

Original article

Neuroprotective Effect of Coffee and Tea on Alzheimer's Disease in a Rat Model

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Keywords

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ABSTRACT

Coffee and Tea are among the most popular beverages in the world. They contain many bioactive substances such as polyphenols and catechins that could have biological activity. For instance, antioxidant activity. The way of preparing tea and coffee drinks is different among societies. Therefore, the method of extraction could influence the type and quality of extracted substances. The goal of the current study was to examine the neuroprotective properties of coffee and tea beverages made by using the boiling technique of preparation on Alzheimer's disease symptoms. Tea and Coffee beverages were prepared by boiling in water and gavaged orally to rats in a concentration of 10% w/v for 1 week followed by i.p injection of scopolamine 2mg/kg, afterward, the behavioral and amnesic parameters for Alzheimer's disease were tested using Open field, Morris water maze, and Object recognition tests. Acetylcholine brain levels were also measured using Ellman's method. Brain histopathological investigation was also carried out to compare changes in both test and control groups. Noted that treated groups that received Coffee and Tea showed significant ($p < 0.05$) improvement in locomotor activity in the open field test and amnesia in the object recognition and Morris water maze tests compared to amnesic rats that were given scopolamine. In addition, biochemical estimation showed that improve in acetylcholine level of treated groups compared to induced group, furthermore in histopathological examination showed a significant difference tissue necrosis in the brains of treatment groups. The results pointed to the possible neuroprotective effect of Coffee and Tea against Alzheimer's disease. The mechanism of protection might be due to antioxidant activity.

Introduction

Coffee and tea are among the most popular beverages in the world for ages. Drinking tea and coffee has become an integral part of the day at work; frequent consumption of tea and coffee has been shown to have many benefits for health [1]. On the contrary, some reports pointed to some harmful effects on health [2]. In Libya drinking tea and coffee is a daily habit among various age groups. However, the Libyans way make tea and coffee is different from most other societies. Hence, the method of preparation significantly impacts the extraction of constituents from tea or coffee [3]. For example, in Libya they prepare tea by prolonged cooking while in most other countries people make tea by macerating tea leaves in water for a short period. Tea is obtained from fermentation of leaves and buds of the *Camellia sinensis* plant; generally, there are three types of tea the differ in processing method and degree of fermentation: black tea (fully fermented), oolong tea (partially fermented), and green tea (not fermented) [4]. As a result of the tea processing technique, black tea leaves are oxidized but green tea leaves are not [3].

Coffee comes in third place after water and tea as the most widely consumed drinks [5]. Coffee comes in two types: roasted and instant. The first type is prepared by roasting green coffee beans. Markedly, the temperature of roasting determines the kind of roasting, while the instant coffee is prepared by hot water extraction of substances from roasted coffee beans [6]. Coffee and tea are rich in bioactive substances importantly, caffeine, and polyphenols, such as flavonoids and catechins [7]. To date, many epidemiological studies have suggested beneficial implications of antioxidants in coffee and tea for protection against neurodegenerative diseases, such as Alzheimer disease and Parkinson's Disease [8].

Alzheimer's Disease (AD) is the most prevalent neurodegenerative condition that causes dementia in the elderly. It is characterized by a progressive decline in memory and other cognitive abilities [9]. A further indication that oxidative stress may play a role in Alzheimer's disease-related synaptic loss is the increased oxidative damage to lipids and proteins in Alzheimer's disease and mild cognitive impairment (MCI) brains, as well as the decline in glutathione and antioxidant enzyme activities, which are more localized to the synapses and correlate with the severity of the disease [10]. Several studies found elevated levels of oxidative stress in mild cognitive impairment (MCI), which is thought to be a transitional stage between normal aging and dementia. These findings imply that oxidative stress may be one of the initial changes that take place during the onset and progression of Alzheimer's disease [11]. Although Oxidative stress has become one of the

key contributors to the pathophysiology of Alzheimer's disease. Nonetheless, using neuroprotective agents from natural origin could be a successful approach in controlling neurodegenerative diseases [12]. It is widely though antioxidant may protect against AD [8]. Hence, consumption of polyphenols may decrease the incidence of oxidative stress related to AD [6]. In this study, we examined whether the coffee and tea made by the Libyan method have a protective effect against AD.

Methods

Drugs and chemicals

Scopolamine hydrobromide obtained from (sigma- Aldrich- Canada), acetylthiocholine iodide obtained from (Sigma- Aldrich- USA). Coffee (*Arabica coffee*), decaffeinated coffee, green tea (Alnaja tea), and black tea (Al Mead tea) were all obtained from the local market in Zawia city. Libya. 0.9% normal saline (Braun Melsungen AG, Germany), donepezil 10mg (Italy) all obtained from a local pharmacy in Zawia city. Libya. Ellman's reagent (5,5-dithiobis (2-nitrobenzoic acid) (DTNB) (BDH Chemical Ltd, Poole – England), phosphate buffer, distilled water, chloroform (Lab Alley Powering Science, Texas, USA), formalin 10% (AQUA Chemical –Turkey), ethanol 100% (Astral Diagnostic – USA), paraffin wax (AMOS Science, Germany), eosin/hematoxylin dye (ORSA tec GmbH – Bobingen) all obtained from Libyan Medical Research Center (LMC), Zawia, Libya.

Preparation of Coffee and Tea Solution

Ten grams of either coffee (caffeinated & decaffeinated) and Tea (black & green) was introduced to beaker containing 100 ml of distilled water and boiled for 5 minutes at 250°C, after cooling, filtrate the mixture by using Whatman filter paper no.1. the filtrate was given by oral gavaging (og) in a volume of 2ml/kg for rats.

Animals

Forty-two male Wister albino rats of 2 months old age, weighting between (150-250 g). All these animals kept in plexiglass cages, under a cycle of 12 h of light and 12 h of dark in temperature about 25°C, animals were approved by ethical committee of the Libyan Medical Research Center (LMC). Before experiment all animals kept with progressively deprived of food and water to maintain their body weight. All experiments were done in accordance with the international principles of laboratory animal care and use.

