

## The Impact of Alpha-lipoic acid on the Monosodium Glutamate Induced Motor Coordination Dysfunction in Rats

Safaa M. Saleh, Gerges S. Yousef, Suzan A. Khodir, Amany F. Elziat\*, Ghana S. Amer

Department of Clinical Physiology, Faculty of Medicine - Menoufia University, Egypt

\*Corresponding author: Amany Fared Elziat, Mobile: (+20) 01007285789, E-Mail: amanifareid7@gmailcom

### ABSTRACT

**Background:** Monosodium Glutamate (MSG) is one of the most commonly used flavors that may lead to motor incoordination. Alpha-lipoic acid (ALA) has antioxidant and anti-inflammatory effects with neuro-protective effects.

**Objectives:** Present work aimed to elucidate possible protective potential of ALA on the motor incoordination induced by MSG and the possible underlying mechanisms. **Materials and Methods:** Seventy male Wister albino rats were randomized into five groups: 1-Control group (10 rats). 2-MSG group (20 rats): MSG 3 wks and MSG 6 wks ten rats each. 3-ALA pretreated group (10 rats). 4-ALA cotreated group (20 rats): ALA cotreated 3 wks and ALA cotreated 6 wks ten rats each. 5-Alpha-Lipoic acid-post- group. Thereafter, motor coordination assessed using Rotarod and Footprint analysis were assessed in addition to serum Malondialdehyde (MDA), total antioxidant capacity (TAC), Tumor necrosis factor- alpha (TNF-  $\alpha$ ), cerebellar brain derived neurotrophic factor (BDNF) and Oxidative DNA damage were measured. Also, histo-pathological examination of cerebellar tissue was performed and number of purkinje cells was assessed. Results: ALA resulted in better improvement for MSG-induced motor incoordination, oxidative impairment and microscopic alterations of cerebellar architecture in cotreated group than that of post-treated group, with insignificant change in pretreated group. **Conclusion:** ALA exhibit protective effect against motor incoordination, oxidative stress and cerebellar damage induced by MSG toxicity best as cotreatment, less when used as post-treatment but this effect useless as pretreatment.

**Keywords:** Alpha-lipoic acid, BDNF and oxidative DNA damage, motor incoordination, MSG.

### INTRODUCTION

Over the world, people's health issues are directly related to unnecessary dietary modifications and delicious food. The majority of them consume fast food from nearby restaurants that contains flavor-enhancing additives such monosodium glutamate (MSG). Changes in eating habits and the body's production of reactive oxygen species (ROS) brought on by MSG ultimately result in systemic abnormalities <sup>(1)</sup>.

According to several studies using animal models, MSG alters the development, metabolism, and activities of many different organs, including the liver, thymus, ovaries, kidney, and several areas of the brain, including the cerebellum. According to investigations, adult rats' Purkinje cells experienced cellular deaths and Purkinje cell degeneration after receiving MSG orally at doses of 3 g/kg BW every day for 14 days straight. Also, young rats given a subcutaneous injection of MSG at a dosage of 4 mg/g BW every other day for ten days had brief impairments in their ability to coordinate their movements <sup>(2)</sup>.

In addition to increasing oxidative stress, MSG also lowers the activity of enzyme antioxidants. Free radical generation or elimination in cells that is either too high or too low, with the bulk of these free radicals being oxygen radicals and other reactive oxygen species (ROS) <sup>(3)</sup>. Oxidative stress is caused by the metabolism of food and a number of extracellular and intracellular components, including hormones, cytokines, and detoxification procedures. Hence, prolonged MSG consumption and excessive renal glutamate metabolism can both be sources of ROS <sup>(4)</sup>. Thiocetic acid, also known as alpha-lipoic acid (ALA), has emerged as one of the most widely utilized antioxidants for protection. A vitamin-like antioxidant called alpha lipoic acid is

known as the "universal antioxidant" because of its special property of being both fat- and water-soluble <sup>(5)</sup>. Due to its crucial involvement in the operation of several enzymes involved in oxidative metabolism, it has an antioxidant effect. It is thought that ALA, or its reduced form, dihydrolipoic acid, performs a variety of biochemical tasks, including functioning as metal chelators, lowering the oxidized forms of other antioxidant agents like vitamin C and E, and glutathione, and acting as biological antioxidants. Moreover, ALA has been demonstrated to lessen oxidative stress during exercise training and to ameliorate endothelial dysfunction <sup>(6)</sup>.

Due to these advantages, ALA has been proposed as a potential therapeutic agent for a number of chronic diseases with significant economic, social, and epidemiological consequences, including diabetes mellitus and its complications, hypertension, Alzheimer's disease, Down syndrome, cognitive impairment, and some types of cancer. In the medical and nutritional care of patients, ALA is suggested as a dietary supplement <sup>(7)</sup>.

It was discovered that ALA dramatically improved muscular coordination and strength, and that its capacity to lower induced lipid peroxidation by scavenging ROS was responsible for its protective impact against tardive dyskinesia <sup>(7)</sup>.

In light of this, the current study aimed to investigate the protective potential of alpha-lipoic acid in different manners, as pretreatment, cotreatment and post treatment, on MSG induced motor coordination deficits in male Wister albino rats and the underlying mechanism involved.

## 2. MATERIAL AND METHODS

### 2.1. Animals and experimental groups.

70 male wister albino rats, weighing 160–200 g apiece and 2–3 months old, were purchased from a nearby animal care facility. Five animals were housed in well ventilated cages (80 x 40 x 30 cm) with unrestricted access to water and a semi-synthetic balanced meal at room temperature and an artificial 12-hour light/dark cycle. The US National Institutes of Health's guidance for the care and use of laboratory animals was followed for animal housing, handling, sampling, and experimental methods <sup>(8)</sup>.

