NO) contents, superoxide dismutase (SOD) activity and Total Antioxidant Capacity (TAC) in hippocampal brain area: Hippocampal MDA, GSH, NO, SOD and TAC were measured according to the methods of Prins and Loose[24], Miranda *et al.*[25], Nishikimi *et al.*[26] and Koracevic *et al.*[27], respectively.

Determination of hippocampal Brain Derived Neurotrophic Factor (BDNF) content: Enzyme Linked Immunosorbent Assay (ELISA) was used to determine hippocampal BDNF by using a test reagent kit (CUSABIO Biotech co., USA), according to the manufacturer's instructions.

Determination of hippocampal amino acids: Hippocampal free amino acids, monoamines and their metabolites were measured by HPLC UV detector[28,29].

Determination of hippocampal acetylcholinesterase (AChE) activity: Hippocampal AChE was determined by using DTNB-phosphate reagent after 10 min incubation of the hippocampal homogenate with acetyl thiocholine iodide[30].

Determination of hippocampal 8-OHDG content: Hippocampal 8OHdG was measured by HPLC UV detector[31].

Determination of hippocampal adenosine tri-phosphate (ATP) content: Hippocampal ATP was measured by HPLC UV detector[32].

Histopathological and immunohistochemical examinations of Bax and Caspase-3 (Casp-3): For histological study, hippocampal brain area were cut and processed for paraffin sections of 5 µm thickness to be stained with H&E, according to Bancroft *et al.*[32]. Immunohistochemical detection of caspase-3 was performed by using primary rabbit anti-rat caspase-3 antibody from Neo Markers Fremont CA, Lab Vision. The steps were applied instead of the primary antibodies[33].

Statistical analysis: All values were presented as Mean±SEM. Statistical analysis was performed by using GraphPad Prism version 5 (Graph-Pad, San Diego, CA). A comparison between different groups was carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. A significant differences were considered when probability less than 0.05.

RESULTS

Behavioral tests for proofing metabolic syndromes associated with aging: The Morris water maze, grid and open field test were applied for determine neuronal metabolic syndrome affected by D-gal and obviate its effects concurrent with natural remedy.

Morris Water Maze (MWM) training course: There were no significant differences between groups in the mean escape latency on the first day of training. On the second, third and fourth days of investigation, D-gal increased escape latency time ($p<0.05$) compared to the control group (Fig. 1a). In contrast, treated group showed markedly amelioration and differ from D-gal group after 1st trail for 2nd, 3rd and 4th test, but didn't differ from normal group except GB group at 3rd and 4th group. In addition, treated group with D-gal deteriorated memory test resembling in the decreasing of swimming time at the selective square of platform (Fig. 1b).

