

*Original Article*

## Neuroprotective Effect of Coffee and Tea on Haloperidol-Induced Parkinson's Disease in Rat Model

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### Abstract

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**Background:** Coffee and Tea are very popular beverages in Libyan society. They contain many bioactive substances such as polyphenols and catchiness that could have some effects for instance; antioxidant activity. The way of preparation of tea and coffee drinks are different among society. For example, in Libya tea drink is prepared by boiling the crude of fresh tea for 10 min. Therefore, the method of extraction could influence the type and quality of extracted substances. **Aims:** The goal of the current study was to examine the neuroprotective properties of coffee and tea beverages made by using traditional Libyan techniques on Parkinson's disease like symptoms induced by haloperidol. **Methods:** Different tea and coffee beverages (treatment) were prepared and given to rats in a concentration 10%w/v for 3 consecutive weeks. Tea and coffee beverages were made as Libyans do. At day 21, rats were injected IP with 1mg/kg of haloperidol, afterward, the behavioral and motor parameters for Parkinson's disease were tested. **Results:** Coffee and Tea treated groups showed significant improvement ( $p > 0.05$ ) in the behavioral activity, and in muscle coordination. Also there was a decrease in oxidation markers as treatment resulted in an elevation of glutathione reductase and decreasing in Malondialdehyde levels. In addition, the histopathological investigation showed a reduction in haloperidol induced damage in substantia nigra. **Conclusion:** The results showed a possible neuroprotective effect of

Coffee and Tea against PD. The mechanism of protection might be due to an antioxidant activity.

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**Keywords:**Coffee,Tea, Parkinson's disease,Haloperidol, Oxidative stress, GSH.MDA.

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## Introduction

Coffee and tea are among the most popular beverages in the world for ages. Drinking tea and coffee showed to have many benefits for health[1]. On the contrary, some reports pointed to some harmful effects on health.[2] .In Libyan drinking tea and coffee is a daily habit among various age groups. However, the way Libyans make tea and coffee is different from most of the 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 crude leaves in water for a short period. Tea is obtained from fermentation of leaves and buds of *Camellia Sinesis* plant,

generally there are three types of tea 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 technique of tea processing, black tea leaves are oxidized but green tea leaves are not [3]. Coffee comes in the 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. To date, many epidemiological studies have suggested beneficial implications of antioxidants in coffee and tea for protection against neurodegenerative diseases as Alzheimer disease and Parkinson's Disease (PD) [7].

Parkinson's disease (PD) is second most common neurodegenerative disease after Alzheimer disease affecting about 1-2% of people older than 60 years [8]. It is marked physically by bradykinesia, postural instability, tremor and muscular rigidity [9]. The neuropathological changes in PD are marked by loss of dopaminergic neurons of substantia nigra pars compacta and the intracytoplasmic eosinophilic inclusions [10]. The etiology of PD is

still unknown relatively, although advancing age, genetic predisposition and environmental exposure are considered as risk factors as a result of increasing the oxidative stress [9]. Yet, symptomatic relief is known therapeutic strategy for treatment of PD. Nonetheless, using neuroprotective agents from natural origin could be a successful approach in controlling neurodegenerative diseases [11].

It is widely thought antioxidant may protect against PD [7]. Hence consumption of polyphenols may decrease the incidence of oxidative stress related to PD [6]. In this study, we examined whether the coffee and tea made by the Libyan method have a protective effect against PD.

## Material and Method

### Preparation of Coffee and Tea solution

Ten grams of either coffee (caffeinated & decaffeinated) or Tea (black & green) were added to a beaker containing 100

ml of distilled water and boiled for 5 minutes at 250 °C. After cooling, the mixture filtered by using Wattman

filter paper no.1. Then the filtrate was used as treatment for various experiments. The route of administration to animals was by oral gavaging (o.g) in a volume of 2ml/kg.