#### Rats were split into five groups at random after two weeks of acclimatization:

##### 1- Control group (C) (n=10 rats):

Control rats received 2 ml of (5% ethanol mixed with olive oil, ethanol/olive oil, 1:19 v/v) orally and 0.5 ml of distilled water was given intragastric gavage, once daily, for six weeks.

##### 2- MSG group (n=20 rats):

2mg/g BW of MSG (Ajinomoto Co. Inc., Tokyo, Japan) was dissolved in 0.5 ml of distilled water and given by intragastric gavage, and 2 ml of (5% ethanol mixed with olive oil, ethanol/olive oil, 1:19 v/v) given orally once daily <sup>(6)</sup>.

All twenty rats received the treatment from the first day. Motor coordination tests were performed for ten rats after three weeks thereafter; rats were sacrificed to investigate the appearance of motor incoordination after this period (**MSG 3 wks**). The remaining ten rats were subjected to motor coordination tests after six weeks then sacrificed for further investigations (**MSG 6 wks**).

##### 3- ALA pretreated MSG-administered group (ALA pretreated) (n=10 rats):

200 mg/kg BW of ALA provided from (Chemical industries development, Giza, Egypt) was dissolved in 2 ml of (5% ethanol mixed with olive oil, ethanol/corn oil, 1:19 v/v) <sup>(7)</sup>, given orally, for three weeks.

Then MSG (as in MSG group) given orally, and 2 ml of (5% ethanol mixed with olive oil, ethanol/olive oil, 1:19 v/v) given orally once daily, for another three weeks.

##### 4- ALA cotreated MSG-administered group (ALA cotreated) (n=20 rats):

MSG given (as in MSG group) with concomitant oral administration of ALA (as in pretreated group), once daily. Ten rats were subjected to motor-coordination tests then scarified after 3 weeks (**ALA cotreated 3 wks**). Te remaining ten rats received ALA concomitant with MSG for six weeks (**ALA cotreated 6 wks**).

##### 5- ALA post-treated MSG-administered group (ALA post-treated) (n=10 rats):

MSG given (as in MSG group) by intragastric gavage, and 2 ml of (5% ethanol mixed with olive oil, ethanol/olive oil, 1:19 v/v) given orally once daily, for three weeks.

Then oral administration of ALA (as in pretreated group) once daily, for another three weeks.

### Study design:

After treatment rats were subjected to motor coordination tests using Rotarod test and Foot print analysis. In order to estimate the serum levels of malondialdehyde (MDA), total antioxidant capacity (TAC), and tumor necrosis factor-alpha (TNF-alpha), all rats were starved overnight before retro-orbital blood samples were taken. Rats were then rendered unconscious by inhaling 70% ethanol for 10 minutes before being killed via cervical elongation and dislocation. Each rat's skull was opened, the cerebellum removed, and it rinsed in ice-cold phosphate buffer saline (pH 7.4) before being weighed and cut into two equal pieces. For the test of cerebellar brain derived neurotrophic factor (BDNF) and oxidative DNA damage (8-hydroxy-2-deoxyguanosine), the left side of the brain was prepared (8-OhdG).

Hematoxylin and Eosin staining (H&E) was used to histopathologically examine the right side, which was preserved in 10% formalin saline.

### Assessment of motor coordination:

**1- Rotarod test <sup>(9)</sup>:** Rats were given four trials per day for three consecutive days as training. Rats were tested in 3 sessions, for maximum of **180 second** each session (**3**). The following parameters were measured:

- 1- The number of falls: The average number of falls for each rat came from the three test sessions that took place that day..
- 2- The latency of the rats on the running surface: were reported as percentages since they were computed by dividing the sum of the two longest durations spent on the revolving rod by 180 seconds.

### 2-Footprint analysis <sup>(10)</sup>:

Manual runway lined with graph paper and enclosed by white paper. The length of the hind stride and the width of the hind base were measured two to four steps into each run (the distance between the right and left hind-limb strides). Statistics were performed using mean values.

### Biochemical assays:

#### Blood sampling <sup>(11)</sup>:

Using a tiny, heparinized capillary tube, blood samples were taken from the rat's retro-orbital venous plexus after an overnight fast of 12 hours. Two milliliters of blood were drawn into a clean graduated centrifuge tube, allowed to clot for 10 minutes at room temperature in a water bath, and then spun for another 10 minutes at a speed of 4000 rpm. When needed for the upcoming examination of the supernatant serum, it was collected in a dry, clean tube and frozen at -20°C: serum MDA (Biodiagnostic Company, Dokki Giza, Egypt), TAC (Biodiagnostic Company, Dokki Giza, Egypt) by colorimetric method, TNF- $\alpha$  (ab46070, abcam, Cambiraidge, UK) by ELISA method according to the manufacturer's instruction.

The left half of a rat's cerebellum was homogenized in 5 ml of cold buffer for every gramme of tissue after being perfused with PBS solution (Biodiagnostic Company, Egypt). After centrifuging the homogenized cerebellum tissue at 4000 rpm for 15 minutes at 4 °C, the supernatant was collected and kept at 80 °C until measurements of: cerebellar BDNF (MBS9139053, MyBiosource, California, USA) and oxidative DNA damage (MBS269902, MyBiosource, California, USA) by ELIZA method according to the manufacturer's instruction.

### **Histopathological assessment of the brain tissue.**

#### **Histological examination:**

Each rat's right hemisphere of the cerebellum was embedded in paraffin and preserved in 10% formalin solution before being cut into successive coronal slices with a freezing sledge microtome. Afterwards, to examine the brain's hippocampus under a light microscope, sections of the brain were stained with hematoxylin and eosin (H&E).

#### **Morphometric analysis** <sup>(12)</sup>:

The information was collected using a colored video camera mounted atop a light microscope (Panasonic color CCTV camera, Matsushita Communication Industry Co. Ltd., Japan) (Olympus BX40, Olympus Optical Co. Ltd., Japan). Each animal's five non-overlapping fields from its five distinct portions were examined. It was determined how many Purkinje cells there were and how thick the molecular cell layer was.

