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Electro-Acupuncture ameliorates memory and learning in induced brain aging via antioxidant, anti-apoptotic and anti-stress properties.

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Keywords

- D-galactose
- Aging
- Electroacupuncture
- learning and memory
- cortisol

Abstract

Background: Aging is a time-dependent multifaceted process accompanied by progressive loss of cognitive function. Electroacupuncture may combat brain oxidative stress, hippocampal damage, spatial learning, and memory impairment. **Objective:** To investigate the effect of electroacupuncture on memory and learning in D-galactose induced rat brain aging and elucidate more underlying mechanisms. **Material and Methods:** Forty male albino rats were divided into four groups; control group (C), Electroacupuncture-treated group (CE), D-galactose treated group (D) and Electroacupuncture and D-galactose-cotreated group (DE). The duration of concomitant administration of D-galactose was 10 weeks and Electroacupuncture was 15 min daily, 6 days/ week, for the last 2 weeks. At the end of the 10th week, spatial learning and memory of all groups were assessed using the Barnes maze test for 5 days. malondialdehyde (MDA), reduced glutathione (GSH) and tumor necrosis factor-alpha (TNF-α) levels were estimated in brain tissue. Cortisol and adrenocorticotropic hormone (ACTH) were estimated in serum. Histopathological changes in rat hippocampus were assessed by hematoxylin and eosin (H&E) and congo red stains to detect amyloid plaques. Results: Electroacupuncture treatment of all rats significantly decreased cortisol and ACTH, while the number of errors and the escape latency per second of the acquisition phase and probe phase of the Barnes maze test, the extent of hippocampal damage, MDA and TNFα were significantly decreased in DE compared to the D group. **Conclusion:** Electroacupuncture ameliorates stress markers and the spatial learning and memory impairment induced by d-galactose through enhancing antioxidant activity, reducing lipid peroxidation, and hippocampal damage.

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INTRODUCTION

Humans underwent a gradual decline of cognitive processes with advancing age. The cognitive decline occurred alone or accompanied by brain disorders [1]. Spatial memory impairment is considered the earliest manifestation of cognitive senescence which has a great effect on the quality of life [2]. Oxidative stress and reactive oxygen species (ROS) are considered the major contributors to aging and its related neurodegenerative diseases, such as Alzheimer's disease (AD) [3]. Stress is considered the common risk factor of 75% to 90% of diseases. While modernization of life has increase lifespan, it also has contributed to the exposure to multiple stressors [4]. High cortisol may also have deleterious effects on the brain structures, including hippocampus, contributing to neurodegeneration pathology [5, 6].

Galactose is a monosaccharide, found in dairy products, sugar beets, and mucilages. It is synthesized in the body and forms part of glycolipids and glycoproteins [7]. Chronic administration of D-galactose was used to induce brain aging in animal models, through oxidative stress and inflammation [1]. D-galactose interacts with free amine via nonenzymatic glycation, resulting in the formation of advanced glycation products which results in the generation of reactive oxygen species (ROS). Also, excessive intake of D-galactose resulted in oxidative metabolism and release of ROS [8]. These effects lead to cognitive impairment and affecting of memory and learning [9].

Acupuncture is considered as traditional Chinese medicine and recognized as a treatment in China and Western countries [10]. It has been used in the treatment of brain diseases and mental disorders. It has provided therapeutic benefits to cognitive disorders and gained recognition from doctors and the general public in China [11]. Electroacupuncture (EA) is considered as a potential strategy for the treatment of memory impairment such as Alzheimer's disease, through its decreasing the oxidative stress and neural injury [12]. However, the effect of EA on cognitive function remains controversial and needs more studying. So, we aimed to investigate the effect of electroacupuncture on memory and learning in D-galactose induced rat brain aging and elucidate the more underlying mechanisms.

2-Materials and Methods

This research protocol was approved by the Local Ethics Committee of Faculty of Medicine, Menoufia University, Egypt. The animals were treated following Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

2.1-Study design and animals

Forty Wistar male albino rats aged two to three months weighing (180±20g) were used in this study. They were kept on standard laboratory chow and water ad libitum and housed in a cage (70X70X60cm, 5per cage) at the animal house of Faculty of Medicine, Menoufia University. The animals were acclimatized to these conditions for one week before the experiment and divided into 4 groups (10 animals each).