### Animals

Male Wistar albino rats at age of 2 months, (weight between 200-250g) were used in this study. All the animals were obtained from animal house facility of Libyan Medical Research Center. Animals were kept in plexiglass cages, under a dark-light cycle of 12 h, and housing temperature around 25 °C. All experiments were undertaken in accordance with Animal Research Advisory Committee (ARAC) guidelines.

### Drugs and chemicals

Haloperidol (Haldol) (Belgium), levodopa (madopar 100/25 mg, Roche company), coffee (Arabica roasting coffee), decaffeinated coffee, green tea (Almead tea- China), black tea (Alnaja Tea- China) coffee and tea were sourced from Al Zawia local market, 0.9% normal saline, 5,5-dithiobis 2-

nitrobenzoic acid (Thermo scientific Company-Sweden) (DTNB), thiobarbituric acid obtained from (Sigma Aldrich). EDTA, sodium dodecylsulphate and trichloroacetic acid obtained from (chemical laboratory of Zawia university).

### Induction of Parkinson Disease

Animal model of PD used in this study is by administering haloperidol the dopamine receptor antagonist to produce PD like symptoms in rats in a dose of 1mg/kg IP [12].

### Experimental design and treatments

The rats were randomly divided into 7 groups of 6 animals each (n=6); group I received normal saline (NS) and worked as a control, animals in group II were received 1mg/kg haloperidol, while group III rats received L-dopa 30mg/kg and worked as a positive control. The rats of groups (4,5,6 and 7) each received one of the 4 tested beverages once daily in a concentration of 10 %w/v extract and volume of 2ml/kg for 21 days. Treatments were; ordinary coffee, decaffeinated coffee,

black tea and green tea. On day 21 animals were injected with 1mg/kg haloperidol, 45 minutes later, various behavioral motor tests were performed in order to assess the parameters of PD. Later, rats were sacrificed by cervical dislocation under mild anesthesia by using chloroform. Brains were taken out for biochemical and histopathological analysis.

#### **Estimation of Behavioral Parameters**

**Catalepsy Bar Test:** -this test was carried out to test muscle rigidity, animals were positioned on their hindquarters on the bench and their forelimb rested 1cm diameter horizontal on the bar (9 cm above the bench). Catalepsy was measured by a time that animal resting on a bar. Whereas the end point of catalepsy was taken when the animal removed their both front paws from the bar. Cut off time of 300 seconds was applied. [13].

**Rotarod Test:** -is to test animal motor coordination, before starting test session, animals were allowed to get trained to remain on the rod rotating at 20rpm constant speed for 5 minutes.

During testing session, the speed accelerated, time of fall off the rotating rod for each animal was recorded [14].

**Grip Strength Test:** -this test assesses muscular strength, by using a gripping force meter (model 47105, Ugo Basile, Italy). The test was performed while the rats held by the tail after it was allowed to hold in a grid attached to a force transducer. The rat was pulled by the tail with increasing firmness until it loses the grip. At this moment the transducer was recorded this as peak force in the equipment [15].

**Wire Hanging Test:** -Is for measuring muscle strength, rats picked up from their tails and put them on the wire (40cm above the surface), the rats catch the wire by their forelimbs paws, rats were forced to hang, time taken when the rat fall (Jansone, nee Opmane et al. 2016, [17].

**Pull-Up Test:** -The test is used to measure catatonia and catalepsy, it is performed by holding rat from its hind legs, then the test parameter is the time taken by the rat to pull itself up and get on the hand of the experimenter [18].

## Estimation of oxidative stress biomarkers

Brain tissue were pulverized under liquid nitrogen, then; they were homogenized with 1 ml of cold PBS on ice. The resultant homogenates were centrifuged for 10 min at 4 °C at 10,000 rpm. Supernatants were separated in a tube for estimation of lipid peroxidation and glutathione reductase.