### **Ethical considerations:**

**Ethics-related matters: The Faculty of Medicine at Menoufia University in Egypt's Research Ethics Committee gave its approval to the study's procedure. The National Institutes of Health (NIH) criteria for the care and handling of animals used in research were followed.**

#### **Statistical analysis**

The SPSS software tool, version 22, was used to evaluate the results (Statistical Package for the Social Science, SPSS Inc., Chicago, Illinois, USA). The information was shown as mean  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare quantitative variables across the groups under study, along with Tukey's post hoc analysis. P value less than 0.05 was regarded as significant.

## **RESULTS**

### **Behavioral Changes:**

The mean value  $\pm$  SD of the number of falls from rotarod apparatus in MSG 3 wks, MSG 6 wks and ALA pretreated groups were significantly higher

( $P < 0.05$ ) when compared with its value of C group. However, it was significantly lower ( $P < 0.05$ ) in ALA cotreated 3wks and ALA cotreated 6 wks groups when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups and insignificantly different ( $P > 0.05$ ) when compared with its value of C group. It was insignificantly different ( $P > 0.05$ ) in ALA cotreated 6 wks group when compared with its value of ALA cotreated 3 wks group. Also, it was insignificantly different ( $P > 0.05$ ) in ALA post-treated group when compared with the corresponding value of ALA cotreated 6 wks and was significantly higher ( $P < 0.05$ ) when compared with its value of ALA cotreated 3 wks and was significantly lower ( $P < 0.05$ ) when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups. **Table (1)**

The mean value  $\pm$  SD of the Latency Period on the running surface of rotarod apparatus in MSG 3 wks and MSG 6 wks groups was significantly lower ( $P < 0.05$ ) when compared with its value of C group . But it was significantly higher ( $P < 0.05$ ) in ALA cotreated 3wks and ALA cotreated 6 wks groups when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups. However, it was significantly lower ( $P < 0.05$ ) in ALA post-treated group when compared with its value of ALA cotreated 6 wks and ALA cotreated 3 wks and was significantly higher ( $P < 0.05$ ) when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups. **Table (1)**

The mean value  $\pm$  SD of the Stride length in MSG 3 wks, MSG 6 wks and ALA pretreated groups was significantly lower ( $P < 0.05$ ) when compared with its value of C group. It was significantly higher ( $P < 0.05$ ) in ALA cotreated 3 wks and ALA cotreated 6 wks groups when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups. Also, it was significantly lower ( $P < 0.05$ ) in ALA post-treated group when compared with its value of ALA cotreated 6 wks and ALA cotreated 3 wks groups and significantly lower ( $P < 0.05$ ) when compared with its value of C group. **Table (1)**

The mean value  $\pm$  SD of the Stride Width in MSG 3 wks and MSG 6 wks groups was significantly higher ( $P < 0.05$ ) when compared with its value of C group . But it was significantly lower ( $P < 0.05$ ) in ALA cotreated 3 wks and ALA cotreated 6 wks groups when compared with its value of ALA pretreated, MSG 6 wks MSG 3 wks groups. Also, it was significantly higher ( $P < 0.05$ ) in ALA post-treated group when compared with its value of ALA cotreated 6 wks , ALA cotreated 3 wks groups, **Table (1).**

**Table (1):** The mean value ± SD of the number of falls from rotarod apparatus, Latency Period on running surface of rotarod (%), Stride length (mm), Stride Width (mm) in all studied groups: Control, MSG 3 wks, MSG 6 wks, ALA pretreated, ALA cotreated 3wks, ALA cotreated 6wks and ALA post-treated groups.

Parameter	Control	MSG		ALA pretreated	ALA cotreated		ALA Post-treated	p-value
		MSG 3wks	MSG 6wks		ALA cotreated 3wks	ALA Cotreated 6wks		
Number of falls from rotarod apparatus	1.5±0.5	6.5±0.5*	7.8±0.76*#	6.1±0.7* Ω	2±0.6 # Ω∞	2.5±0.5# Ω∞	3.3±0.8*# Ω∞\$	<0.05
Latency Period on running surface of rotarod (%)	83.8±6.3	40.5±3*	36.5±4.7*	39.3±4.3*	81.4±5.6# Ω∞	71.8±8.6*# Ω∞	56.5±5*# Ω∞\$¥	<0.05
Stride length (mm)	66.1±3.4	45.6±4.7*	38±3.7*	45.1±5.1*	66±2.8# Ω∞	61.6±3.8# Ω∞	39.6±6.1*# \$¥	<0.05*
Stride Width (mm)	13.5±1	25±1.4*	28.5±1.8*#	23.8±0.9* Ω	16±0.89 # Ω∞	15.6±1.2# Ω∞	21.1±1.2*# Ω∞\$¥	<0.05*

Data are expressed as mean ± SD. Number of rats in each group was 10 (n=10). One-way analysis of variance was applied, and significant level was set on P < 0.05. The marks \*, #, Ω, ∞, \$ and ¥ indicate significant differences when values are compared with control, MSG 3wks, MSG 6wks, ALA pre-treated, ALA cotreated 3wks, ALA cotreated 6wks groups respectively.