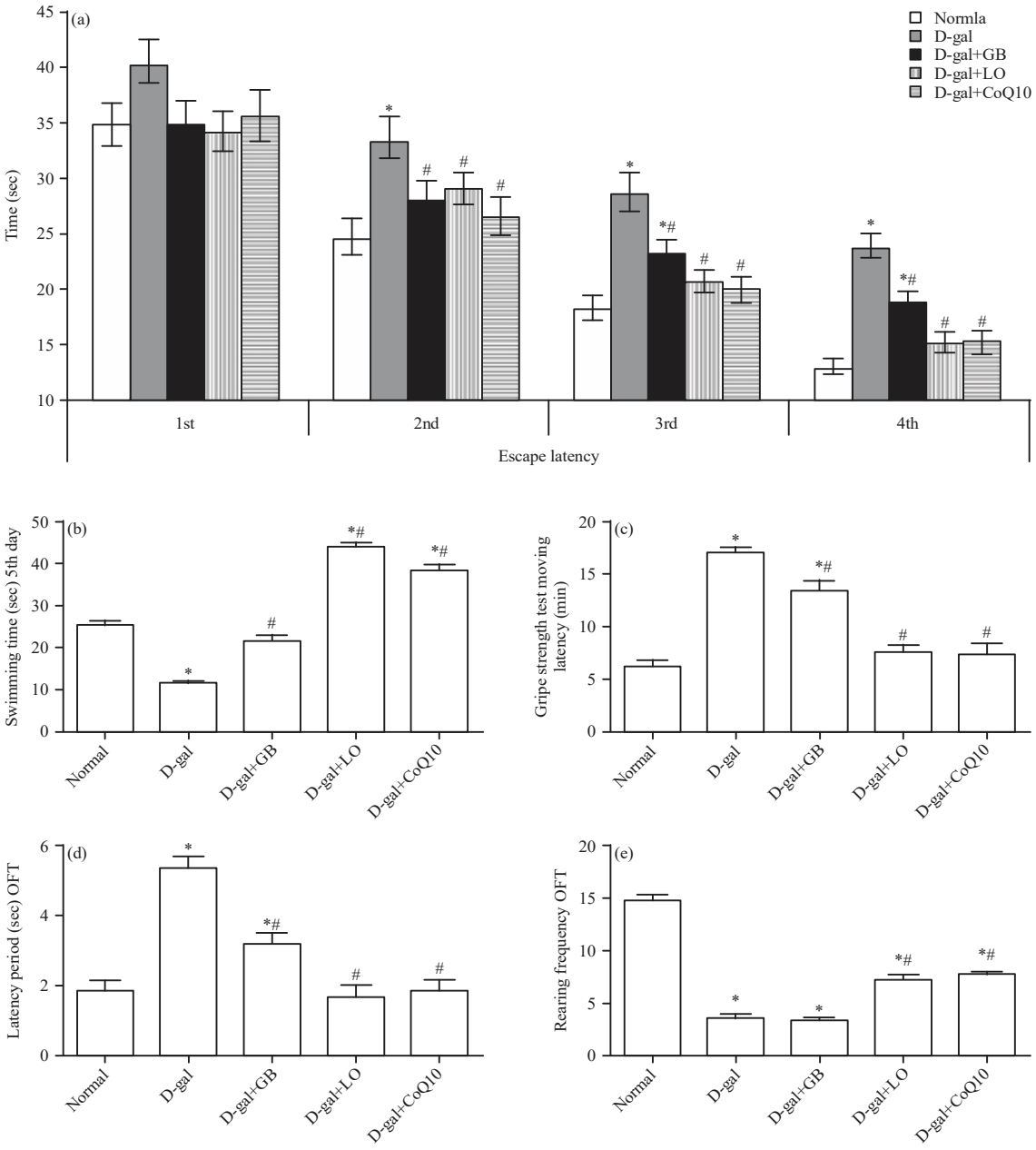

Fig. 1(a-e): Behavioral effects of Linseed Oil (LO) or Coenzyme Q10 (CoQ10) and *Ginko biloba* (GB) extract on D-galactose (D-gal)-induced brain aging in rats on morris water maze test (a) Escape latency and (b) Swimming time, (c) Moving latency, (d) Open field test and latency time and (e) Rearing frequencies

Each bar represents means (n = 10)±standard error. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test. *Significantly different from normal group at $p<0.05$, #Significantly different from control (D-gal) group at $p<0.05$

On the other hand, treated groups with GB, LO and CoQ10 showed significant amelioration via increase swimming time in comparing with D-gal group, but LO and CoQ10 showed superior amelioration from control group. In the grid test, as illustrated in Fig. 1c, the administration of D-gal to rats induced significant increase in catalepsy score of grid test compared to the normal group. Administration of GB and D-gal decreased moving latency of grid test compared to D-gal-treated group. Concomitant administration of either LO plus D-gal or CoQ10 plus D-gal induced significant normalize catalepsy score, respectively as compared to D-gal treated group. In the OFT, as illustrated in Fig. 1d-e, administration of D-gal significantly increased latency time and decrease the rearing respectively in comparing with normal group. In

Table 1: Neuroprotective effect of linseed oil as compared with *Ginko biloba* on oxidative stress parameters in hippocampal homogenate in D-galactose

Groups	MDA (nmol g^{-1} tissue)	NO (μmol g^{-1} tissue)	GSH (mg g^{-1} tissue)	SOD (U g^{-1} tissue)	TAC (mmol g^{-1} tissue)
Normal	4.11±0.06	12.40±0.13	8.92±0.37	3.50±0.03	2.90±0.06
D-gal	5.27±0.09*	15.18±0.13*	2.00±0.18*	2.75±0.01*	2.30±0.06*
D-gal + GB	3.91±0.09#	11.60±0.24*#	7.08±0.52*#	2.75±0.02*	3.10±0.06#
D-gal + LO	3.79±0.03*#	12.10±0.14#	5.67±0.38*#	2.84±0.03*	3.65±0.06*#
D-gal+CoQ10	3.72±0.03*#	12.25±0.14#	4.58±0.32*#	2.94±0.04*#	3.59±0.07*#

Each value represents means (n = 10)±standard error. Statistical analysis was carried out by one way ANOVA followed by Tukey–Kramer multiple comparison test. *Significantly different from normal group at p<0.05. # Significantly different from control (D-gal) group at p<0.05, GB: *Ginko biloba*, LO: Linseed oil, CoQ10: Coenzyme Q10, MDA: Malondialdehyde, NO: Nitric oxide, GSH: Reduced glutathione, SOD: Superoxide dismutase, TAC: Total antioxidant capacity

contrast, treated group with GB, LO and CoQ10 significantly decreased the latency and increase the rearing respectively compared to D-gal group except GB didn't differ for rearing only. Finally, treated group with LO and CoQ10 showed significant amelioration resembling in latency period only, but the other parameter and groups showed markedly disrupt via increase the latency and decrease rearing in comparing with normal group.

Hippocampus MDA, NO, GSH, SOD and TAC contents: As shown in Table 1, D-gal induced a significant increase in hippocampus MDA and NO content to be 5.27±0.09 and 15.18±0.13, respectively with significant decrease in hippocampus GSH, TAC contents and SOD activity respectively as compared with normal rats. Administration of GB, LO and CoQ10 significantly decrease the MDA (3.91±0.09, 3.79±0.03 and 3.72±0.03) and NO contents (11.60±0.24, 12.10±0.13 and 12.25±0.14), respectively as compared with aging rats. While, GB, LO and COQ10 significantly increased GSH content (7.08±0.52, 5.66±0.38 and 4.58±0.32), respectively and TAC by means of 3.10±0.06, 3.65±0.06 and 3.59±0.07 respectively, however only CoQ10 nearly normalize SOD content as compared with aging animals..