Group 1: Control group (C), rats were injected by 0.9% saline intraperitoneally (i.p.) (0.5ml/rat) once daily for 10 weeks, and anesthetized with 5%

isoflurane for 15 minutes, six days/week, for the last 2 weeks.

Group 2: Electroacupuncture group (CE), rats were injected with 0.9% saline i.p. (0.5 ml / rat) once daily for 8 weeks. On day 57, rats have received EA at acupuncture points, 15 minutes daily, six days/week, for the last 2 weeks [13].

Group 3: D-galactose group (D), D-galactose powder was dissolved in 0.5 ml 0.9% saline and injected i.p in a dose of 100 mg/ kg once daily for 10 weeks [14]**,** and anesthetized with isoflurane for 15 minutes, six days/week, for the last 2 weeks. D-galactose powder was purchased from (S D Fine-CHEM Ltd, Mumbai, India).

Group 4: Electroacupuncture -D-galactose group (DE). D-galactose was given in a dose of 100 mg/ kg i.p. once daily for 8 weeks. On day 57 rats have received EA at acupuncture points, 15 minutes daily, six days/week, for the last 2 weeks**.**

At the end of the study period, spatial learning and memory of all groups were assessed Then, rats were sacrificed by cervical decapitation. retroorbital blood samples were collected to measure cortisol and Adrenocorticotropic hormone (ACTH) levels. Whole brains were extracted and separated into two halves; half brain samples prepared for histopathological assessment. The remaining brain halves were homogenized to assess malondialdehyde (MDA), reduced glutathione (GSH) and tumor necrosis factor-alpha (TNF- α) levels.

2.2- Electrical acupuncture technique

Rats were anesthetized with isoflurane to minimize stress during EA stimulation sessions. Two bilateral stainless-stain needles (0.18 mm diameter) were inserted to a depth of approximately 2 mm at the murine acupoints corresponding to Baihui acupoint (GV20, the midpoint of the line connecting the apexes of both ears on the parietal bone) and Dazhui acupoint (GV14, the posterior midline in the depression below the spinous process of the 7th cervical vertebra) and were connected to a Grass S88 electro stimulator [13]. EA stimulation lasted for 15 min at a frequency of 2 Hz and the output voltage was set at 2 V following previous studies six days/ week, for 2 weeks. Fig. 1a

2.3-Assessment of spatial learning and memory -Barnes maze test [15]

Barnes maze is formed of a circular platform (90 cm in diameter) with 18 spaced holes (5 cm diameter; 7.5 cm between holes) along the perimeter and is placed 100 cm above the floor. In the Barnes maze, rats received bright light and noise as sources of reinforcement to escape from the open platform surface to a small dark recessed chamber located under the platform called (target box) or (escape box) (28 × 22 × 21cm) through the target hole (7 cm diameter). All the experimental conditions should be the same, as Animals had spatial visual cues endogenous to the room (for example a door, a desk or a computer). These cues should not be moved during the whole experiment as these are the animal's reference points for locating the target hole. Escape latency (time interval to reach the target hole in seconds) and number of errors (total number of head deflections into incorrect holes) during training days of the acquisition phase and trial of probe phase were analyzed and calculated using Sony Digital Still Camera DSC-HX350, Japan. Fig. 1b. To void odor cues of the animals, 70% ethanol was used to clean the apparatuses.

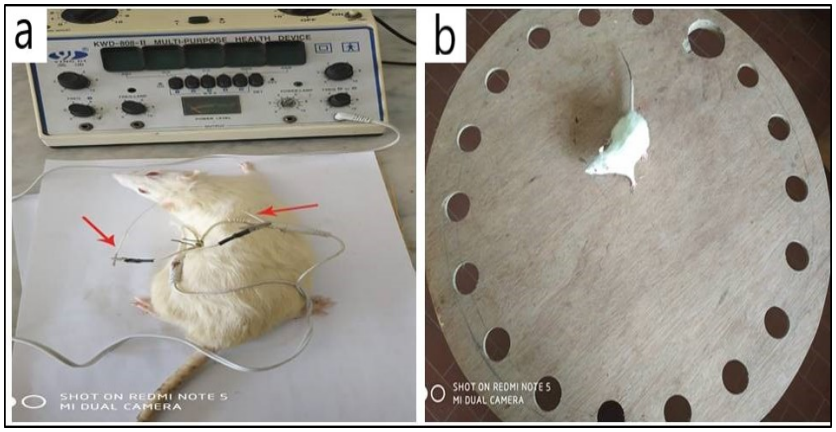

Fig. 1 a: Rat on Electrical acupuncture device **b**: Rat on Barnes maze

2.3.1-Acquisition phase: (for assessment of spatial acquisition or learning):