**Changes of biochemical markers:**

The mean value ± SD of the serum MDA and Cerebellar Oxidative DNA damage (8-OhdG) in MSG 3 wks, MSG 6 wks and ALA pretreated groups was significantly higher (P<0.05) when compared with its value of C group . However, it was significantly lower (P<0.05) in ALA cotreated 6 wks groups when compared with its value of ALA pretreated, MSG 6 wks group and MSG 3 wks groups. But it was significantly higher (P<0.05) in ALA post-treated group when compared with its value of ALA cotreated 6 wks and ALA cotreated 3 wks and was significantly lower (P<0.05) when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups, **Table (2).**

The mean value ± SD of the Total Anti-oxidant Capacity in MSG 3 wks, MSG 6 wks and ALA pretreated groups was significantly lower (P<0.05) when compared with its value of C group . But it was significantly higher (P<0.05) ALA cotreated 3 wks and ALA cotreated 6 w ks groups when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups. However, it was significantly lower (P<0.05) in ALA post-treated group when compared with its value of ALA cotreated 6 wks and ALA cotreated 3 wks groups and was significantly higher (P<0.05) when

compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups, **Table (2).**

The mean value ± SD of the serum TNF-alpha in MSG 3 wks, MSG 6 wks and ALA pretreated groups was significantly higher (P<0.05) when compared with its value of C group. However, it was significantly lower (P<0.05) in ALA cotreated 3 wks and ALA cotreated 6 wks groups when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups. And it was insignificantly different in ALA post-treated group (P>0.05) when compared with its value of ALA cotreated 6 wks and was significantly higher (P<0.05) when compared with its value of ALA cotreated 3 weeks, **Table (2).**

The mean value ± SD of the cerebellar BDNF in MSG 3 wks, MSG 6 wks and ALA pretreated groups was significantly lower (P<0.05) when compared with its value of C group. But it was significantly higher (P<0.05) in ALA cotreated 3 wks and ALA cotreated 6 w ks groups when compared with its value of ALA pretreated, MSG 6 wks group and MSG 3 wks groups. Also, it was insignificantly different (P>0.05) in ALA post-treated group when compared with its value of ALA cotreated 6 wks and ALA cotreated 3 wks groups **Table (2).**

**Table (2):** The mean value ± SD of the serum MDA, Cerebellar BDNF, Cerebellar Oxidative DNA damage (8-OhdG) (ng/mg) in all studied groups: Control, MSG 3 wks, MSG 6 wks, ALA pretreated, ALA cotreated 3wks, ALA cotreated 6wks and ALA post-treated groups.

Parameter	Control (No.=10)	MSG		ALA pretreated	ALA cotreated (No. = 20)		ALA Post treated	P-value
		MSG 3wks	MSG 6wks		ALA cotreated 3wks	ALA Cotreated 6wks		
MDA (nmol/ml)	1.18±0.3	7.5±0.8*	9.3±0.5*#	7.45±0.8* Ω	2.1±0.3 # Ω∞	2.15±0.9 #Ω∞	4.1±0.7 *#Ω∞\$¥	<0.05*
Total Anti-oxidant Capacity (µmol/L)	1.7±0.17	0.9±0.2*	0.7±0.1*	0.8±0.1*	1.6±0.4# Ω∞	1.7±0.17#Ω∞	1.2±0.3*# Ω∞\$¥	<0.05*
Serum TNF-alpha (pg/ml)	48.5±4.1	166.6±16.3*	179.8±12.5*	153.3±17.5* Ω	51.6±5 # Ω∞	69.8±4.6 #Ω∞	77.3±8.5*# Ω∞\$	<0.05*
Cerebellar BDNF (pg/mg)	376.6±41.3	50±4.4*	37.3±5.5*#	*62.8±2.3* Ω	295±45 # Ω∞	203.8±24 #Ω∞	195.2±6.6 *#Ω∞	<0.05*
Cerebellar (8-OhdG) (ng/mg)	5.7±1.3	67.8±6.9*	81.6±3.7*#	62.5±9.3* Ω	19.3±3.5*# Ω∞	23.5±3.2*# Ω∞	36±4.4 *#Ω∞\$¥	<0.05*

Data are expressed as mean ± SD. Number of rats in each group was 10 (n=10). One-way analysis of variance was applied, and significant level was set on P < 0.05. The marks \*, #, Ω, ∞, \$ and ¥ indicate significant differences when values are compared with control, MSG 3wks, MSG 6wks, ALA pretreated, ALA cotreated 3wks, ALA cotreated 6wks groups respectively.

**Histo-pathological results:**

**A) Statistical results:**

The mean value ± SD of the cerebellar weight, number of purkinjie cells and molecular layer length in MSG 3 wks, MSG 6 wks and ALA pretreated groups was significantly lower (P<0.05) when compared with its value of C group. However, it was significantly higher (P<0.05) in ALA cotreated 3 wks and ALA cotreated 6 w ks groups when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups. Also, it was significantly lower (P<0.05) in ALA post-treated group when compared with its value of ALA cotreated 6 wks and ALA cotreated 3 wks and was significantly higher (P<0.05) when compared with its value of ALA pretreated, MSG 6 wks and MSG 3 wks groups, **Table (3).**

**Table (3):** The mean value ± SD of the **Cerebellar weight (mg), Number of purkinjie cells** and the Molecular layer length (µm) in all studied groups: Control, MSG3wks, MSG6wks, ALA pretreated, ALA cotreated 3wks, ALA cotreated 6wks and ALApost-treated groups.

Parameter	Control	MSG		ALA pretreated	ALA cotreated		ALA Post-treated	p-value
		MSG 3 wks	MSG 6 wks		ALA cotreated 3wks	ALA Cotreated 6wks		
Cerebellar weight (mg)	72.5±9.3	39.1±5.6*	35±6*	39.6±6.3*	69.1±5.8# Ω∞	68.3±5.1# Ω∞	66±7.4 #Ω∞	<0.05*
Number of purkinjie cells	12.8±2.2	6.8±1.4*	4.9±0.8*	6.7±0.9*	12±0.9 #Ω∞	10±0.9 #Ω∞	8.9 ±1.4 *#Ω∞\$¥	<0.05*
Molecular layer length(µm)	182.5±9.3	72.5±9.4*	53.3±8.7*#	80.3±12.5* Ω	172.5±2.7# Ω∞	165.8±5.8*# Ω∞	164.1 ±4.9*# Ω∞	<0.05

Data are expressed as mean ± SD. Number of rats in each group was 10 (n=10). One-way analysis of variance was applied, and significant level was set on P < 0.05. The marks \*, #, Ω, ∞, \$ and ¥ indicate significant differences when values are compared with control, MSG 3wks, MSG 6wks, ALA pretreated, ALA cotreated 3wks, ALA cotreated 6wks groups respectively.