Hippocampus BDNF content: As illustrated in Fig. 2, D-gal induced a significant decrease in hippocampus BDNF content (1.48±0.03) as compared with normal rats (10.28±0.31). Oral administration of GB, LO and CoQ10 significantly increased the content of BDNF (4.08±0.04, 5.13±0.13 and 7.68±0.35), respectively as compared with aging animals.

Hippocampus neurotransmitters contents: As illustrated in Fig. 3a-f, D-gal deteriorated hippocampus monoamines and amino acids contents were manifested by reduction in 5-HT content (0.38±0.01), DA (1.09±0.03) and NE (0.55±0.03) contents as compared with values of normal rats. This reduction accompanied by significant increment in GABA (5.50±0.11), GLU (1.12±0.02) and HVA (0.49±0.02) contents as compared with normal rats, respectively. Administration of

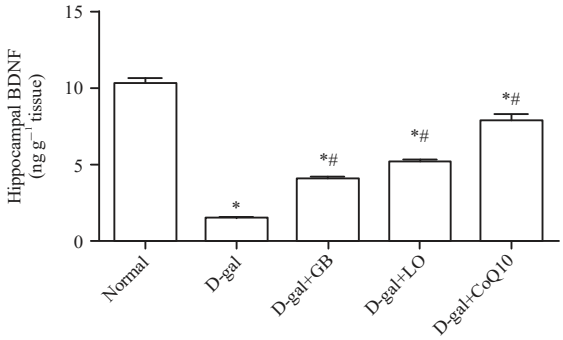

Fig. 2: Neuroprotective effect of Linseed Oil (LO), Coenzyme Q10 (CoQ10), and *Ginko biloba* (GB) extract on hippocampal Brain Derived Neurotrophic Factor (BDNF) in D-galactose (D-gal) treated rats

Each bar represents means (n = 10)±standard error. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test, *Significantly different from normal group at p<0.05, #Significantly different from control (D-gal) group at p<0.05

CoQ10 markedly increased the reduced hippocampus contents of 5-HT (0.74±0.04), DA (1.47±0.06) and NE (0.92±0.04) accompanied with significant decrease in GABA (4.51±0.11), GLU (0.87±0.01) and HVA (0.23±0.01) contents as compared with aging animals. Moreover, treatment of aging animals with GB markedly increased the reduced hippocampus contents of DA (1.46±0.09) with significant decrease in GABA, GLU and HVA contents as compared with D-gal treated animals. In addition, treatment of aging rats with LO significant decrease in GABA, GLU and normalized HVA contents as compared with D-gal treated animals.

Hippocampus AChE content: As illustrated in Fig. 3g, D-gal markedly increased hippocampus AChE content as compared with that of normal rats. In contrast, administration of LO and CoQ10 similarly markedly decreased the elevated hippocampus AChE content to 1.76±0.02 and 1.76±0.03 as compared with D-gal treated rats.