The rat was placed in the center of the maze. After 10 seconds had elapsed, the buzzer was switched on and the rat was allowed to explore the maze for 3 minutes. The numbers of errors and escape latency were recorded by the experimenter. Immediately after the rat entered the box, the buzzer was turned off and the rat was allowed to stay in it for 1 minute. Rats may go out of the escape box during the time they stayed in it, so the escape hole was covered once the rat was inside it for 1 minute. Rats were placed in their home cage until the next trial. Rats received 4 trials per day with an inter-trial interval of 15 minutes during 4 days of the acquisition phase.

2.3.2. Probe phase: (for assessment of short term spatial memory retention):

On day 5, 24 hours after the last training day, the rat was allowed to explore the maze for 90 seconds. The probe trial is done to determine if the animal remembers where the target hole was located. Numbers of pokes (errors) in each hole and escape latency to reach the virtually target hole were measured.

2.4 Blood sampling

retroorbital blood samples (about 2 ml) were collected into ice-cooled centrifugal tubes and centrifuged (1700 ×g, 10 min, 4°C). The serum collected was stored at −80°C until hormone assay for cortisol and Adrenocorticotropic hormone (ACTH) levels.

2.4.1 Measurement of serum cortisol and ACTH hormones

Blood serum was used to measure cortisol and ACTH by Enzyme-linked immunosorbent assay (ELISA) technique (Catalogue Number: SE120082) as described by McComb et al. [16] for cortisol and according to Makrigiannakis et al. [17], for ACTH **(**Catalogue Number: SE120081), they were purchased from Sigma Aldrich, Louis, MO 73103, USA

2.5. Measurement of MDA, GSH, and TNF-α levels

The brain tissue was homogenized using a test tube and metal probe in 5-10 ml cold buffer (i.e. 50 mM potassium phosphate, pH 7.5) per gram tissue and centrifuged at 4000 r.p.m for 15 minutes at 4˚C. The supernatant was used for measurement of MDA by colorimetric method using thiobarbituric acid reaction according to the method described by Draper et al. [18]. GSH was

measured by the colorimetric method as described by Beutler et al. [19]. MDA and GSH kits were purchased from Biodiagnostic Company, Egypt. TNF-α was measured by ELISA using kits from Assaypro LLC, Charles, MO, USA as described by Taylor [20].

2.6-Histopathology assessment:

2.6.1. Hematoxylin and Eosin (H&E) staining [21]

Brain halves from each rat were placed in 10% formaldehyde for 2 hours. The brains were removed and placed in a new formaldehyde solution for 24 hours before being dehydrated using ethanol (70% for 24 hours, 90% for 1 hour and 100% for 1 hour) then cleaned in xylene and embedded in paraffin. Coronal sections were cut using a microtome (Leica RM 2025, Germany) at 5 µm thicknesses, mounted on glass slides and stained with the routine hematoxylin and eosin technique for assessment of structural changes.

2.6.2. Morphometric study

For quantitative assessment, We made three sections of each rat and obtained two nonoverlapped fields in each section. So, six fields were obtained from each rat. The two nonoverlapping fields of H and E-stained slides at a magnification of ×400 per section were randomly captured using a digital camera (Olympus, Japan) from regions (CA1, CA3and dentate gyrus) of the hippocampus , the number of pyramidal cells and apoptotic cells were counted in CA1 and CA3 regions and the number of granular cells and apoptotic cells were counted in the dentate gyrus. Fields taken from at least three anatomically comparable sections were assessed using image J-analyzer software (NIH image, Maryland, USA) and the number for each cell type was averaged per field for each animal. The number calculated for at least five animals/ experimental group were considered for comparison and statistical analyses.