### B) Light Microscopic Examination Results:

There is marked histological changes in MSG 3 wks and MSG 6 wks groups when compared to control group. These changes were in the form of sever congestion of the cortical, medullary and subdural blood vessels in MSG 3 wks and MSG 6 wks groups. The purkinje cells appeared with different degrees of degeneration; most of cells appeared shrunken with darkly stained nuclei, few cells showed marked distortion in shape with ghost-shape appearance Plate (1a).

The cerebellar sections MSG 6 wks showed more sever changes than that found in the MSG 3 wks group. The single row of purkinje cells showed disarrangement Plate (1b,c).

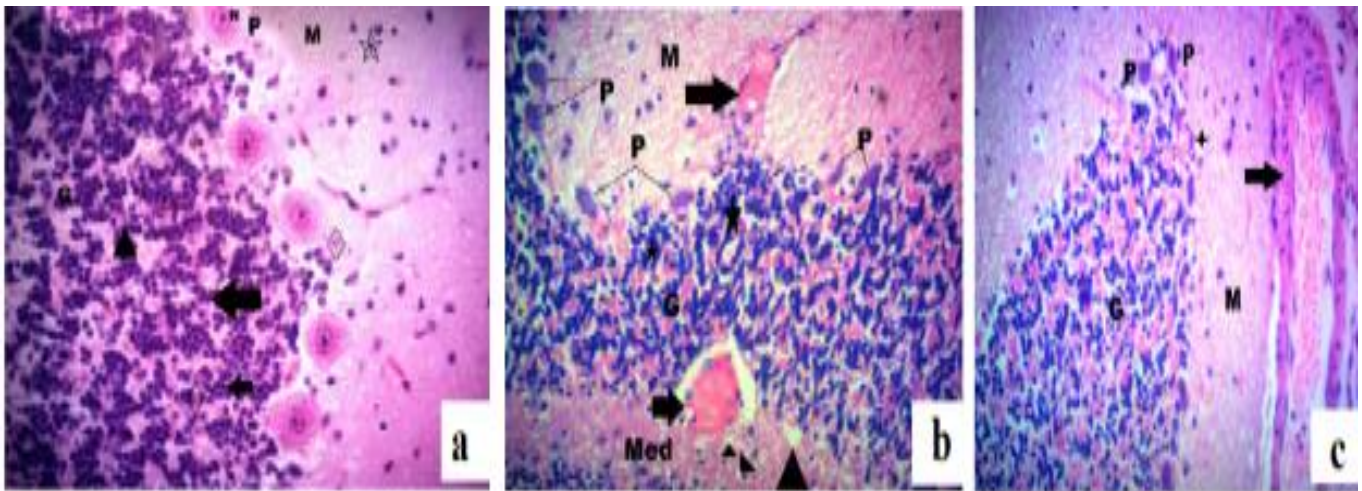
The cerebellar sections of rats of pretreated group showed histological picture similar to much extent to that of MSG 3 wks group. There was thinning of granular layer and hypocellularity. Most of cells were shrunken with pyknotic darkly stained nuclei while some others of cells appeared normal in size and shape having vesicular nuclei with prominent nucleoli. There was evidence of infiltration with edema and congestion.

These changes were to high extent improved in ALA cotreated group. There was mild congestion. Many purkinje cells were nearly normal in size, flask in shape with central rounded vesicular nuclei. Very few cells showed degeneration and there was clumping in some cells and small spaces in the granular layer.

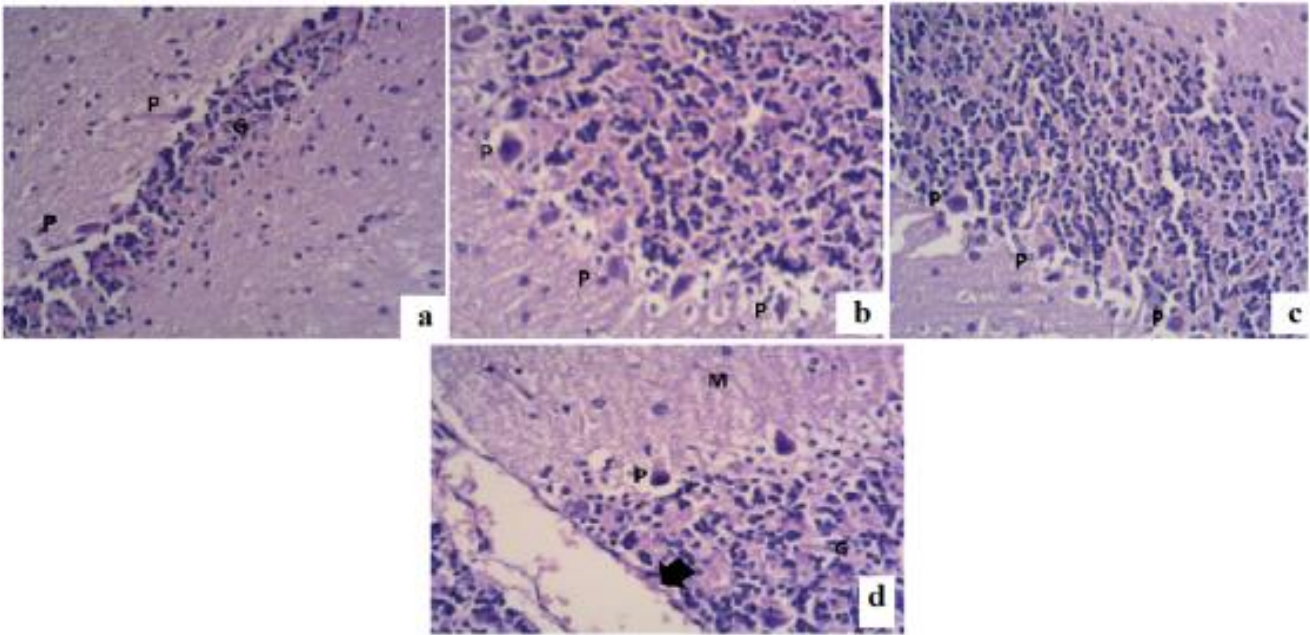
But ALA post-treated group revealed mild improvement. There were some areas of congestion and edema. The purkinje cells were still degenerated with shrinkage of their cytoplasm and pyknotic of their nuclei with peripheral hallow around them. Evidence of edema and sloughing of capillary endothelium was still present.