## Estimation of Malondialdehyde (MDA) level

Malondialdehyde level was estimated by Ohkawa method (an indirect method for determining lipid peroxidation) [19]. The brain tissues are homogenized in 0.1M buffer PH 7.4. Tissue homogenate (0.2 ml), 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 8% TBA are added to aqueous solution of thiobarbituric acid. The volume of the mixture is made up to 4 ml with distilled water and then heated at 95°C for 60 min on water bath. After incubation the tubes are cooled to room temperature and final volume was

made to 5ml in each tube. Five mL of butanol: pyridine (15:1) mixture is added and the contents are vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer is taken and measured at 532 nm. [20].

## Estimation of Glutathione Reductase (GSH) level

The assay procedure was; 1ml of tissue homogenate was mixed with 1ml of trichloroacetic acid (TCA) and 1ml of EDTA to precipitate the proteins, 200µl of the aliquot of the supernatant was added with phosphate buffer and 1.8ml of DTNB reagent (Ellman's reagent). The sulfhydryl group reacts with DTNB to produce a yellow color, then the absorbance was measured for the solution at 412nm. [21].

## Histopathology analysis

Substantia nigra brain tissue was segregated and isolated [22] followed by immediate preservation in 10% formalin solution, 5µm thickness paraffin embedded tissue slides were prepared, then stained with eosin and

hematoxylin for further microscopically investigation of information including; the pattern, shape, and structure of cells in a tissue sample.

### Statistical Analysis

Descriptive statistical analysis was applied on the parameters of different samples using SPSS (software package, version 25) to find out whether the observed samples normally distributed using Kolmogorov-

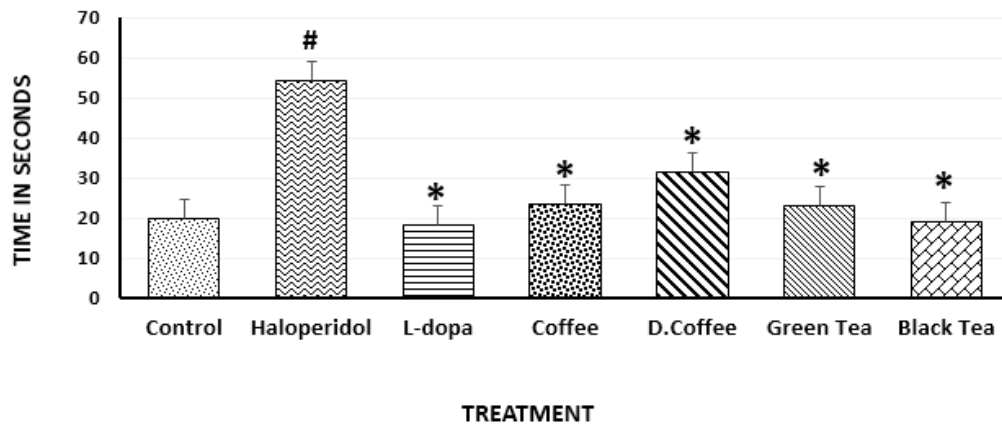
### Results

#### Catalepsy test

Bar test data (figure. 1), indicating all the tested treatments; coffee, decaffeinated coffee, black tea, green tea and L-dopa were able to produce a decrease in the time at which animal successfully removes its forelimbs from

Smirnov maximum deviation test for goodness of fit. If the parameters were normally distributed, treatments were compared by applying One-way ANOVA (one dependent variable). Post-Hoc tests (LSD and Duncan tests) was applied. If the parameters are not normally distributed, treatments were compared by applying the Mann-Whitney two samples (non-matched) test. The differences were considered to be significant at  $p < 0.05$ .

the bar in comparison to haloperidol group. Haloperidol group significantly increases the cataleptic score as compared to the vehicle control group that received normal saline.