2.6.3. Congo red stain

The Amyloid Stain (Congo Red) is intended for use in the histological visualization of amyloid in brain tissue sections according to the method described by Garg and Nigam [22].

The procedure of staining:

1. Deparaffinize and hydrate sections to distilled water.
2. Stain in Congo red solution for 30-60 minutes.
3. Rinse in distilled water.
4. Differentiate rapidly (5-10 dips) in alkaline alcohol solution.
5. Rinse in running tap water for 5 minutes.
6. Counterstain in hematoxylin for 30 seconds.
7. Rinse in tap water for 1 minute.
8. Dip in ammonia water for 30 seconds or until sections turn blue.
9. Rinse in tap water for 5 minutes.
10. Dehydrate through 95% alcohol, 100% alcohol
11. Clear in xylene and mount with resinous mounting medium.

Interpretation of staining results: Amyloid, elastic fibers, eosinophil granules stain red and the nuclei stain blue.

Examination of slides, photography and morphometric studies were done at Pathology Department, Faculty of Medicine, Menoufia University.

2.7-Statistical Analysis

The results of the experiments were expressed as the means ± standard error of the mean (S.E.M.). We used a one-way analysis of variance (ANOVA) followed by post hoc (Tukey test) to

determine the significance of differences between groups using SPSS (version, 22). P<0.05 is considered significant.

3. Results

3.1. Barnez maze

3.1.1. acquisition phase (Learning)

The mean number of errors in D group was significantly higher (P <0.05) compared to C and CE groups (on days 1, 2, 3 and 4). In CE group, it was significantly higher (P <0.05) when compared to C group (on day 3). In DE group, it was significantly lower (P <0.05) compared to the corresponding value in D group (on day1, 2 and 3) while it was still significantly higher (P<0.05) compared to the C group (on days 1 and 2). Fig. 2a

The mean of escape latency was significantly higher (P<0.05) in D group when compared to C and CE groups (on day 2, 3 and 4). In the DE group, it was significantly lower (P<0.05) compared to D group (on days 1, 2, 3 and 4), while it was significantly higher (P<0.05) when compared to C and CE groups (on day 4). Fig. 2b

3.1.2. probe phase (Short term Memory)

The short term memory was carried 24 hours after the last training day (day 5). The mean number of errors and the mean of escape latency in D group were significantly higher (P <0.05) when compared to C and CE groups. While in DE group they were significantly lower (P<0.05) compared to D group. In DE group, the mean of escape latency was still significantly higher (P<0.05) compared to C and CE groups. Fig. 2c, 2d

3.2. Biochemical results

MDA (nm/gm tissue) and TNFα (nm/gm. tissue) levels in D group were significantly higher (P<0.05) compared to C and CE groups. In DE group, they were significantly higher (P<0.05) compared to CE group and significantly lower (P<0.05) compared to D group. TNFα level in DE group was significantly higher (P <0.05) compared to the corresponding value in C group. Tab. 1

GSH (nm/gm tissue) levels in D group was significantly higher (P<0.05) when compared to C and CE groups. In DE group, it was significantly higher (P<0.05) when compared to the C and CE groups. Tab. 1

Serum cortisol (ug/dl) and ACTH (pg/ml.serum) levels in D group were significantly higher (P<0.05) compared to C and CE groups. In DE group, they were significantly lower (P<0.05) compared to D group while, significantly higher (P<0.05) compared to C and CE groups. In CE group, they were significantly lower (P<0.05) when compared to C group. Tab.1

3.3. Histological Results

3.3.1. Hematoxylin and Eosin (H&E) staining

Sections of the hippocampus and dentate gyrus of DE group have the same structure as C group.

Sections of the hippocampus of D group showed a decreasing number of pyramidal cells (hypocellularity) with disarrangement. The dentate gyrus of this group revealed marked disarrangement of the granular layer cells with many apoptotic cells with pyknotic darkly stained nuclei. Apparent increased number of astrocytes and microglia was observed denoting gliosis.

Sections of the hippocampus of DE group revealed that most of the pyramidal cells were arranged at the same line. The dentate gyrus of this group revealed few numbers of immature granular cells. However, the hilar cells appeared well organized with vesicular rounded nuclei; some astrocytes appeared among hilar cells. astrocytes and microglia (gliosis) were also seen in the field. Fig. 3.