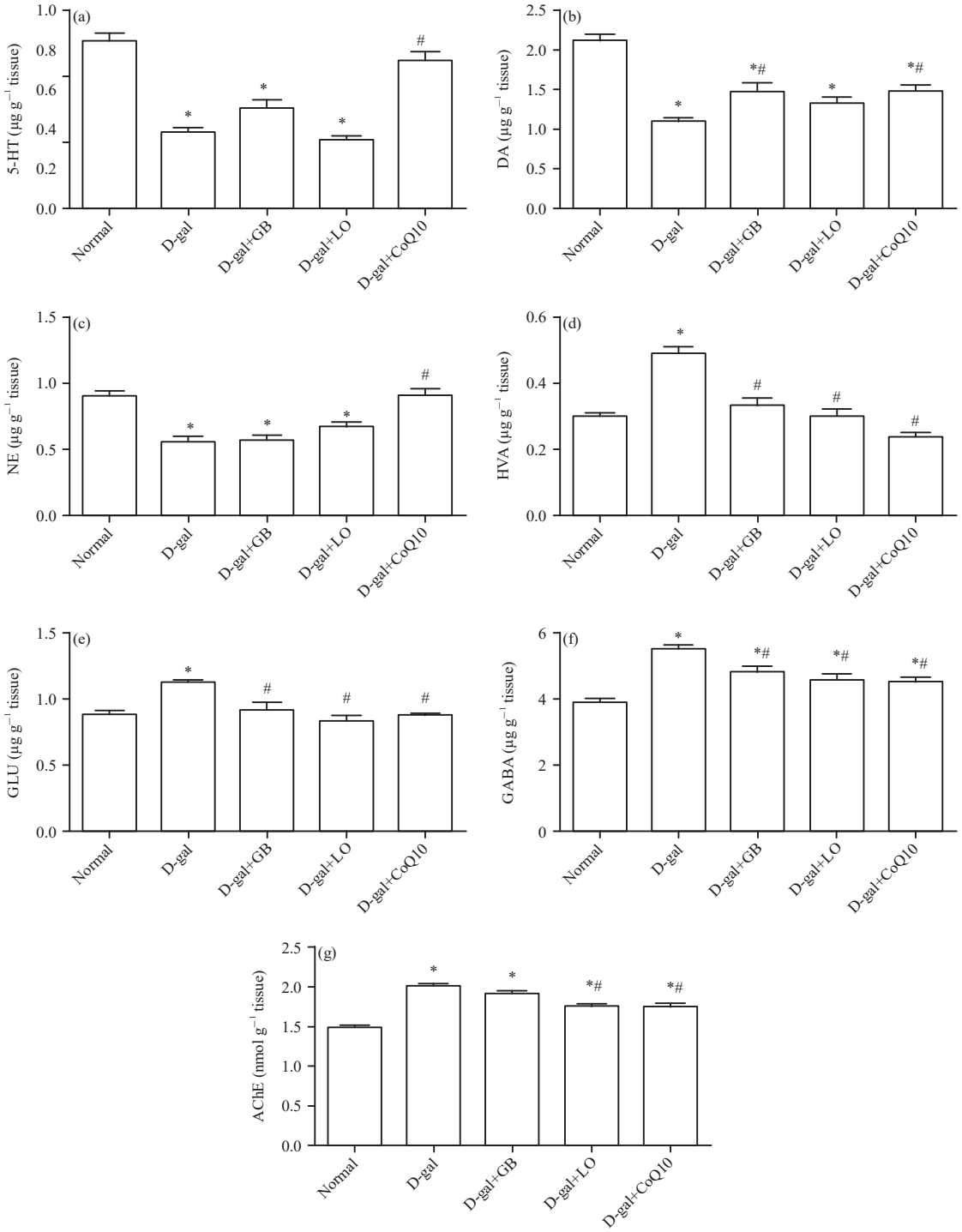

Fig. 3(a-g): Neuroprotective effect of Linseed Oil (LO), Coenzyme Q10 (CoQ10), and *Ginko biloba* (GB) extract on (a) Hippocampal serotonin (5-HT), (b) Dopamine (DA), (c) Norepinephrine (NE), (d) Homovanillic acid (HVA), (e) Glutamate (GLU), (f) Gamma aminobutyric acid (GABA) and (g) Acetylcholinesterase (AChE) contents in D-galactose (D-gal) treated rats

Each bar represents means (n = 10)±standard error. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test, *Significantly different from normal group at $p<0.05$. #Significantly different from control (D-gal) group at $p<0.05$

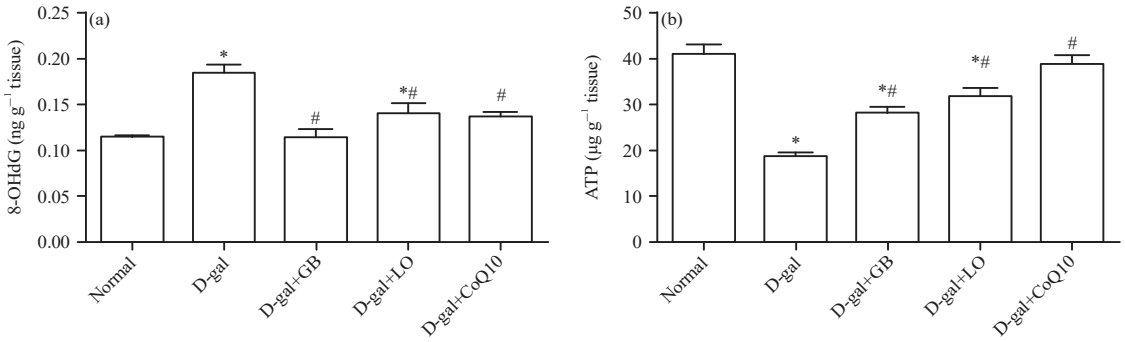

Fig. 4(a-b): Please replace title to this :Neuroprotective effect of Linseed Oil (LO), Coenzyme Q10 (CoQ10) and *Ginkgo biloba* (GB) extract on (a) Hippocampus 8-Hydroxy-2-deoxyguanosine (8-OHdG) and (b) Adenosine tri-phosphate (ATP) contents in D-galactose (D-gal) treated rats

Each bar represents means (n = 10)±standard error, Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test. *Significantly different from normal group at $p<0.05$. #Significantly different from control (D-gal) group at $p<0.05$