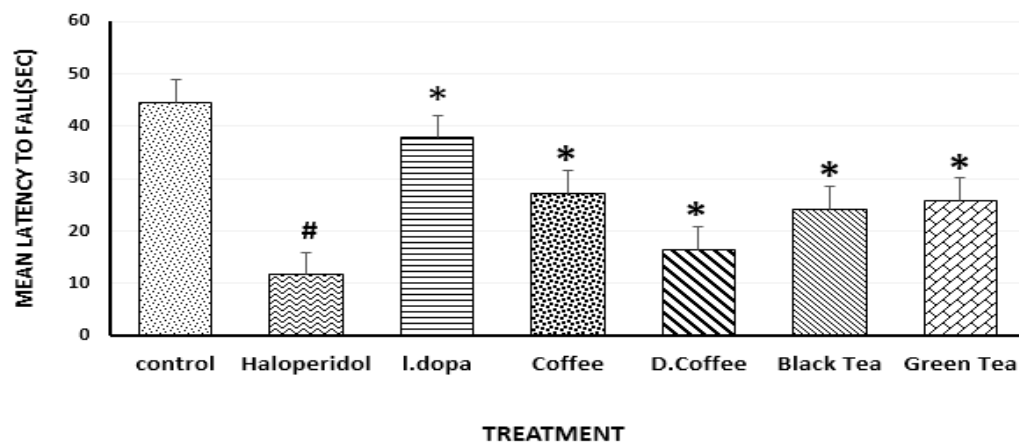


**Figure 1:** Study of cataleptic effect of haloperidol in rats treated for 3 weeks with; L-dopa coffee, decaffeinated coffee, black tea and green tea. The values represented in graph are the means  $\pm$  S.D of the times of rat forelimbs removal off bar. (n=6). \* indicate the result is statistically significant from haloperidol group. # indicate the result is statistically significant from control group ( $P \leq 0.05$ ).

## Rotarod Test

Fall off time from rotarod (Motor coordination test) was significantly decreased in haloperidol treated group as compared to the vehicle control group and it was significantly

improved with L-dopa, coffee, decaffeinated coffee, green tea and black tea in comparison to haloperidol group. (figure 2).

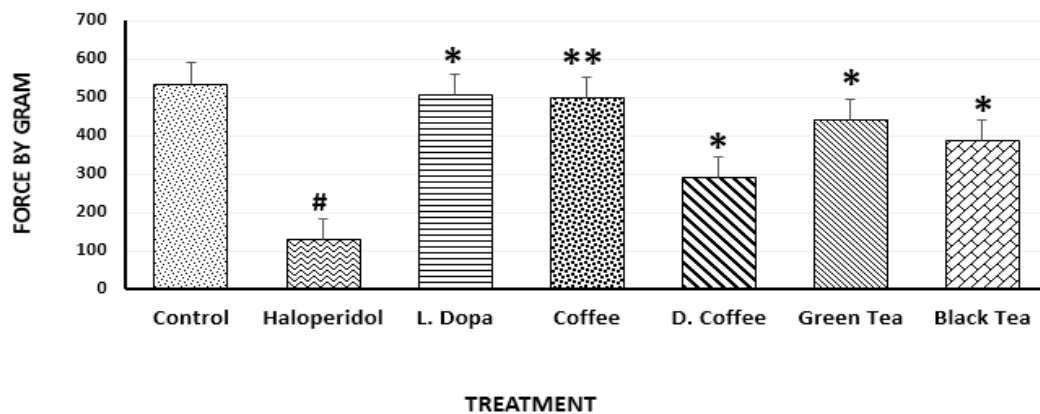


**Figure. 2.** Study the effect of haloperidol on musclecoordination in rats by rotarodpre-treated for 3 weeks with; L-dopa, coffee, decaffeinated coffee, black tea and green tea. The values represented in graph are the means  $\pm$  S.D of the times of rat falling off. (n=6). \* indicate the result is statistically significant from haloperidol group. # indicate the result is statistically significant from control group ( $P \leq 0.05$ ).