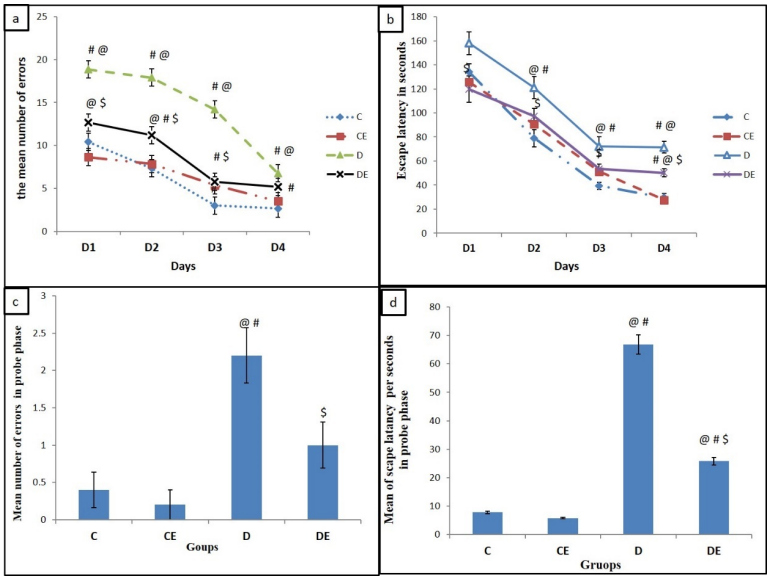

Fig. 2 Illustration of results of the **acquisition phase** of Barnes maze test for all groups; **a-** the mean number of errors; **b-** the mean of escape latency in seconds per day in 4 days (D1-D4), the results of **probe phase** of Barnes maze test for all groups; **c-** the mean number of errors; **d-** the mean of escape latency in seconds (s) in day 5. Data were expressed as mean ±SE (n=10).one way ANOVA: #p<0.05 versus the C group;@ p<0.05 versus the CE group;$p<0.05 versus the D group