Induction of Alzheimer's disease

The principle to induce Alzheimer's disease like symptoms by intraperitoneal (i.p) injection of scopolamine hydro bromide (2mg/kg) for one week [13].

Experimental Design

The animals were divided into 7 groups of 6 rats each: a control group receiving distilled water; a scopolamine group (SCP) receiving scopolamine 2mg/kg; 4 treated groups receiving 10% w/v of coffee, decaffeinated coffee, black tea, and green tea respectively followed by the injection of scopolamine (2mg/kg i.p), and a positive control group receiving donepezil (30 mg/kg) followed by injection of scopolamine (2mg/kg i.p). Donepezil and treated groups were administered orally 1h before scopolamine injection. All treatments were daily administered for one-week of consecutive days. On the last day, 45 minutes after scopolamine injection, all groups used for behavioral, histopathological, and biochemical parameters were estimated using suitable tests. Later, rats were sacrificed by cervical dislocation under mild anesthesia using chloroform. Brains were taken out for biochemical and histopathological analysis.

Estimation of Behavioral and Exploratory Parameters

Locomotion Activity in Open Field.

The objective of the open field test is to assess locomotor and exploratory behavior in rats. The test is based on the concept of an open novel space from which escape is blocked by a surrounding wall [14]. The apparatus used in the study was a square (50×50 cm) with 42 cm-high opaque walls. The floor was divided into 25 equally spaced squares. Testing was conducted in a silent room under white light [15]. To observe the activity, animals were taken out of their home cages one at a time. Activity in the open field was measured by monitoring the latency period in the center and counting the number of squares crossed (all four paws) and the number of rearing for five minutes, as previously described by [16]. To avoid order effect, activities of control rats were monitored as well as those of drug treated rats.

Novel Object Recognition Test

A novel object recognition (NOR) test is a cognitive test for animals that was first developed by [17]. It was used to test a rat's ability to recognize a new object in a known environment. This test is a recognition memory test. The method used in this experiment was the same as Okuda et al, [18], which used two similar objects to be exposed to the rat and then recorded the discriminating ability of the rat to discriminate a novel object placed simultaneously with one of the familiar objects. The apparatus used in this experiment is a square box made from grey-painted wood with dimensions of

45×45×45cm³. To saturate the box with odor, cleaning of the box was not permitted throughout the experiment. The objects used in this experiment were two similar opaque glasses filled with white cement (familiar A1, familiar A2) that were heavy enough that rats couldn't move them. The rats were placed in the middle of the box, facing the wall. The apparatus and the objects were explored. The test was performed in three phases: pre-exposure, training, and test session. The rats were pre-exposed to the testing chamber on day 1 for 10 minutes. The following day, 24 hours later, rats were placed inside the pre-exposure box with two analogous objects (A1, A2) and allowed to explore for 5 minutes. At the end of the 5-minute exploration period, rats were removed from the NOR box and returned to their home cage. They were also removed from the NOR boxes for 20 minutes before the 3-minute test phase began. The rats were removed from their home cages and returned to the NOR box, where they were exposed to one novel object (N) for 3 minutes. The sniffing time was measured for the new and familiar objects. Exploration of an object is when the rat directs its nose toward the object at <2cm distance. Total exploration time for the familiar and novel objects was used to calculate the discrimination index to measure cognitive performance as described by Okuda et. al. 2004. The discrimination index is a measure of the discrimination between the familiar and the new objects corrected for exploration activity. It can be calculated as follows:

$$DI = \frac{(\text{time spent on novel object} - \text{time spent on familiar object})}{(\text{time spent on novel object} + \text{time spent on familiar object})}$$

The discrimination index can range from -1 to 1, with -1 indicating complete preference for the familiar object, 0 indicating no preference for either object, and 1 indicating complete preference for the novel object.

Morris Water Maze Test

The Morris water maze test is a behavioral test used to test many aspects of learning and spatial memory for rodents. The MWM test was designed to assess the effects of spatial memory on hippocampus-dependent spatial learning and memory. Richard G. Morris developed the Morris water maze in 1981. In the Morris water maze test, an animal is asked to locate a hidden platform just below the surface of the circular pool of water. It is also asked to remember the location of the hidden platform as in the previous trial. The maze used in the Morris water maze training for rats is the same as the one used in the study with some modifications [19]. The circular pool of water has a diameter of 45 cm, a height of 37 cm, and a depth of 12 cm. The inner surface of the pool is a metal cylinder painted white. The escape platform is also a metal cylinder with a flat metallic top. The surface diameter of the cylinder is 8 cm, and the platform is 2 cm below the surface of the water. The pool filled with water is made opaque with milk to obscure the platform.

For four days, the rats had four consecutive trials spaced five minutes apart. They were gently positioned with their backs to the tank wall in one of the four apparently equal quadrants. Each time a new trial was conducted, the animals were placed in the water in the following quadrant, and the amount of time it took them to realize they were on a platform was noted as "escape latency". Any rat that discovered the platform was permitted to stay on it for 20 seconds, and escape latency data were recorded for up to 120 seconds (cut-off). When the rat didn't find the platform after 120 seconds, it had to be manually led to the platform by hand. After the procedure of the acquisition trials, the rats were towel-dried and put in their cages. The platform was taken out of the tank on day 5, the last day of the acquisition trials, and the animals were free to swim around in the tank for 120 seconds. The amount of time spent in the target quadrant was then recorded.

Active Avoidance Shuttle Box

Every rat was acclimated to the device for two minutes prior to avoidance training. Each session began with the placement of a rat in the left compartment, facing the end wall and close to it. The animal is given a sound stimulus for 10 seconds after a light stimulation for 30 seconds in each session. Immediately the rat was shocked once with a low intensity foot shock (0.5 mA; 3 s) through the grid floor just after the auditory stimulus. For five days, each animal had a daily session of 15 trials with a 15-second inter-trial interval, for a total of 75 trials [20].

Time spent moving between compartments, and the number of avoidances to the stimulus were recorded. A considerable rise in the avoidance reaction on the fifth session (retention) compared to the first session (training) was considered to be a marker of improved cognitive activity. To avoid outside interferences with the experiment, all behavioral models were conducted in a semi-dark, soundproof room [21].