### Grip strength test

Muscle strength results (figure 3) show a significant decrease in muscle force tone in the haloperidol group as compared to vehicle control group, and

significant increase in muscle strength in groups treated with L-dopa, coffee, decaffeinated coffee, black tea and green tea.

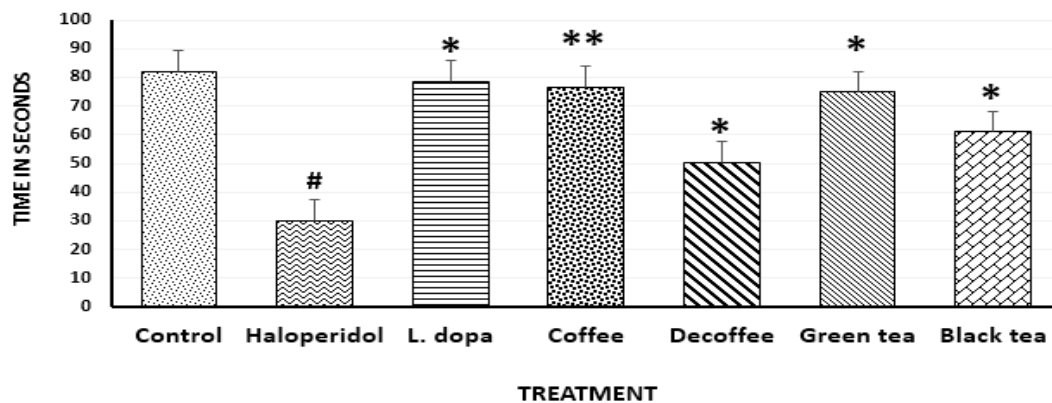


**Figure. 3.** Studying the effect of haloperidol on muscle strength in rats by grip test in rats pre-treated for 3 weeks with; L.dopa, coffee, decaffeinated coffee, black tea and green tea. The values represented in graph are the means  $\pm$  S.D of the force required to make rat lose the grip. (n=6). \* indicate the result is statistically significant from haloperidol group. # indicate the result is statistically significant from control group ( $P \leq 0.05$ ). \*\* means the  $p \leq 0.005$ .

### Wire hanging Test

Wire hanging data are represented in figure 4. Groups treated with L-Dopa,

coffee and tea had higher times in comparison to the haloperidol group.

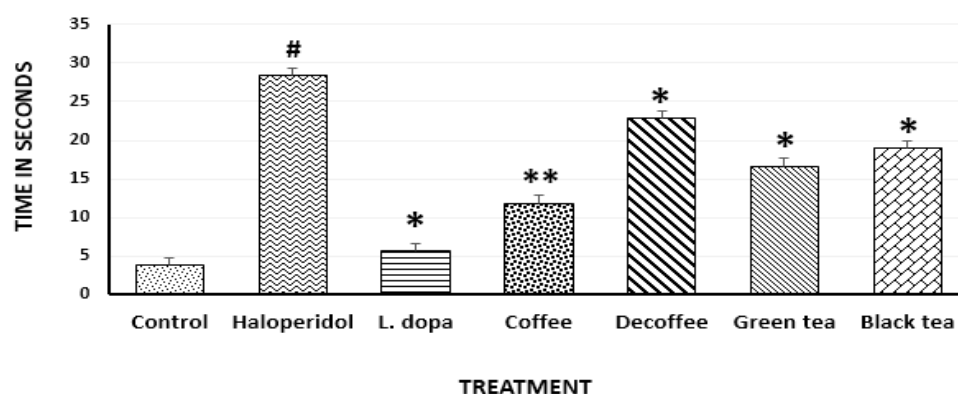


**Figure 4.** Studying the effect of haloperidol on muscle strength in rats by wire hanging test in rats pre-treated for 3 weeks with; L-dopa, coffee, decaffeinated coffee, black tea and green tea. The values represented in graph are the means  $\pm$  S.D of time before falling down from the wire. (n=6). \* indicate the result is statistically significant from haloperidol group. # indicate the result is statistically significant from control group ( $P \leq 0.05$ ). \*\* means  $p \leq 0.005$ .