As shown in Plate (3); the cerebellar sections of ALA cotreated 3 wks and ALA cotreated 6 wks rats showed a picture more or less similar to control. Most purkinje cells were nearly normal with central rounded vesicular nuclei.

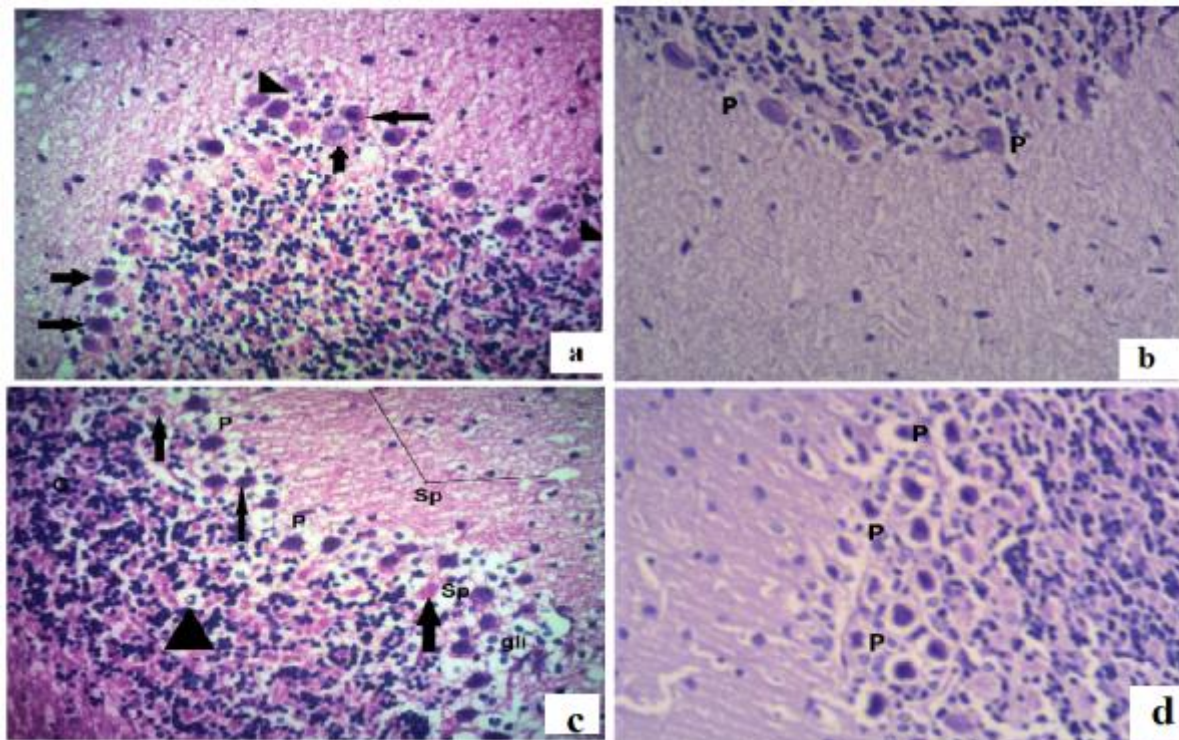
The cerebellar sections of ALA cotreated 3 wks rats showed a picture with better improvement when compared to MSG 3 wks group. ALA cotreated 6 wks rats showed a picture with better improvement when compared to MSG 6 wks group.



**Plate (1):** Histo-pathological assessment of rat cerebellum showing the purkinje cells degeneration (a) (C) group showing the three layers of cerebellar cortex; the outer molecular layer (M), the middle Purkinje cell layer (P), the granular layer (G); (b) (MSG 3 wks) group showing congestion of cortical and medullary blood vessels (arrow) and different degrees of purkinje cell (P) degeneration. Most of cells appear shrunken with darkly stained pyknotic nuclei; (C) (MSG 6 wks) group showing congestion of subdural blood vessel (arrow) and different degrees of purkinje cell degeneration (P). Most of cells appear shrunken with darkly stained pyknotic nuclei. Few cells show marked distortion in shape with ghost shape appearance (star). (Examined under a light microscope; Olympus bx41, H&E x200 for all)



**Plate (2):** Histo-pathological assessment of rat cerebellum showing (a) (ALA pretreated) group showing degenerated Purkinje cells with hypocellularity of Granular cells; (b) (ALA cotreated) group showing most of cells appeared normal in size and shape having vesicular nuclei with prominent nucleoli and showed the characteristic flask shape appearance, while some others were shrunken darkly stained nuclei; (c) (ALA post-treated) group showing the Purkinje cells were still degenerated with shrinkage of their cytoplasm. (Examined under a light microscope; Olympus bx41, H&E x200 for all)



**Plate (3):** Histo-pathological assessment of rat cerebellum showing (a) (ALA cotreated 3 wks) group showing some Purkinje cells nearly normal in size and shape with vesicular nucleus and prominent nucleolus (arrow). Other cells appear shrunken with deeply stained pyknotic nuclei (arrow head). The granular and molecular cell layer appeared nearly normal.; (b) (ALA Cotreated 6 wks) group showing most of cells appeared normal in size and shape having vesicular nuclei with prominent nucleoli and showed the characteristic flask shape appearance; (c) (MSG 3 wks) showing most of cells appear shrunken with darkly stained pyknotic nuclei and arranged in more than one layer. Some cells show complete degeneration with disappearance of their nuclei having ghost shape appearance (arrow); (d) (MSG 6 wks) showing Purkinje cells were arranged in more than one layer. (Examined under a light microscope; Olympus bx41, H&E x200 for all).