On the 8th day, after checking the behavioral parameters, rats were at that point decapitated, and their brains were removed from the skull within 30 s after finishing. The layer covering the brain was removed with the aid of fine forceps. The brain at that point was taken out utilizing the spatula was immersed in ice-cold saline, and each brain was divided into two portions. The first portion was put away at -70 °C until examination of the oxidative status of the neurochemical profile, and the second portion was fixed in formalin buffer (10%) for histopathological examination.

Estimation of Oxidative Stress Biomarkers

Brain tissue were powdered by liquid nitrogen. Then; they were homogenized with 1 ml of cold PBS (phosphate buffer solution) on ice. The resultant homogenates were centrifuged for 10 min at 4 °C at 10,000 rpm. Supernatants were moved to another tube for estimation of acetylcholinesterase activity.

Estimation of Acetylcholinesterase Activity

The amount of acetylcholinesterase was estimated by the method described by Ellman [22]. Acetylcholinesterase (AChE) activity was estimated in the brain homogenates according to Ellman's method [23]. Briefly, the brain homogenate was incubated for 5 min with 2.7 ml of phosphate buffer and 0.1 ml of 5, 5-dithiobis (2-nitrobenzoate) (DTNB). Then, 0.1 ml of freshly prepared acetylthiocholine iodide (pH 8) was added, and the difference in absorbance was recorded at 412 nm.

Histopathological Examination

Autopsy samples from the brains of the rats were fixed in 10 formalin for 24 h. Washing was done in tap water, then periodic dilutions of alcohol were used for dehydration. Samples were cleared in xylene and embedded in paraffin at 56°C in an oven for 24 h. Paraffin wax tissue blocks were prepared for sectioning at 4 microns consistence by a Sledge microtome. The tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin & eosin stain for examination under the light electric microscope [24].

Statistical Analysis

All the data are presented as mean $\pm$ S.D. The statistical analysis was done using SPSS software (version 25.0 for windows programmed on a computer).

All the parameters were tested for normal distribution by using the Kolmogorov-Smirnov maximum deviation test for goodness of fit and were found to be normally distributed at ($p < 0.05$). Therefore, in instances of multiple mean comparisons, the results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons between groups. The level of significance for all comparisons was set at ($p < 0.05$).

Results

Open Field Results:

The rats were evaluated for their locomotor activity in the open field due to the administration of Scopolamine. (Figure 1) showed the time spent in the center of the open field decreased significantly in animals given Scopolamine ($p < 0.05$). An increasing of this time was observed when the animals were treated with green tea, coffee, black tea, and decaffeinated coffee, and donepezil compared to the Scopolamine group.

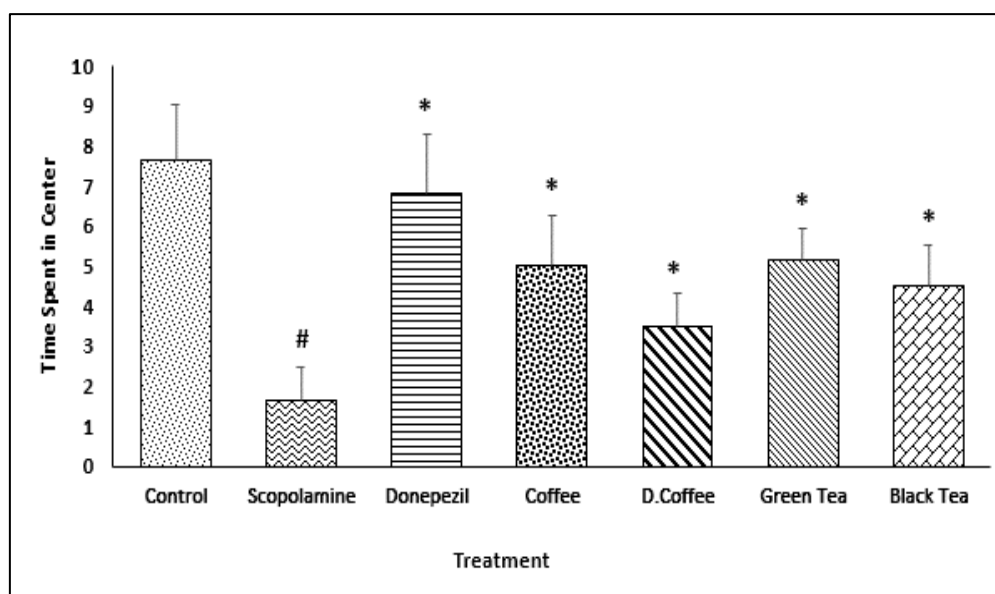


Figure.1. Effect of coffee and tea groups against scopolamine group on exploration in rats by using open field test.

The values represented in the graph are the means $\pm$ S.D of the times of rat stay in the center. ($n=6$). The symbol* indicates the result is statistically significant from scopolamine group, the symbol# indicates the result is statistically significant from control group ($P < 0.05$).

In (Figure 2), significant increase ($p < 0.05$) in locomotor activity by the number of crossings, in the rat receiving green tea, coffee, black tea and decaffeinated coffee and receiving donepezil, compared to the Scopolamine group.