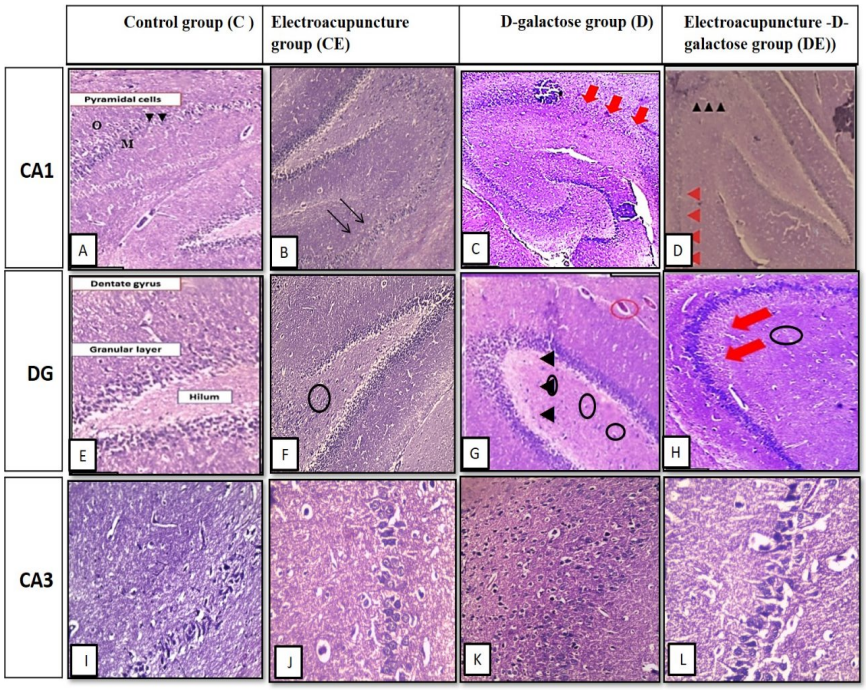

Fig. 3 section of CA1of hippocampus and dentate gyrus for all group showing; **A-** The three layers of CA1 area in the control group: outer polymorphic (O), middle pyramidal (P) and inner molecular layer (M). The pyramidal cells are closely packed together and normal cellularity; **B-**The pyramidal cells of Electroacupuncture-treated group (CE) are closely packed together and normal distrupution; **C-** showing significant hypocellularity of the pyramidal cell layer in D-galactose treated group(D) (red arrows); **D-** (CA1) area with many of pyramidal cells appear on the same line(red arrows). Section of (CA_3) area of rat hippocampus of (DE) group showing many of pyramidal cells with nearly normal appearance(black arrows) **(H and E; a, b, c and d: x40); E-** Section of rat dentate gyrus of (C) group showing the apex and parts of the 2 blades. Granular layer and hilar well organized (**H and E ×200**); **F-** Section of rat dentate gyrus of (C E) group showing Granular layer and hilar are well-organized (**H and E: × 100**); **G-** D-Galactose Group reveals severe congestion of the blood vessels (red circles) and severe fluid accumulation in the granular cell layer of the dentate gyrus with abnormal arranged in the granular cell layer(black arrows) increase in astrocytes (gliosis)in hilum (black circles) (**H and E: × 200**); **H-** Section of the hilar region of (DE) group showing nearly normal well organized hilar cells and Mild fluid accumulation in the granular cell layer of the dentate gyrus in (DE) group (red arrows) and astrocytes (gliosis)in hilum (black circles)**.** Section of CA3 region of hippocampus showing no significant microscopic change in both the control group (I) (H and E × 400) and in the electro-accupuncture group (J). D galactose group (K) shows significant increase in the cellularity. A decrease in the cellularity was noticed in the Electro-acupuncture D-Galactose Group (L) **(H and E; I, J, K and L: x400)**

Table 1: MDA, GSH, TNFα, serum cortisol and ACTH in the control group (C), Electroacupuncture-treated group (CE), D-galactose treated group (D)and Electroacupuncture and D-galactose cotreated group (DE)

	C	CE	D	DE
MDA(nm/gm.tissue)	24.2±1.31	15.40±2.50	54.40±3.12$^{\#\,@}$	37±5.04$^{@\$}$
GSH (nm/gm.tissue)	17.29±0.57	17.62±2.19	7.35±0.71# @	9.69±0.74$^{\#\,@}$
TNFα (nm/gm.Tissue)	38.31±2.74	44.23±2.86	118.07±0.88$^{\#\,@}$	71.52±2.47$^{\#\,@\,\$}$
SerumCortisol (ug/dl)	5.67±0.29	2.02±0.14$^{\#}$	8.31±0.33$^{\#\,@}$	3.33±0.30$^{\#\,@\,\$}$
Serum ACTH(pg/ml)	67.20±2.43	49.80±1.019$^{\#}$	110.60±1.63$^{\#@}$	78.20±1.061$^{\#\,@\,\$}$

Data are expressed as mean ±S.E.M. (n=10).one way ANOVA: #p<0.05 versus the C group;@ p<0.05 versus the CE group;$ p<0.05 versus the D group.

3.3.2. Morphometric results

Granular cell count for area % in the dentate gyrus and Pyramidal cell count for area % in (CA1 and CA3) regions in D group were significantly lower (P<0.05) when compared to C and CE groups. In DE group, it was significantly higher (P<0.05) compared to D group, while it was significantly lower (P<0.05) compared to CE and C groups. Only granular cell count for area % in the dentate gyrus in CE group was significantly higher (P<0.05) compared to C group. Tab. 2

Apoptotic cell count for area % in (CA1, CA3 and dentate gyrus) regions in D group were significantly higher (P<0.05) compared to C and CE groups. In DE group, it was significantly lower (P<0.05) compared to D group while still significantly higher (P<0.05) compared to CE and C groups and. Tab. 2

3.3.3. Congo red stain

Congo red stain of brain sections in C group revealed normal thickness of the vascular wall without narrowing in lumen or structure abnormality. Fig. 4a

In CE group, sections revealed no significant change in the thickness of the vascular wall whit the same results as in C group. Fig. 4b

In D group, sections showed structureless homogenous thickening of the vessel wall leading to narrowing of the lumen due to deposition of amyloid plaque in the wall of the blood vessel. Fig. 4c

In DE group, sections of the hippocampus of this group showed normal vessel wall thickness present in some area and in other area appear structureless homogenous red material causing thickening of the vessel wall leading to narrowing of the lumen due to deposition of amyloid. Fig. 4d