## DISCUSSION

MSG, a naturally occurring substance found in many protein-rich foods, has been linked to neurotoxic consequences, most likely as a result of oxidative stress<sup>(13)</sup>. According to a research by **Mekkawy et al.**<sup>(14)</sup> published in 2020, MSG can affect the cerebellum's structural, histopathologic architecture, and tissue integrity and function in Wister albino rats.

Alpha Lipoic Acid, is a potent antioxidant, it possesses a wide array of the metabolic benefits with neuroprotective properties<sup>(15)</sup>.

The aim of the present work was to study the possible beneficial role of ALA on the MSG-induced motor coordination deficits in male Wister albino rats when used as pretreatment, cotreatment and post-treatment to MSG, by assessment of motor coordination and to investigate the possible mechanisms behind the actions of ALA. Furthermore, H&E staining of the cerebellum was assessed.

Our study showed deterioration in motor coordination, as demonstrated by significant decrease in the performance of the rats in the behavioral tests in MSG3wks and MSG6wks groups when compared to control group. And these results was in agreement with previously reported researches of **Aminuddin et al.**<sup>(16)</sup> and **Lau and Tymianski**<sup>(17)</sup>.

And this may be explained by: As MSG mostly consists of glutamate, it may have killed Purkinje cells by too activating glutamate receptors<sup>(18)</sup>.

Our results of behavioral tests demonstrated significant improvement in motor incoordination in ALA cotreated 3wks and ALA cotreated 6wks groups when compared with MSG 3 wks and MSG 6 wks groups. And these results was in agreement with previously reported researches of: **Liu et al.**<sup>(18)</sup> study that observed that ALA can protect against cognitive dysfunction and **Elsawy et al.**<sup>(19)</sup> study. However, our results show no improvement in pretreated group when compared to ALA cotreated 3wks and ALA cotreated 6wks. And this can be explained by: the ALA's short half-life, low solubility, and instability in the stomach, as well as its low bioavailability because of these processes, which include hepatic breakdown<sup>(20)</sup>.

However, post-treated group showed significant improvement in motor-coordination when compared to MSG 3 wks and MSG 6 wks groups but this improvement is still significantly lower than that of ALA cotreated 3 wks and ALA cotreated 6 wks groups and this is well supported by our histo-pathological results; this may be due to neural necrosis. **Goldsmith**<sup>(21)</sup>, **Daniels and Brown**<sup>(22)</sup> and **Jiang et al.**<sup>(23)</sup> Increased calcium entry, internal oxidative stress with the production of free radicals, mitochondrial dysfunction, and eventually apoptosis result from increased glutamate levels when it is not cleared by the body, according to studies. It appears that alpha lipoic acid cannot stop this necrosis and apoptosis that has already taken place.

Monosodium glutamate causes oxidative stress as demonstrated in our study which revealed a significant increase in the serum MDA in MSG3wks, MSG6wks groups when compared to control group and also significant decrease in the Total antioxidant capacity in MSG3wks, MSG6wks groups when compared to control group. And these result agree with **Heil et al.**<sup>(24)</sup> study. This can be explained by: According to MSG, there is a reduction in the activity of enzyme antioxidants such superoxide dismutase and catalase **Onaolapo et al.**<sup>(25)</sup>.

This is in contrary to **Abd-Elkareem et al.**<sup>(26)</sup> that revealed increase in TAC level with MSG administration and returned it to an adaptive compensating reaction to oxidative instability after MSG supplementation.

ALA supplementation to MSG group revealed significant improvement in oxidative stress markers, MDA and TAC levels, in ALA cotreated 3wks and ALA cotreated 6wks groups compared to MSG3wks, MSG6wks groups as shown in our results and this is in agree with<sup>(27)</sup>. The bioactivity of lipoic acid to directly react with oxidation and its capacity to block the oxidation processes in the lipid and aqueous cellular compartments may be responsible for ALA's protective function on lipid peroxidation state<sup>(28)</sup>. Also, it appears that maintaining mitochondrial function is necessary for neural resilience to excitotoxic shocks, and ALA is crucial for maintaining mitochondrial metabolism. The majority of cell death caused by glutamate and oxidants was reduced by ALA, along with oxidative damage, antioxidant defense, and mitochondrial function<sup>(27)</sup>.

Neuro-inflammation plays an important role in MSG induced motor incoordination. Our results revealed significant increase in TNF- $\alpha$  in MSG 3 wks, MSG 6 wks groups when compared to control group, and this is in agree with **Abdou et al.**<sup>(27)</sup>. And this can explained by excessive increase in ROS produced by MSG leads to overexpression of TNF-  $\alpha$  in microglia and astrocytes<sup>(29)</sup>.

Continually, the present results demonstrated significant improvement in the levels of TNF- $\alpha$  in the serum samples of ALA cotreated 3wks and ALA cotreated 6wks groups when compared to MSG 3 wks, MSG 6 wks groups. And this is in agreement with **Çakır et al.**<sup>(29)</sup>.

Our results showed decrease in BDNF in MSG 3 wks, MSG 6 wks when compared to control group and this is in agree with **Rosa et al.**<sup>(30)</sup> study revealed that MSG can induce down regulation of BDNF mRNA and protein levels and this can explain the decrease in cerebellar BDNF levels.