### Pull- Up test

The results from pull-up test are represented in figure 5, L-dopa, coffee, black tea and green tea were all

decreased the time the rat take to get on hand while has been hanged up from tail.



**Figure 5.** Studying the effect of haloperidol on muscle strength and muscle coordination by pull up test in rats pre-treated for 3 weeks with; L-dopa, coffee, decaffeinated coffee, black tea and green tea. The values represented in graph are the means  $\pm$  S.D of time taken by rat to get on tester's hand (n=6). \*

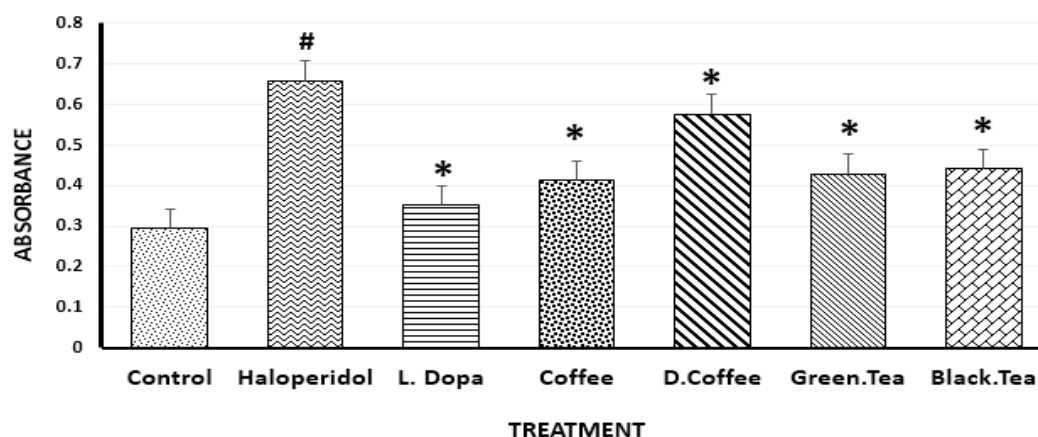
indicate the result is statistically significant from haloperidol group. # indicate the result is statistically significant from control group ( $P \leq 0.05$ ). \*\* means  $p \leq 0.005$ .

## Biochemical assay results

### Lipid peroxidase assay

Malonedialdehyde(MDA) levels which were produced by lipid peroxidation in brain tissue of rats are significantly lower in treated groups when

compared to the haloperidol control group (figure 6) except for the group which received decaffeinated coffee.