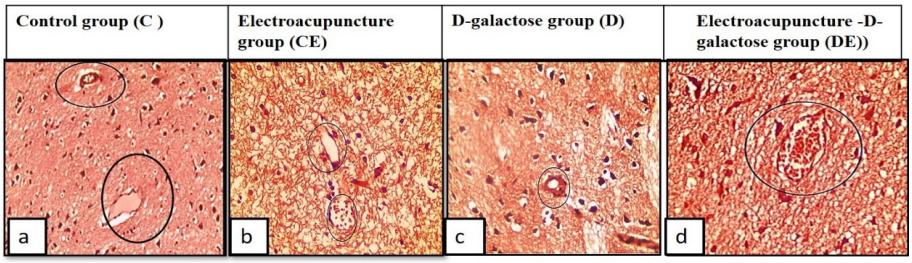

Fig. 4 Congo red stain of brain slices show; **a**- The normal thickness of the vascular wall (black circles) in the control group; **b**- No significant change in the thickness of the vascular wall (black circles) in control-treated with the Electroacupuncture group; **c**- Structureless homogenous thickening of the vessel wall leading to narrowing of the lumen in the D-galactose treated group; **d**- Normal vessel wall thickness while other areas displayed structureless homogenous red material causing thickening of the vessel wall leading to narrowing of the lumen (black circle) in Electroacupuncture and D-galactose cotreated group (D) **(Congo red stain 400x)**

Table 2: Pyramidal cell and Apoptotic cell count for area % in (CA1, CA3)and Granular cell and Apoptotic cell count for area % dentate gyrus of in the control group (C), Electroacupuncture-treated group (CE), D-galactose treated group (D)and Electroacupuncture and D-galactose cotreated group (DE).

Count of area %	C	CE	D	DE
CA1				
Pyramidal cell	90±3.53	93±2.69	31±3.69 # @	66±4.30 # @ $
Apoptotic cell	1.80±0.37	2±0.44	16.40±2.3 # @	8.60±0.67 # @ $
CA3				
Pyramidal cell	70±2.62	69.8±5.20	29±5.56 # @	47.80±3.44 # @ $
Apoptotic cell	2.20±0.58	3±0.31	16±1.14 # @	7.40±0.76 # @
Dentate gyrus				
Granular cell	159±4.30	183±4.63 #	73±3.47 # @	104±4.30 # @ $
Apoptotic cell	2± 0.44	2.4±0 .50	30.6 ± 2.85 # @	10.8±0 .73 # @ $

Data are expressed as mean ±S.E.M. (n=10).one way ANOVA: #p<0.05 versus the C group;@ p<0.05 versus the CE group;$ p<0.05 versus the D group.

4. Discussion

The aging-induced degenerative functional and morphological changes in hippocampal neurons may affect learning and memory function [23], also high cortisol may exert neurotoxic effects on the hippocampus, and contribute to the neurodegenerative pathology, via promoting oxidative stress, inflammatory mediators and amyloid β peptide toxicity [6].

In the present work and concordance with several studies [24, 9, 25, 1], chronic injection of D-galactose (100 mg/kg) i.p for 10 weeks induced spatial learning and memory-related changes assessed by Barnes maze test in the form of increases in the mean number of errors and mean of escape latency per day denoting short term spatial memory retention impairment. This impairment of spatial learning and memory may be contributed to the induction of brain oxidative stress, caused by D-galactose that led to hippocampal damage. Our results revealed that D-galactose caused a significant increase in MDA, TNFα levels and a significant decrease in GSH level in brain tissue that caused neuronal damage, especially in the hippocampus.

Also, it caused a significant increase in serum ACTH and cortisol levels that induced chronic stress. The hippocampus and it's mnemonic functions are deemed particularly sensitive to stress because hippocampal cells pack high concentration of receptors for corticosteroids (glucocorticoids and mineralocorticoids), whose synthesis and secretion by the adrenal cortex are augmented by stress [26].

Our results revealed that D-galactose significantly decreased the pyramidal cell count for area % in CA1, CA3 and granular cell count for area % in DG and increase apoptotic cell count in CA1, CA3 and DG comparing to control group. Chronic stress increased the oxidant level and inflammatory mediators may be related to these changes. Cortisol has neurotoxic effects by

stimulating neuronal degeneration through increased susceptibility to metabolic and vascular injuries, reduction of dendritic length, and cell death possibly associated with apoptosis [27]. It is associated with aging-related outcomes at cognitive, emotional, mental, and neurobiological levels [28].