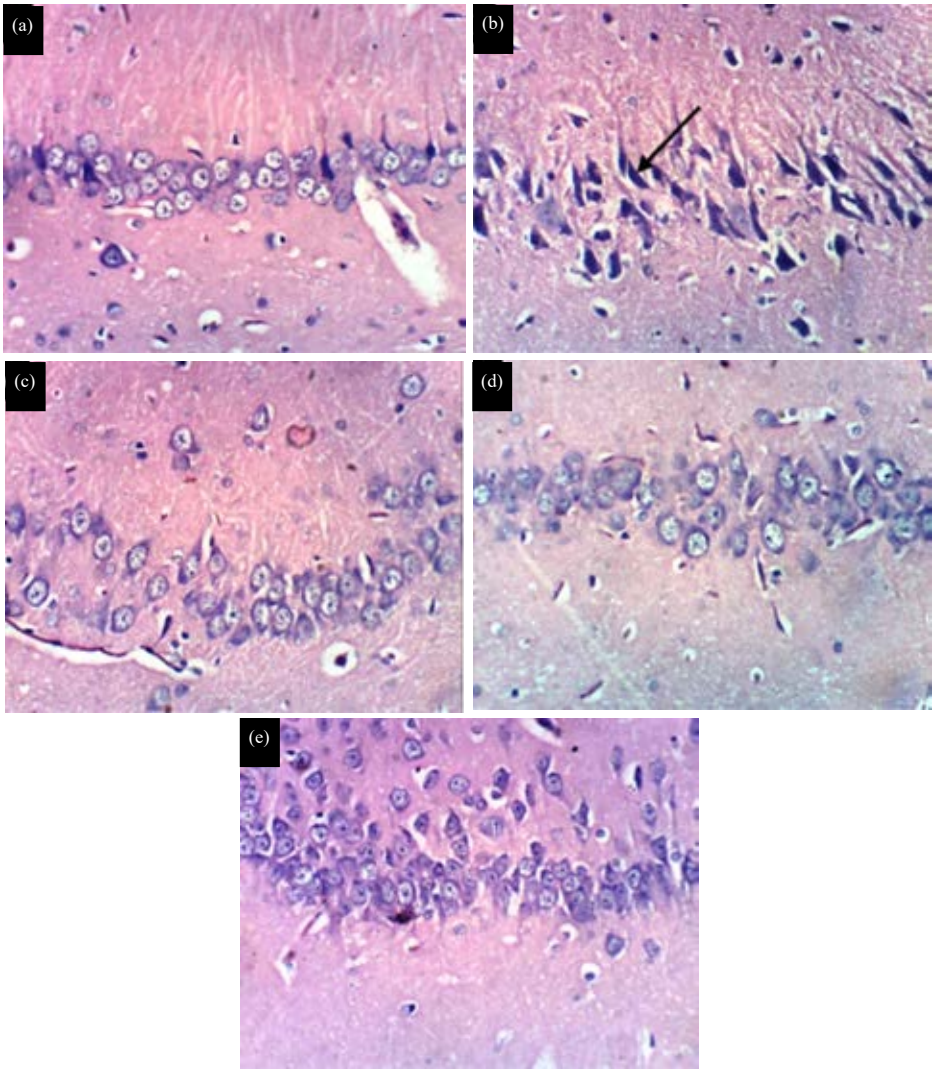

Fig. 5(a-e): Photomicrographs of rat hippocampus sections of, (a) Normal control group, (b) D-gel treated and (c-e) GB, LO and CoQ10 treated, stained with H&E (X400)

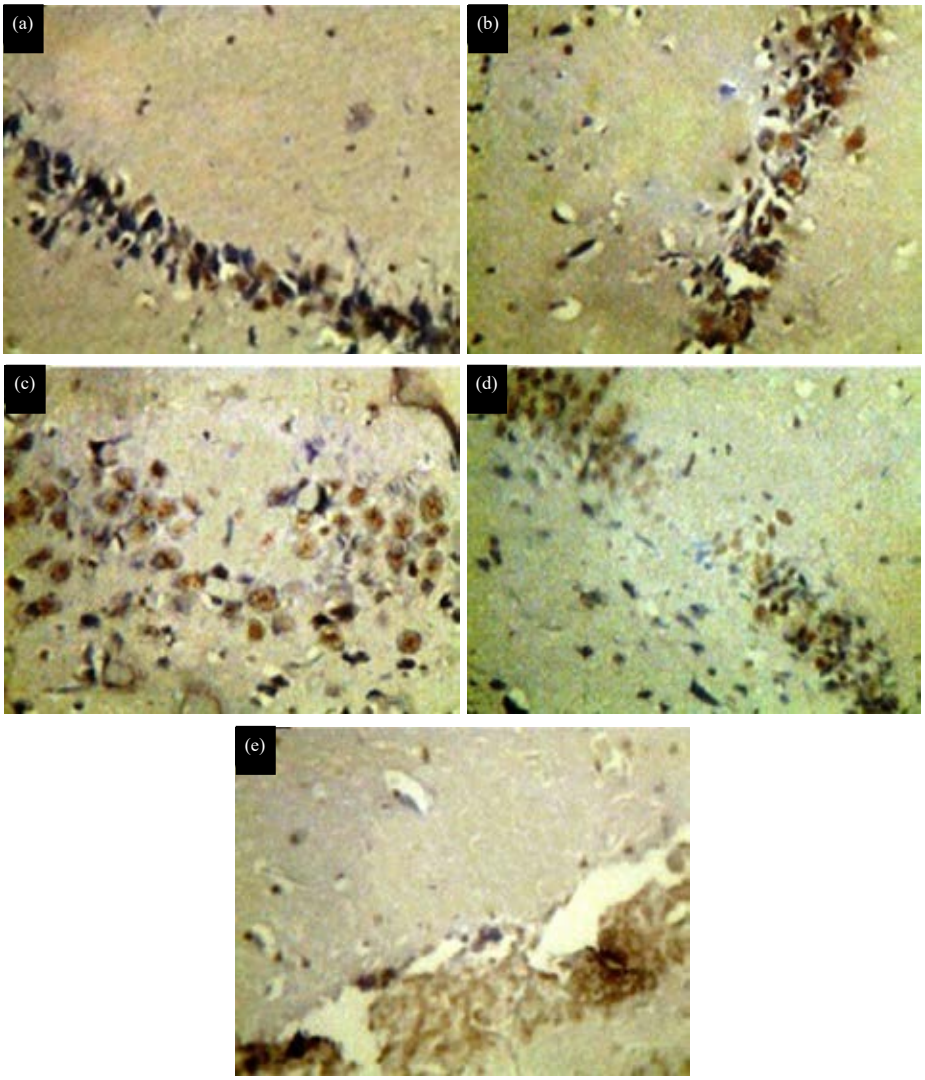

Fig. 6(a-e): Photomicrographs staining of box in hippocampus sections of (a) Normal control group, (b) D-gel treated and (c-e) GB, LO and CoQ10 treated, stained with H&E (X200)

Hippocampus 8-OHdG and ATP contents: As illustrated in Fig. 4a,b D-gal deteriorated hippocampal ATP and 8-OHDG contents were manifested by reduction in ATP accompanied by significant increment in 8-OHDG as compared with normal rat, respectively. Oral administration of GB, LO and CoQ10 induced a significant increase in ATP, respectively accompanied with significant reduction in 8-OHDG, respectively as compared with aging animals.