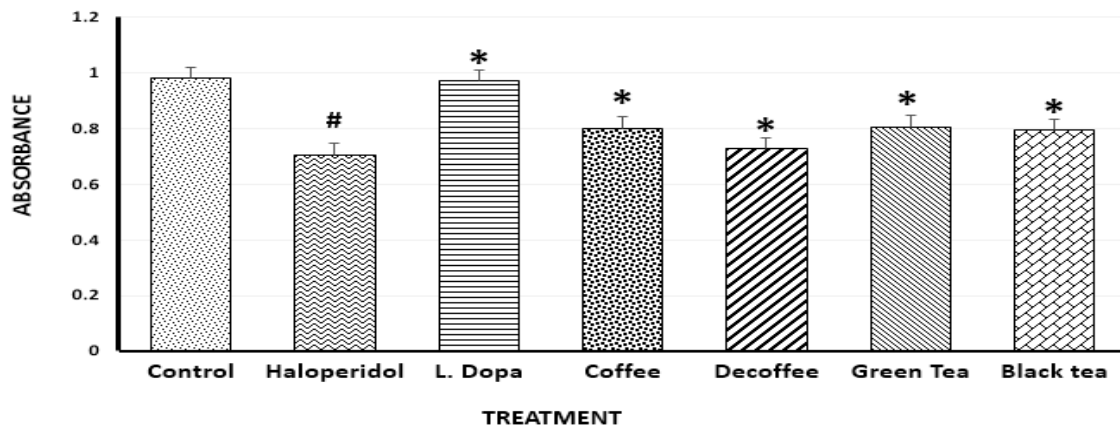


**Figure 6:** Effect of haloperidol on brain tissue lipid peroxidase levels for rats pre-treated for 3 weeks with; L-dopa, coffee, decaffeinated coffee, black tea and green tea. The values represented in graph are the means  $\pm$  S.D of absorbance reading for tissue samples ( $n=6$ ). \* indicate the result is statistically significant from haloperidol group. # indicate the result is statistically significant from control group

### Glutathione reductase assay

Results from this assay (figure 7) showed that significant enhancement in glutathione activity in tissues taken from treated rats; L-dopa, black tea,

green tea, coffee, decaffeinated coffee. However, tissue from the haloperidol group has reduced activity in comparison to controls.

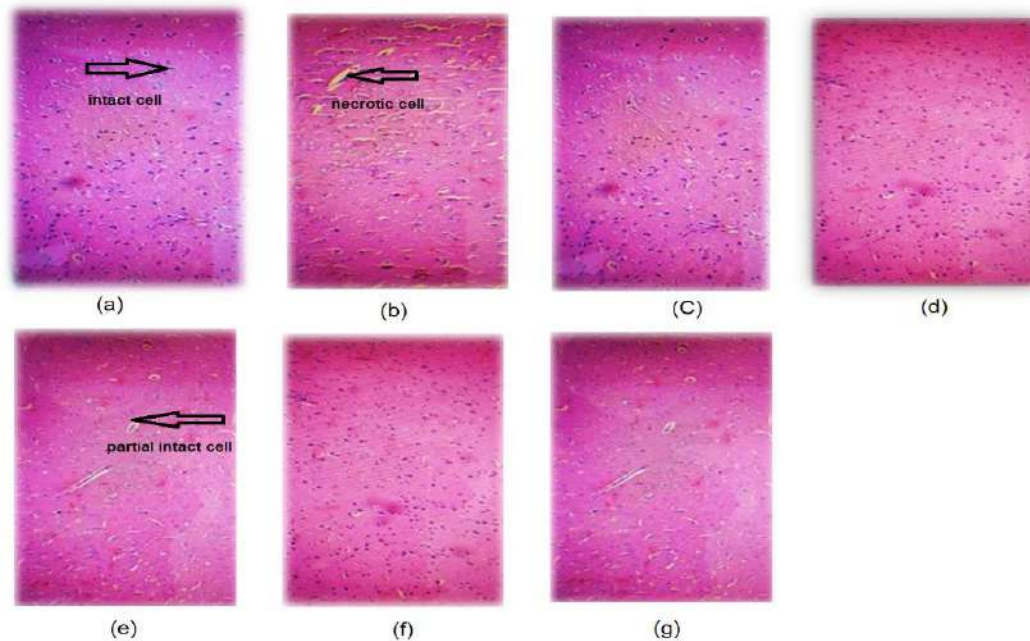


**Figure 7:** Effect of haloperidol on brain tissue GSH for rats pre-treated for 3 weeks with; L-dopa, coffee, decaffeinated coffee, black tea and green tea. The values represented in graph are the means  $\pm$  S.D of absorbance reading for tissue samples (n=6). \* indicate the result is statistically significant from haloperidol group. # indicate the result is statistically significant from control group ( $P \leq 0.05$ ).