Sections of the hippocampus of the D-galactose treated group showed structureless homogenous thickening of the vessel wall leading to narrowing of the lumen due to deposition of amyloid plaque in the wall of the blood vessel. Barage and Sonawane [29] reported the presence of amyloid-β (Aβ) senile plaques in the brains of Alzheimer's disease (AD) patients which are directly or indirectly responsible for the ensuing neurodegeneration and memory loss

Electroacupuncture and D-galactose co-treated group (DE) showed a significant improvement in spatial learning and memory performance when compared to (D) group that was apparent in day1 of acquisition phase and day5 of probe phase of Barnes maze test as the mean number of errors and mean of escape latency per day in (DE) group were significantly lower when compared to (D) group and significantly higher when compared to the control values. These results were in agreement with a study conducted by Liu et al. [30]. The improvement in spatial learning and memory performance in (DE) group may be due to the antioxidant and anti-inflammatory effect of electro-Acupuncture as MDA and TNFα level in brain tissue homogenate of (DE) group was significantly lower when compared to (D) group and GSH level in brain tissue homogenate was significantly higher when compared to (D) group this was in agreement with Phunchago et al. [31]. Electroacupuncture and D-galactose co-treated group significantly increased the pyramidal cell count for area % in CA1, CA3 and granular cell count for area % in DG and decrease the apoptotic cell count in CA1, CA3 and DG comparing to D-galactose group. These results may be contributed to the antioxidant effect, anti-inflammatory, and anti-stress effects of electroacupuncture that caused improvement in the hippocampal histological and morphometric picture of this group than the (D) group. Xu et al. [32] detected that acupuncture reduces apoptosis, necrosis of the neurons, and protect the hippocampus and cortex through decreasing the level of cytokines in the hippocampus and adjusting the cholinergic system and neurotrophic factors.

Additionally, it has been suggested that acupuncture can increase the activity of superoxide dismutase (SOD) and decrease the level of malondialdehyde (MDA) in the brain to improve the antioxidant capacity and reduce brain tissue damage caused by free radical [33]. Acupuncture can improve the learning-memory ability of Alzheimer's disease mice, which may be related to its effects in up-regulating the contents of serum Aβ internalizing enzymes and promoting the clearance of hippocampal Aβ by inhibiting the phosphorylation level of the mammalian target of rapamycin (mTOR) [34]. Acupuncture on specific acupoints improves the cerebral blood flow and hippocampal connectivity [35].

Electroacupuncture treatment has an antistress effect as our results showed a significant decrease in cortisol and ACTH levels comparing to the control and D-galactose groups. Previous studies have shown that acupuncture may reduce the

stress-induced by excess cortisol [36] and the mechanism of action of acupuncture has been associated with the blockade of the stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation [37].

Corticotropin-releasing hormone (CRH) concentrations in the hypothalamus were significantly decreased after EA [38]. AD involves progressive dysregulation of the HPA which is governed by the secretion of CRH from the hypothalamus, which in turn activates the secretion of ACTH from the anterior pituitary that finally stimulates the secretion of cortisol [39].

Sections of the hippocampus of Electroacupuncture and D-galactose co-treated group showed significant increase in the number of pyramidal and granular cells with decrease in apoptotic cells also showed normal vessel wall thickness present in some area and other areas appear structureless homogenous red material causing thickening of the vessel wall leading to narrowing of the lumen due to deposition of amyloid plaque in the wall of the blood vessel. Tang et al. [34] reported that Electroacupuncture treatment of transgenic mice decreased the amyloid precursor protein (APP) and β-Site amyloid precursor protein cleaving enzyme 1 (BACE1) in the hippocampus. So, EA exerted neuronal protection and induced improvement of memory and learning abilities.

Conclusion

Electroacupuncture has a beneficial effect against oxidative stress-induced spatial learning and memory impairment in D-galactose-induced rat brain aging through antioxidant, anti-apoptotic and anti-stress properties.

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Conflict of interest

The authors declare no conflict of interest.

Reference

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