Histopathological and immunohistochemical examinations of Bax and Caspase-3 (Casp-3): Photomicrographs of rat hippocampus sections stained with hematoxylin and eosin (H&E) were illustrated in Fig. 5a-e. Normal control group showed no histopathological changes (Fig. 5a). Sections of rat treated with D-galactose showed necrosis and pyknosis of neurons (Fig. 5b). Sections of rat treated with *Ginkgo biloba* extract, linseed oil and CoQ10, respectively showed normal histopathological appearance (Fig. 5c-e).

Photomicrographs staining of Bax in hippocampus sections are illustrated in Fig. 6a-e, normal control group showed normal expression of Bax (Fig. 6a) and sections of rat treated with D-galactose showed salient raise in the number of positive Bax immunoreactive cells (Fig. 6b). Daily administration of *Ginkgo biloba* extract showed moderate highly positive expression of Bax which induced by D-galactose (Fig. 6c). Daily administration of linseed oil and CoQ10 showed weak positive expression of Bax, respectively as compared with D-galactose treated rats (Fig. 6d-e).

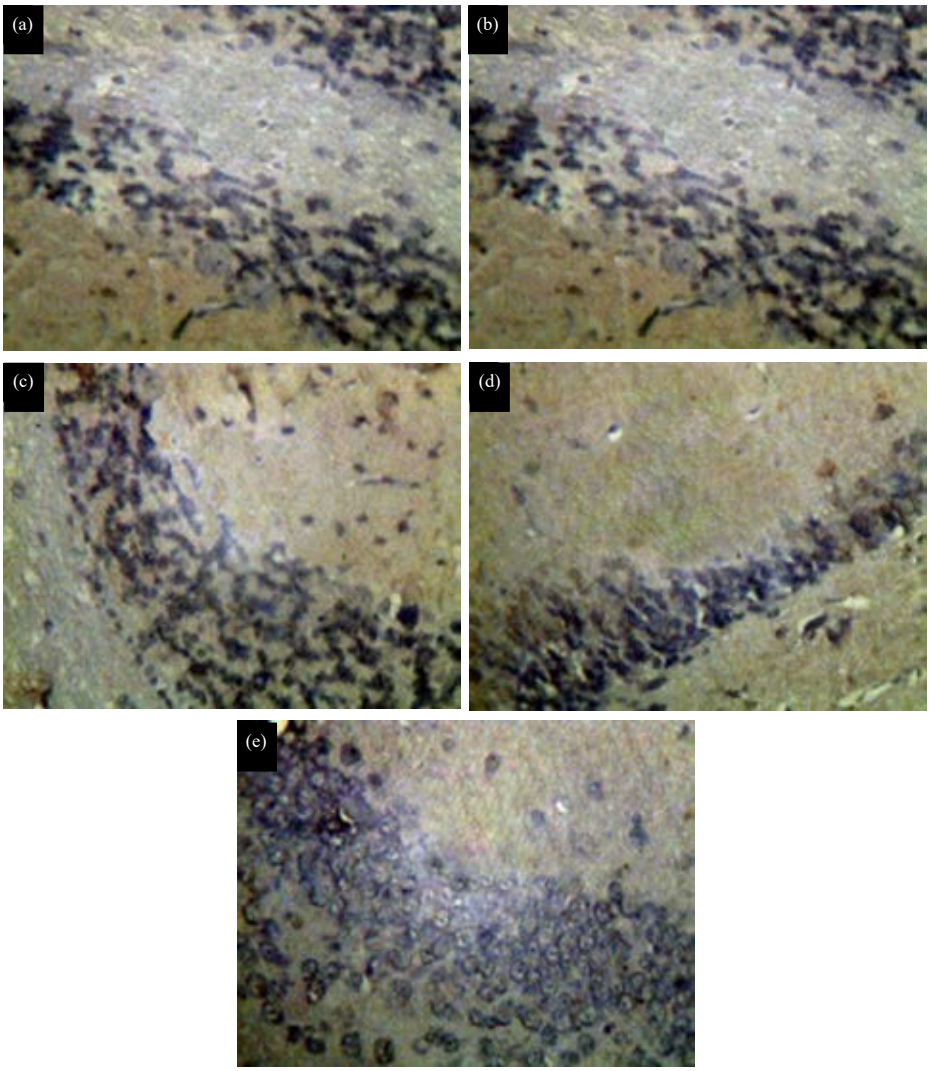

Fig. 7(a-e): Photomicrographs staining of Casp-3 (x200) in hippocampus sections of (a) Normal control group, (b) D-gel treated and (c-e) GB, LO and CoQ10 treated, stained with H&E (X200)

Photomicrographs staining of Casp-3 in hippocampus sections are illustrated in Fig. 7a-e, normal control group showed normal expression of Casp-3 (Fig. 7a), sections of rat treated with D-galactose showed increment in the number of positive Casp-3 immunoreactive cells (Fig. 7b). Daily administration of *Ginkgo biloba* extract showed moderate positive expression of Casp-3 which induced by D-galactose (Fig. 7c). Daily administration of linseed oil and CoQ10 showing almost normal expression of Casp-3, respectively as compared with D-galactose treated rats (Fig. 7d-e).