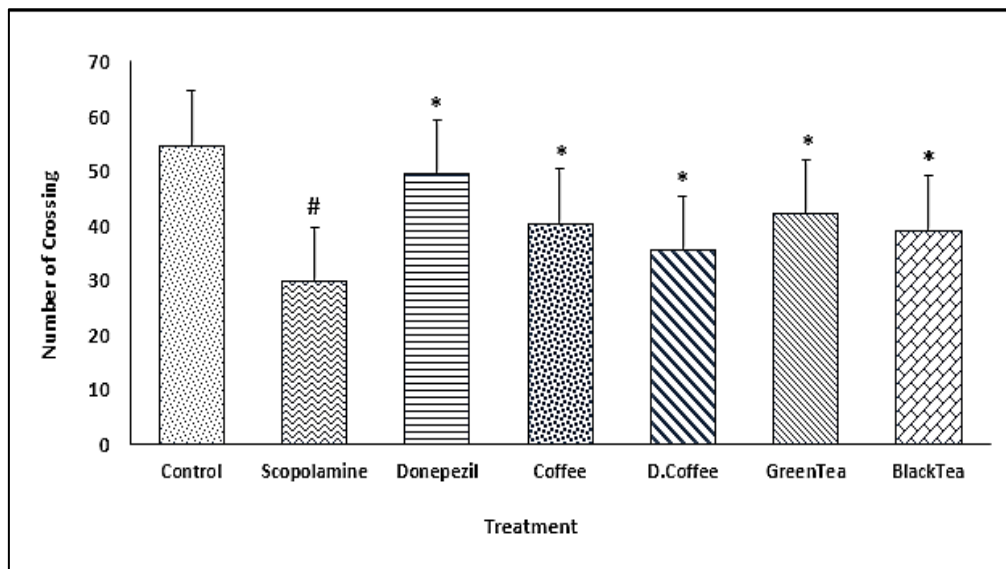


Figure 2. Effect of coffee and tea groups against the scopolamine group on the number of crossings using opened field test in rats. Data were represented by means $\pm$ S.D, of number of crossings. The symbol * indicates significantly higher compared to scopolamine group ($p > 0.05$). The symbol # indicates significantly different from the control group.

Furthermore, results of rearing number in the open field test showed the activity of rats increased in the animals receiving green tea, coffee, black tea, and decaffeinated coffee, and donepezil ($p < 0.05$), contrary to the Scopolamine group ($p < 0.05$) that decreased.

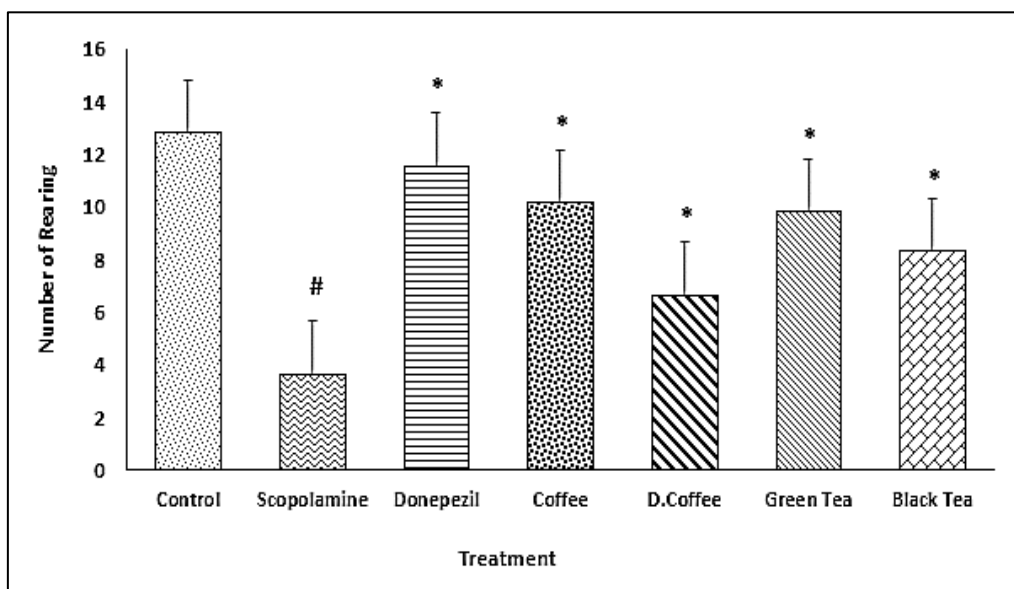


Figure 3. Effect of coffee and tea groups against the scopolamine group on the number of rearing by using open field test in rats. The values are represented by means $\pm$ S.D, treated groups received coffee, decaffeinated coffee, green tea, black tea, and donepezil, and showed a significant increase in the number of rears compared to the scopolamine group. The symbol * indicates significant to scopolamine, the symbol # indicates significant scopolamine to control.

Object Recognition Results

Effects of green tea, black tea, coffee, decaffeinated coffee, and donepezil on Scopolamine-Induced Memory Impairment in Novel Object Recognition increase the latency to discover the novel object compared to the familiar object (Table1). The scopolamine group shows a significant increase ($p < 0.05$) in the time spent with the familiar object compared to the control group. The standard group, that is received donepezil, showed a significant increase in exploration time spent with the new object N ($p < 0.05$) compared to the old object (Figure 4).

Table.1. Effect of different treated groups on scopolamine induced memory impairment in Object Recognition Task.

Sniff Time	Control	Scopolamine	Donepezil	Coffee	D. Coffee	Green Tea	Black Te
Sniff Time (new)	13.5± 2.5	3.83±0.75	12.5±1.65	11.82±2.91	8.83±1.16	11.6±1.03	9.3± 1.21
Sniff Time (old)	2.83±0.75	9.33±2.25	3.33±0.51	4.83±1.83	7± 0.06	3.1± 0.75	5.5± 1.04
DI	0.65±0.03	- 0.39±0.04	0.58±0.07	0.43±0.08	0.12±0.06	0.57±0.06	0.25±0.14

Sniff time(new): sniffing time to novel object, sniff time (old): sniffing time to familiar object, DI: discriminative index between two objects.