### Histopathological examination

Eosin-hematoxylin stained, slides from brain tissues treated with various treatments are represented in figure 8. They are represented as 400x magnification pictured microscopic views. Control (NS) group exhibit normal structure, and morphology and

intact cells, On the other hand, haloperidol samples showed necrotic neurons with intracellular spacing showing oedema. Nevertheless, treated groups showed minimal changes in cell integrity.



**Figure 8:** -A light microscope 400X images of eosin heamatoxylin stained rat brain samples for rats treated with normal saline (a), haloperidol (b) or recived haloperidol but pretreated for 3 weeks with; L-dopa (c), coffee (d), decaffeinated coffee (e), green tea (f), black tea (g). The arrows indicate the site of tissue alteration or necrosis.

## Discussion

Parkinson's disease (PD) is a common disabling neurodegenerative disorder. It is characterized by clinical features including triad of motor symptoms; tremor, rigidity, and bradykinesia [23]. According to studies PD symptoms don't develop in patients until 80% or greater loss of dopamine-producing cells occurs in substantia nigra [22]. Environmental influences such as smoking, consumption of coffee, and pesticide exposure have been

postulated to effect the risk of PD development, importantly oxidative stress process is shown to lead to the degeneration of dopaminergic neurons in PD [24]

Haloperidol induced catalepsy is a widely used rodent model for PD as the disease results from depletion in dopamine in substantia nigra [25]. Therefore, haloperidol the dopamine receptor antagonist mimics the depletion of dopamine. When

systemically given to rodents, haloperidol can induce catalepsy, rigidity and a behavioural state of bradykinesia, leading to the inability of animal correct externally imposed posture [26]. In this study haloperidol model was used to induce PD symptoms.

Studies have looked into how oxidative stress and free radicals play a role in the chain of events that cause dopamine cell degeneration in Parkinson's disease (PD). In summary, the CNS's built-in defense mechanisms, which include enzymatic and non-enzymatic antioxidants, are responsible for avoiding the loss of neuronal cells as a result of free radicals actions [27]. Tea and coffee contain various bioactive substances such as caffeine and polyphenols that have been shown to have a significant neuroprotective effects [28]. However, the method by which crude tea and coffee are prepared could have a great influence on amount and type of extracted substances. Tea (green and black), coffee and decaffeinated coffee were prepared using the Libyan method.

They were given for 21 days before induction of PD to give some means of protection against possible damage expected to be produced by haloperidol. Catalepsy, motor coordination and motor strength tests were undertaken in this study, noticeably, ordinary coffee, decaffeinated coffee, black tea and green tea all resulted in improvement of all the aspects of motor performance tested. These results point to presence of compounds in tea and coffee extract able to reverse the effects of haloperidol on motor activity. As coffee and tea rich in antioxidants, in addition, haloperidol induces the generation of ROS [29], therefore, further studies were carried out to investigate the potential role of antioxidants in decreasing of PD symptoms.

The oxidative state of brain tissue by measuring Malonedialdehyde (MDA) levels indicated a reduction of MDA in the treated group which points to an antioxidant action. Hence, this could explain the reversing of oxidative effects of haloperidol [30] produced in treatment groups. Further, the levels of

Glutathione reductase enzyme that play role in preventing oxidative stress were measured. Data from MDA test indicate the ability of tea and coffee to raise its levels. This data was consistent with what was previously shown [31]. Brain substantia nigra tissues were also examined for possible improvement in the histopathological changes produced by haloperidol, the

## Conclusion

In this study, it can be concluded that consumption of tea and coffee in general and in Libyan society could have a protective action against PD. However, more investigation needed

results showed normalization in the necrosis, damage and the histopathological changes in tea and coffee treated groups.

This study gave us somewhat tangible results for the benefit of drinking coffee and tea to people who are prone to PD, but we still need more details about the mechanism.

to elucidate and identify the active compounds behind this effect. Also, more pharmacological studies in other models of PD may be required to confirm the exact mechanism of action.

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