DISCUSSION

According to dietitians, dieting has a significant impact on the body's homeostasis and subsequent to brain and neurotransmitters stability. Therefore, proper nutrition can eliminate signs of aging and maintain public health and in particular nervous system. On the basis of these facts, many studies have shown that carbohydrate treatment, especially D-galactose has a negative effect and causes significant changes, including reduced neuromuscular activity, increased free radical production, reduced antioxidant activity and reduced immune responses and all these changes match the symptoms of natural aging[34]. D-galactose is a reducing sugar and has the ability to easily interact with free amines and peptides to form Advanced Glycation End-product (AGE). The AGE is increasingly formed during aging and has been associated with the emergence of many diseases such as; diabetes, arteriosclerosis, nephropathy and Alzheimer's disease[35]. The hypothesis suggested that accumulation of

D-galactose may interact with proteins, peptides and increases the composition of AGEs and accelerates the onset of aging. In the present study long term administration of D-gal induced impairment in working and reference memories in senescence rats by spent a longer time in finding the hidden platform during the retrieval trial in the Morris Water Maze (MWM) in task with a decline in coordination skills has been reported as an increased falling rate and weak grip strength on the grid strength test that used to screen for neuromuscular function and remarkably decreased the number of squares passed and prolonged latency to move in the open field test suggested the anxiogenic effects of D-gal when compared with the normal rats[36]. These finding are in accordance with previous studies[1,37-40]. Memory and learning impairment is thought to be the result of increased neurodegeneration and decline in the neuronal function. The resulting cognitive decline is a multifactorial process caused by excessive levels of D-gal[40] and involves oxidative stress, altered brain neurotransmitters and apoptosis[41,35]. In this study, obtained data demonstrated that D-galactose, treated rats had a significant decrease in brain monoamines (NE, DA and 5HT) and increase turnover of DA metabolites (HVA). The present data are conferment with previous study of Ho *et al.*[2], who reported that D-gal induced changes that resemble natural aging in rodents, including neurodegeneration and cognitive dysfunction and thus becomes a routinely used method to induce aging in rodents. In addition, excitatory amino acid was markedly increase and in the same way inhibitory amino acid GABA was elevate in comparing with control group although its mechanism needs further research. D-galactose induced a lead to aging progressive deterioration of cell and DNA, accompanied with severity metabolites and subsequent increase 8-OHdG. worthily, 8-OHdG can be seen as results from the mimetic ageing. These finding are in consistent with Evans *et al.*[42], who reported that D-Gal which occupied terminal positions in N-linked oligosaccharides of glycoproteins and play important roles in altering various biological functions which leads to emergence of pathologies in nucleus DNA structure and function. Increase of 8-OHdG formation in brain, may be due to large numbers of mitochondria that are the most reliant upon oxidative phosphorylation[43]. On the same manner results showed that D-galactose markedly decreases ATP which remarkable for cell energy and dysfunctional of cell carrying capacity in comparing with control group. However, glycation presses didn't need ATP for lipid or protein deterioration only it the cell energy was depleted or declined. Because the accumulation of redox status progressively exhausting ATP for endogenous balancing reaction. Presented data found that D-gal induced a significant increase in hippocampal MDA and NO content with significant decrease in hippocampal rGSH, TAC contents and SOD activity these results compatible with previous studies[1,7,44] and Anand *et al.*[45], who explained that D-gal at the physiological level can metabolize into glucose. So, an over-supply of D-gal is converted into aldos, hydrogen peroxide and formation of advanced glycation end products (AGEs) resulting in production and accumulation of ROS, which is widely recognized as a primary mechanism of natural age-related changes. On the one hand, the brain is the largest organ for oxygen consumption and subjected to deficiency of antioxidant enzymes[46]. In addition, BDNF encourage the survival and differentiation of neurons, protected neurons against neurodegeneration[47]. Presented data found that BDNF is decreased in the hippocampus of aging rats and is in agreement with Woo *et al.*[48], who found that repeated administration of D-gal decline BDNF production in the brain resulting in cognitive impairment. In normal aging and increased oxidative stress conditions, memory function declines due to decreases in BDNF[48,49]. In the present study, D-gal induced hippocampal neurotransmitters deterioration, which may be an important cause of memory decline that appeared with aging. This may be due to increase degradation of monoamine neurotransmitters. These results are compatible with Kou *et al.*[50], who found that AChE increased so decrease cholinergic functions associated with cognitive decline with normal aging. In addition, D-gal raised GLU as Zhou *et al.*[51] who found that GLU increment mediated neurotoxicity and oxidative stress due to reduction of the SOD level that decreased glutamate transporter 1 which removed GLU from synapse. Oxidative damage to DNA has a strong association with the molecular process of aging[52,53]. Valavanidis *et al.*[54], study showed that 8-OHdG is the general forms of free radical-induced DNA oxidative injury. Obtained results are in accordance with many lines of evidence that emphasize that expression of 8-OHdG was observed centrally in the cytoplasm of cells suggested that D-gal induced DNA oxidative injury in the hippocampus[54-57]. Apoptosis is one of the mechanisms involved in D-galactose-induced neurodegeneration, these results are compatible with Yu *et al.*[58]. Apoptosis is modified by many proteins, so the Bax/Bcl2 ratio is a crucial indicator of apoptosis[59], also caspase-3 is known as an apoptosis stimulus[60]. Administration of D-gal increased the Bax and caspase-3 in hippocampus tissues and apoptosis was developed in these tissues[61,62]. In the present study, treatments with linseed oil and CoQ10 improved working and reference memories in MWM, muscle coordination as well as locomotor function as compared with D-gal treated rats. At first, linseed oil could improve the D-gal-