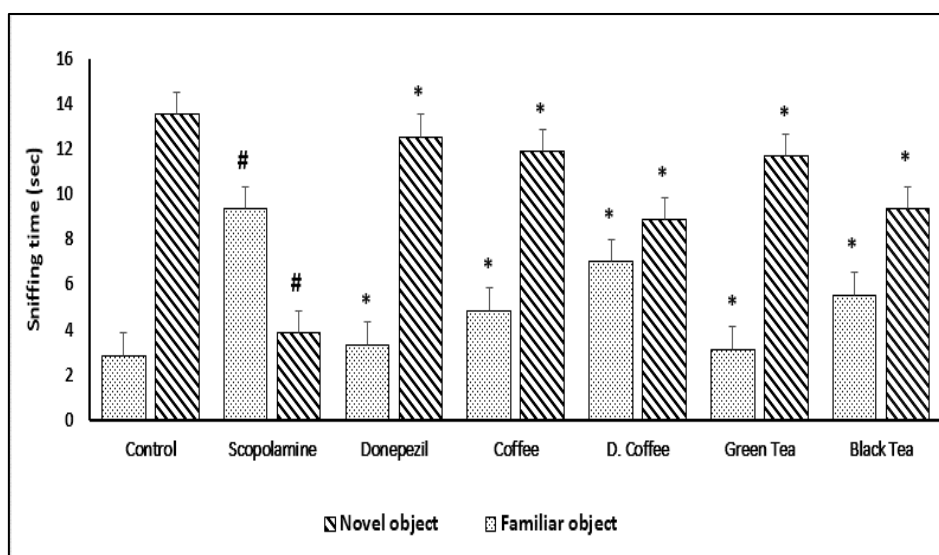


Figure.4. Effect of coffee and tea groups against scopolamine group on the latency time to discover the different objects by using object recognition test in rats.

Each bar represents the mean ± S.D (n = 6). Latency time to the novel object increases in the treated groups compared to Scopolamine group ($p > 0.05$). The symbol * indicates significantly difference treated groups to the scopolamine group. The symbol # indicates a significant difference of scopolamine to the control group.

In addition, (Figure 5) showed the results of the discrimination index to the objects. A decrease in the discrimination index to the novel object was observed, which was - 0.3933 for the rats in the Scopolamine group compared to 0.6566 in the control group. A significant increase ($p < 0.05$) in this discrimination was observed in the groups of animals receiving green tea, coffee, black tea, and decaffeinated coffee, respectively, to novel object than familiar object. Donepezil, the reference molecule, also increased this index of 0.5899 ($p < 0.05$).

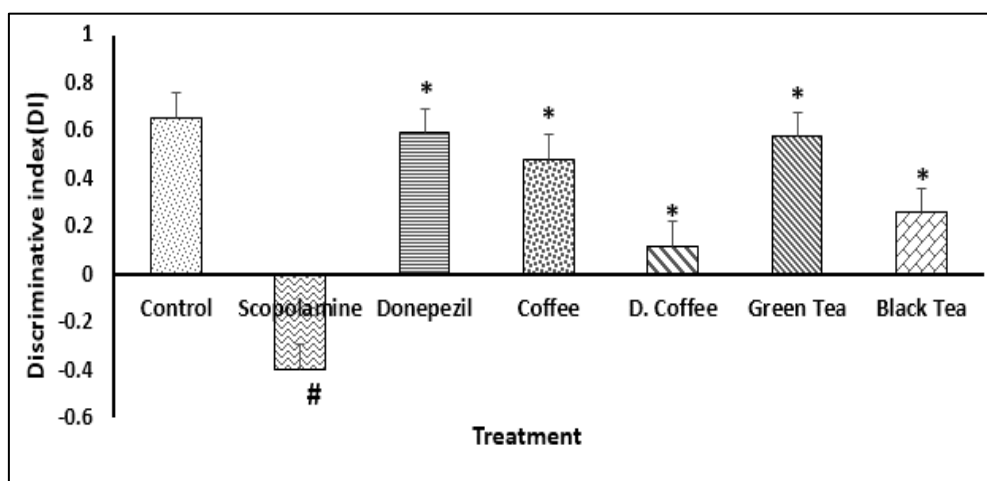


Figure 5. Effect of coffee and tea groups against scopolamine group on the discrimination index by using Novel Object Recognition Test in rats.

Each bar represents the mean ± S.D (n = 6) of discrimination index between two objects. The symbol * indicates difference between treated group and Scopolamine group. The symbol # indicates difference of Scopolamine group to control group, Significant results at ($p > 0.05$).

Morris Water Maze Test (MWM) Results

Acquisition Phase

Escape latency values measured in the Morris Water Maze to found platform shows significant changes between the 1st day and the 4th day in different treated groups which received green tea, black tea, coffee, and decaffeinated coffee compared to the scopolamine group ($p > 0.05$). and scopolamine is significantly different to the control group (Figure 6).

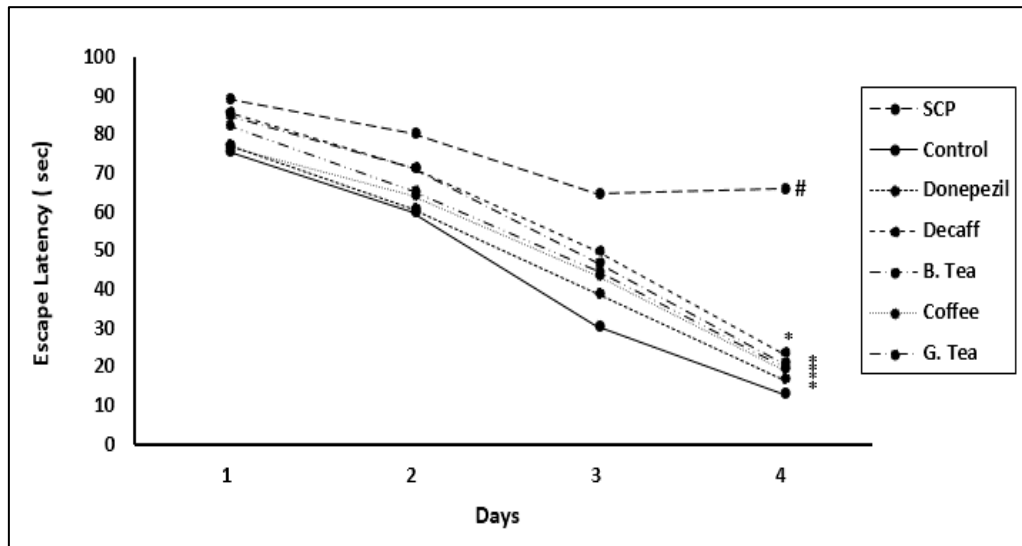


Figure 6. Effect of coffee and tea groups against scopolamine group on escape latency of acquisition phase by using Morris Water Maze Test in rats.

Shown that changed in the “escape latency” values measured by means $\pm$ S. D, between treated groups and induced group. The symbol * indicates significant difference between treated groups and scopolamine group ($p > 0.05$). The symbol # indicates significant difference of scopolamine group to control group.

Probe Phase

At the end of the acquisition trial, the platform was removed and shows the time spent in the “target quadrant” was shown in the Morris water maze test (Figure 7). Amnesic rats that received scopolamine spent significantly shorter time in the target quadrant than the healthy controls ($p > 0.05$), and treated groups spent longer time in target quadrant compared to the scopolamine group ($p > 0.05$).

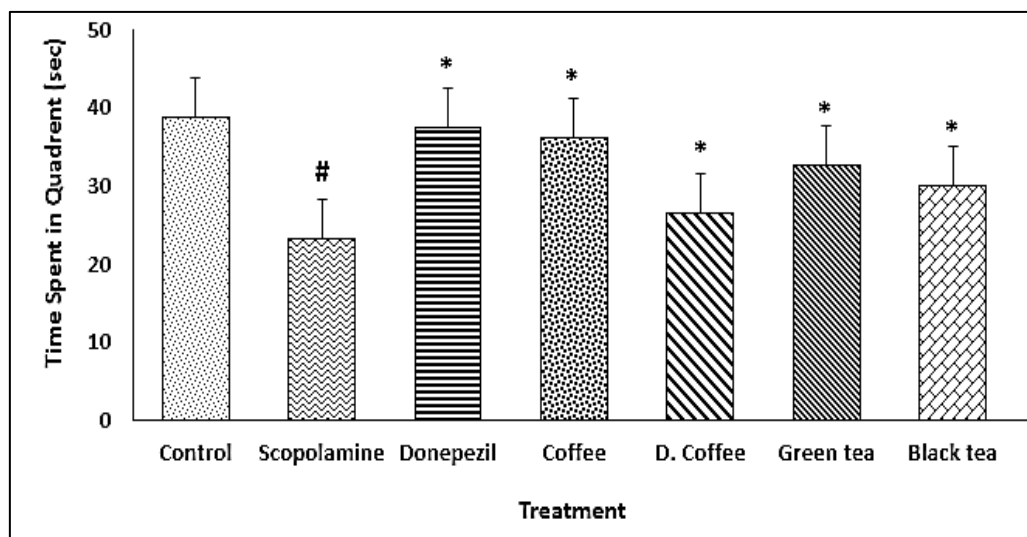


Figure .7. Effect of coffee and tea groups against scopolamine group on time spent in target quadrant of probe phase trial by using Morris Water Maze Test in rats.

Values represented by means $\pm$ S.D of time spent in quadrant after platform is removed compared to the scopolamine group ($p > 0.05$). The symbol * indicates significant in compare to scopolamine group. The symbol # indicates scopolamine significant difference to control group.

In addition, the number of crossings over the quadrant was significantly higher in treated groups that received green tea, black tea, coffee, and decaffeinated coffee (Figure 8), in compare to the scopolamine group, furthermore scopolamine has lower number of crossings compared to the control group.

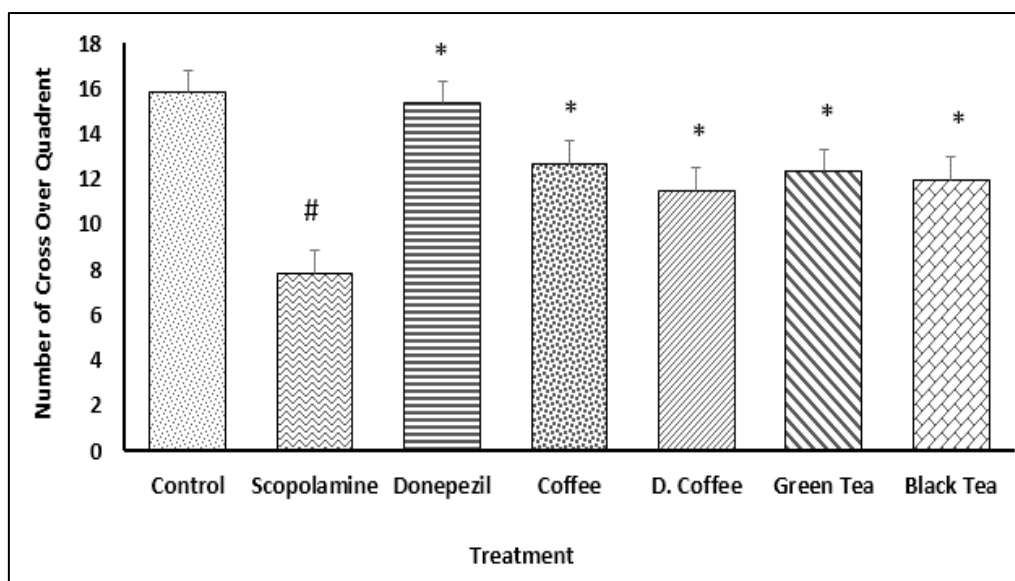


Figure 8. Effect of coffee and tea groups against scopolamine group on the number of crossings over quadrant by using Morris Water Maze Test in rats.

Were data presented by means $\pm$ S.D(n=6), The symbol * indicates significantly higher in compare to scopolamine group ($p > 0.05$). The symbol # indicates scopolamine significantly different in compare to control group.

Active Avoidance Shuttle Box Results

(Figure 9) showed that significantly difference in the number of avoidances of receiving the shock from day1 to day4 in the treated groups, which significantly increased compared to the amnesic group. Whereas the amnesic group showed significantly decrease in the number of avoidances of the shock compared to the control group.