induced neurotoxic effects may be due to the free radical scavenging activity and its Omega-3 fatty acid content. Taken together presented results are compatible with study of Freemantle *et al.*[63], linseed oil containing ALA is a good substrate for ketogenesis as well as β-oxidation. It has been hypothesized that ALA improved cognitive function by providing the brain with a fast available source of energy and facilitate astrocyte development[64]. A higher level of antioxidant reserve in linseed oil combined with D-gal treated rats as compared to rats of D-gal group only could be due to reduced oxidative stress because of antioxidant activity of flax lignan complex, so that no need for induction of antioxidant reserve[9]. The decrease in MDA, NO and 8-OHdG content correlated well with the increase of glutathione. Obtained study, linseed oil increased catecholamine content in brain of rats due to its Omeg-3 fatty acids content that may modify brain function by affecting production and function of neurotransmitters such as 5-HT and DA[65]. In addition, decreasing in Omega-3 fatty acids predicted decreasing 5-hydroxyindoleacetic acid, the major metabolite of 5-HT, which is known as anti-depressive and Omega-3 fatty acid deficiency is associated with decrements in 5-HT, DA and acetylcholine release[66]. In the present study, chronic treatment with CoQ10 in D-gal-treated rats significantly attenuated an impairment of spatial learning and memory task performance, CoQ10 is an essential cofactor of the electron transport chain, the neuroprotective effects of CoQ10 lie in the blocking of neuronal lesions produced by the mitochondrial toxin malonate and blocks activation of the mitochondrial permeability transition[67]. The study observed significant improvement in CoQ10 group which recover the low neurotransmission and energy metabolic concentration in the brain tissue after D-galactose exposure. CoQ10 has been shown to enhance neurotransmitters (monoamines) and reduce their turn-over. Schiefer *et al.*[68] observed increased neurotransmission by activating serotonin receptors in chronic unpredictable mild stress rat model. In addition, Schiefer *et al.*[68] reported that the CoQ10 treatment increased the concentration of 5-HT and NE in diabetic rats model. On the other hand, the use of CoQ10 reduced the activity of high tyrosine hydroxylase lead to increase of NE concentration. In addition, the concentration of the increase of monoamines in paraventricular nucleus in the hypertension model is due to the high activity in the transfer of electrons in the electron transport chain during oxidative phosphorylation and ATP production in the mitochondria[69]. Swomley *et al.*[70] has linked between an exaggerated production of ROS/RONS and development of hippocampal degeneration. Thus, CoQ10 protected against oxidation of DNA damage and increased lifespan in rats. In addition, other studies have showed a direct association between longevity and mitochondrial levels of CoQ10 in the model of aging-accelerated animals[71]. Supplementation of CoQ10 increases brain endogenous Q10 content and affords as an antioxidant against free radical generation and has been reported to improve cognitive function, ATP synthesis and provides significant improvement in patients with neurological disorders[72]. Fischer *et al.*[73] confirmed that a high CoQ10 is accompanied by an increase in muscle strength that in line with results of present study.

CONCLUSION

Present study concluded that, oxidative stress is a major cause in the initiation of neurobehavioral dysfunction in aging. The findings suggested that linseed oil or CoQ10 improved these changes and attenuated the effect of aging on learning and memory in D-gal treated rats. They exhibited beneficial effects on the cognition of aging rats by enhancing antioxidant capacity and anti-apoptotic, promoting the survival and neurogenesis, normalized hippocampal neurotransmitters. Therefore, they may be considered as healthcare anti-aging products to decrease incidence of age related brain diseases such as; Alzheimer which may be due to their free radical scavenger affinity.

This study discover the possible role of linseed oil and coenzyme Q10 as compared with standard neuroprophylactic *Ginkgo biloba* extract to protect hippocampal cells against toxic effects induced by D-galactose where the dietary intake of natural substances may be beneficial in common aging troubles.

SIGNIFICANCE STATEMENT

This study discover the comparison between the most common neuroprotective herb and diet rich with linseed oil or CoQ10 on D-galactose induced metabolic syndrome and neuronal destruction, that can be beneficial for attention to medicinal herbs and nutritional supplements with ageing. This study will help the researcher to uncover the critical areas of the neuronal monoamines turn-over and subsequently apoptotic stimulation after aging combined with D-gal that many researchers were not able to explore. Thus, a new theory on neuronal apoptosis may be arrived at.

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