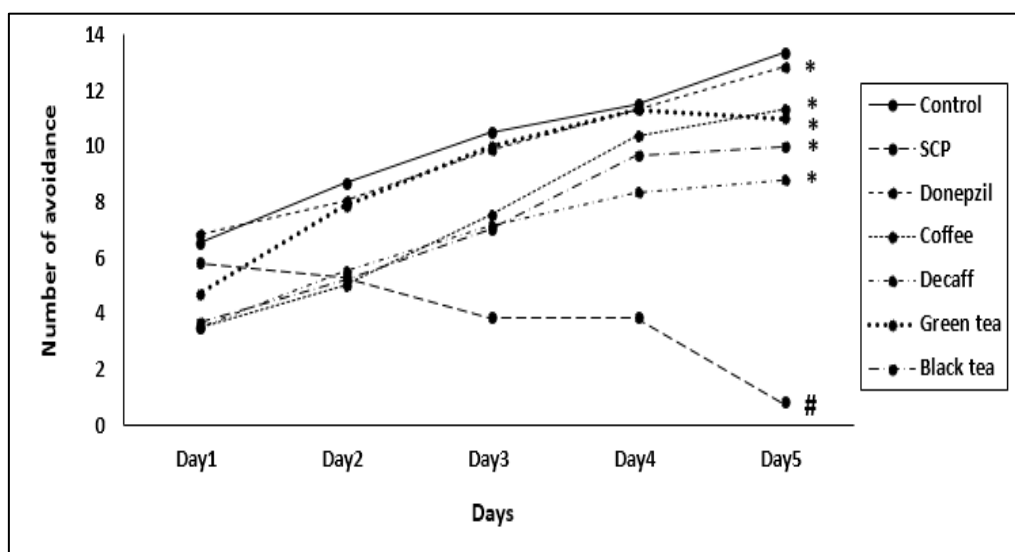


Figure .9. Effect of coffee and tea groups against scopolamine group on the number of avoidances between day 1 and day5 by using Active Avoidance Shuttle Box in rats.

Values represented by means $\pm$ S.D, the symbol * indicates that significantly difference between treated groups (coffee, decaffeinated coffee, green tea, black tea, donepezil) and scopolamine group is in day 5 test session ($p > 0.05$), and the symbol # indicates scopolamine group significant difference to control group.

Estimation of Oxidative Stress Biomarker Results

Acetylcholinesterase Activity Results

The effect of administration of coffee, decaffeinated coffee, green tea, black tea, and donepezil on Acetylcholinesterase inhibitory activity is shown in (Figure 10), and the results indicate that the treated groups showed a significant reduction in comparison to the negative control group. The negative control animals expressed a significantly ($p < 0.05$) higher level of Acetylcholinesterase enzyme upon comparison with the control group after the induction of amnesia. In the treatment group, the enzyme levels have reduced as indicative of neuroprotection.

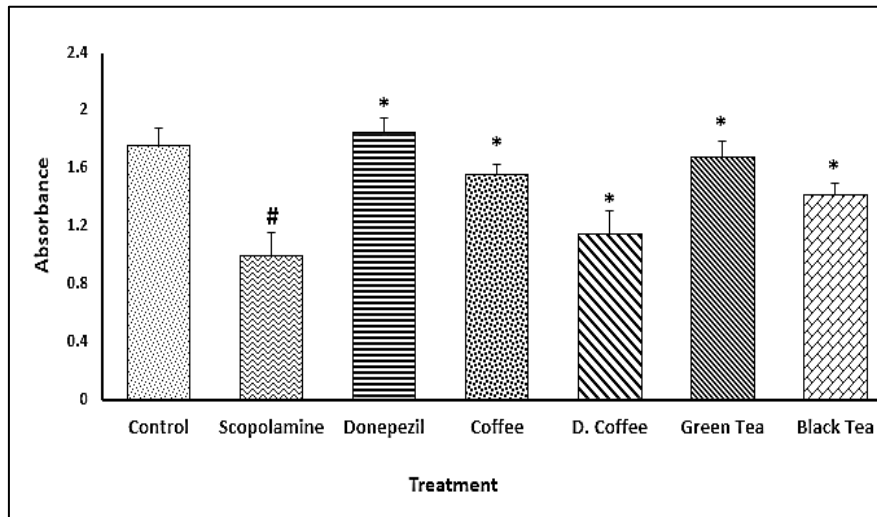


Figure .10. Effect of coffee and tea groups against scopolamine group on the difference of absorbance means of Acetylcholinesterase activity in rats.

Data represented by means $\pm$ S.D of absorbance at 412 nm of Acetylcholinesterase enzyme. The symbol * indicates significant difference of coffee and tea groups to scopolamine group, the symbol # indicates significant difference of scopolamine group to control group.

Histopathological examination Results

Histopathological alteration in the hippocampus section by scopolamine in the induced group(b) (Figure 11) showed edema, and active inflammation in brain tissue. The brain tissues of control animals showed normal, intact cellular arrangements (a). Treated animals (c to g) showed no significant pathology and displayed reduced cellular alterations compared to the scopolamine group.

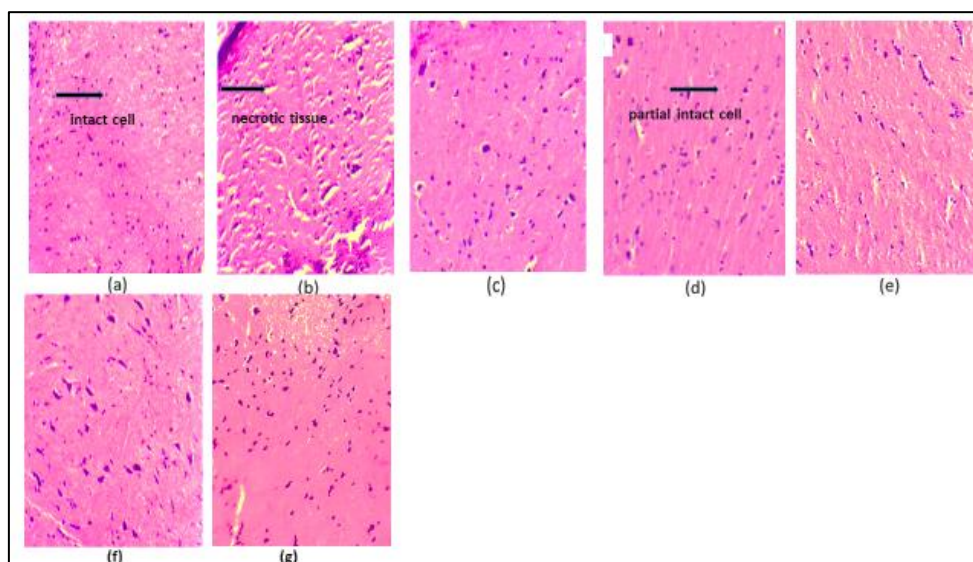


Figure11. A light microscopic 400x images of coffee and tea groups against scopolamine group for histopathological examination of hippocampus of rats' brain.

(a): group received normal saline, (b): group received scopolamine showed necrotic cell with inflammation, (c) group received donepezil showed intact cell as control group, (d) to (g): treated groups received coffee, decaffeinated coffee, green tea and black tea respectively showed partial intact cell in compare to scopolamine group.

Discussion

Alzheimer's disease (AD) is now one of the leading causes of disability and death among elderly people [25]. The precise etiology and pathogenesis of Alzheimer's disease are not fully understood [26]. Currently, there is no cure for Alzheimer's disease, despite the enormous progress that has been achieved in Alzheimer's disease research over the past few decades, there are medications now available to relieve symptoms, such as acetylcholinesterase (AChE) inhibitors, which increasing the level of acetylcholine in the brain helps nerve cells communicate with each other [27]. Therefore, it is necessary to find novel preventive and therapeutic neuroprotective agents. An increasing body of evidence indicates that oxidative stress may be one of the initial changes that take place during the onset and progression of Alzheimer's disease [28], although oxidative stress has been suggested to be one of the key contributors in the pathophysiology of Alzheimer's disease [29]. It is still unclear how the redox balance in the brain is affected and where the free radicals come from. Accordingly, the importance of antioxidants as protection against oxidative stress is now a hot topic [30]. It is well documented that coffee and tea are rich in polyphenols that have a clear effectiveness on oxidative stress [31]. The ways of preparation of tea and coffee could affect the drinkable extract of these two beverages. Therefore, the present study was undertaken to investigate whether drinking coffee and tea as prepared by the Libyan way has a positive effect on protecting nerve cell damage related to the incidence of Alzheimer's disease.

On the other hand, scopolamine hydrobromide is a very widely used chemical to induce Alzheimer's disease-like symptoms in order to investigate and screen the potential anti-Alzheimer's disease remedies [13]. Scopolamine, a nonselective antagonist of muscarinic receptors, blocks cholinergic signaling which leads to cognitive and memory dysfunction. Hence, learning and memory impairment may occur [32]. Scopolamine has been found to increase oxidative stress and cause disruption in the anti-oxidative defense system [33]. Numerous clinical studies have shown that oxidative stress is included in the pathogenesis of Alzheimer's disease [34].

In this study, the results obtained from the open field test on behavior and locomotor activity are significant with all treated groups; in comparison to the scopolamine group, moreover, in the Object Recognition Test (ORT), exposure to scopolamine for diminishing recognition capacity and episodic memory. The results showed that the exploration and discrimination index decreased in the scopolamine group which may be due to an impairment of learning and recognition caused by blocking muscarinic receptors, which have been responsible for disrupting learning and memory performance in animals and humans [35], but pretreatment with coffee and tea protected animals, as it showed an increase in exploration time to the novel object in comparison to the old object.

As the Morris Water Maze Test (MWM) model demonstrated deficiency in reference memory and cognitive performance, the time spent in the target quadrant and crossing over the target quadrant decreased significantly throughout both the one-hour and 24-hour probe trials in the scopolamine group in comparison to the treated group with coffee and tea. Indicating improvement in reference memory that is normally correlated in Alzheimer's disease patients [36].

Active Avoidance Shuttle Box Test showed that the scopolamine group had a lower number of avoidances to shock, where to stay in the chamber to receive the shock, but other groups (coffee, tea, and positive control) showed a significant increase in the number of avoidances in comparison to the scopolamine group. These results are in accordance with what has been found previously, as polyphenols improved memory in Alzheimer's disease patients [37].

Further biochemical and histopathological tests were carried out to confirm what has been found in the scopolamine model: a significant rise in Acetylcholinesterase (AChE), which might be related to the prevalence of Alzheimer's disease by increasing the breakdown of acetylcholine (ACh) [38]. A decreased level of ACh in the hippocampus was reported in Alzheimer's disease patients [39]. So, from the results of this study, the coffee and green tea groups showed that a decrease in AChE activity (Figure 1) may influence the process of cholinergic transmission, producing deficits in learning and memory performance and enhancing ACh activity [40].

The histopathological examination of the hippocampus of rat brain tissue showed a significant difference in cell integrity between the scopolamine group and the treated groups coffee and tea treatment decreased the disruption of the nucleus of the cell and sheets of amyloid beta in comparison to the scopolamine group.

Therefore, the results obtained from this study indicate the effect of administration of coffee and tea prepared by the Libyan technique on Alzheimer's disease-like symptoms induced by scopolamine, related to various effects of active components in coffee and tea [31]. In addition, found that caffeinated coffee has a more significant neuroprotective effect than decaffeinated coffee; on the other hand, green tea had a more significant neuroprotective effect than black tea due to the fermentation method. Furthermore, in this study, the Libyan technique does not influence on the extraction of coffee and tea components, as the methods of extraction mentioned earlier had an influence on the activity of these components.

Conclusion

This study gave us somewhat noticeable results for the benefit of drinking coffee and tea in our society to people who are prone to Alzheimer's disease, but we still need more details about the mechanism of the effect of coffee and tea and the extent of its protection for nerves. To fully understand coffee and tea constituents in the numerous biochemical pathways linked to neurological illnesses, more research, in particularly clinical trials in humans, is needed. Future research should

look at the control of neurological mechanisms, specific brain areas where neuronal failure occurs, bioavailability, and dose for the best results with no toxicity or adverse effects when consuming coffee and tea.

Conflict of interest. Nil

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