However, ALA supplementation to MSG group showed increase in BDNF in ALA cotreated 3 wks, ALA cotreated 3 wks and ALA post-treated groups when compared to MSG 3 wks, MSG 6 wks and pretreated groups and this is in agree with **Filho et al.**<sup>(31)</sup> that found that treatment with ALA significantly



increased BDNF levels in the prefrontal cortex. And this may be due to the antioxidant and anti-inflammatory effects demonstrated in our results.

And this is in contrary to **Yusha'u *et al.*** <sup>(32)</sup> that reported that ALA did not significantly affect the brain BDNF level of the mice.

Our study showed a significant elevation in 8-Hydroxyguanine Levels in in MSG3wks and MSG6wks groups when compared to control group. And this is in agreement with <sup>(33)</sup>.

Also, our study showed a significant decrease in 8-Hydroxyguanine Levels in ALA cotreated 3 wks, ALA cotreated 6wks and ALA post-treated groups when compared to MSG3wks, MSG6wks, ALA pre-treated groups. The ability of ALA to chelate the transitional metal ions, raise levels of cytosolic glutathione and vitamin C, and avoid related toxicity as a result of their loss can be used to explain this. Moreover, ALA is an efficient free radical scavenger in both the aqueous and lipid phases <sup>(34)</sup>.

Our histological results proved the motor coordination deficits in MSG3wks, MSG6wks, ALA pre-treated groups which revealed purkinje cells degeneration with significant decrease in their number and also significant decrease in molecular layer length when compared to control group. And this is in agree with **Hashem *et al.*** <sup>(35)</sup> investigations who concluded that MSG plays a key role in neurons cell death due to its effect in increase of oxidative damage.

Also, the histological results have showed significant improvement in the purkinje cells number and degeneration in ALA cotreated 3 wks, ALA cotreated 6 wks and ALA post-treated groups when compared to MSG3wks, MSG6wks, ALA pretreated groups that prove that ALA has protected against these neurotoxic and degenerative effects observed by the antioxidant, anti-inflammatory and neuro-protective and neuro-therapeutic effect of ALA against DNA damage.

## CONCLUSION

Our findings support that MSG despite its flavoring functions may be harmful to cerebellar function and structure. We demonstrated that concomitant oral administration of ALA offered protection from MSG-induced oxidative impairment and microscopic alterations of cerebellar purkinje neurons and its use as post-treatment can achieve some improvement but using it for a period and then it is stopped (as pretreatment) cannot protect against further use of MSG.

**Supporting and sponsoring financially:** Nil.

**Competing interests:** Nil.

## REFERENCES

1. **Banerjee A, Mukherjee S, Maji B (2021):** Monosodium glutamate causes hepato-cardiac derangement in male rats. doi:10.1177/09603271211049550
2. **Singh P, Mann K, Mangat H *et al.* (2003):** Prolonged glutamate excitotoxicity: effects on mitochondrial antioxidants and antioxidant enzymes. *Mol Cell Biochem.*, 243: 139–145.
3. **Prastiwi D, Djunaidi A, Partadiredja G (2015):** High dosage of monosodium glutamate causes deficits of the motor coordination and the number of cerebellar Purkinje cells of rats. *Human & Experimental Toxicology*, 34(11):1171-1179.
4. **Paul M, Abhilash M, Varghese M *et al.* (2012):** Protective effects of alpha-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. *Toxicol Mech Methods*, 22(8):625–30.
5. **Moura F, de and rade K, dos Santos J *et al.* (2015):** Lipoic Acid: its antioxidant and anti-inflammatory role and clinical applications. *Curr Top Med Chem.*, 15(5):458-83.
6. **Narayanan S, Kumar R, Paval J *et al.* (2010):** Effect of ascorbic acid on the monosodium glutamate-induced neurobehavioral changes in periadolescent rats. *Bratislava Med J.*, 111:170–175.
7. **Alsemeh A, Ibrahim A, Mohammed H *et al.* (2020):** Structural and Behavioural Changes in Rat Hippocampus Induced by Methotrexate and The potential ameliorative Effect of Alpha lipoic acid. *Egyptian Journal of Histology*, 43(1): 301-324.
8. **Garber J, Barbee R (2021):** Guide for the care and use of laboratory animals: Institute for laboratory animal research, division on earth and life studies. Washington DC, USA: National Academies Press; eighth. Pp. 220. [https://www.orc.msstate.edu/sites/www.orc.msstate.edu/files/inline-files/guide-for-the-care-and-use-of-laboratory-animals\\_0.pdf](https://www.orc.msstate.edu/sites/www.orc.msstate.edu/files/inline-files/guide-for-the-care-and-use-of-laboratory-animals_0.pdf)
9. **Deacon R (2013):** Measuring Motor Coordination in Mice. *J Vis Exp.*, 75: e2609. doi: 10.3791/2609.
10. **Kara H, Çağlar C, Asiltürk M *et al.* (2021):** Comparison of a manual walking platform and the CatWalk gait analysis system in a rat osteoarthritis model. *Adv Clin Exp Med.*, 30(9):949-956.
11. **Parasuraman S, Raveendran R, Kesavan R (2010):** Blood sample collection in small laboratory animals. *Journal of Pharmacology & Pharmacotherapeutics*, 2: 87-93.
12. **Mohammed H, Hassan N, Aidaros A *et al.* (2020):** Effect of monosodium glutamate on the cerebellar cortex microscopic structure in suckling rats, and possible protective role of vitamin C. *Journal of Medical Histology*, 4(1): 97-118.
13. **Eweka A, Iniabohs O (2007):** Histological studies of the effects of monosodium glutamate on the cerebellum of adult Wistar rats. *Internet J Neurol.*, 8: 1–4.
14. **Mekki A, Ahmed Y, Khalaf A *et al.* (2020):** Ameliorative effect of Nigella sativa oil and vitamin C on the thyroid gland and cerebellum of adult male albino rats exposed to Monosodium glutamate (histological, immunohistochemical and biochemical studies). *Tissue Cell*, 66:101391. doi: 10.1016/j.tice.2020.101391.
15. **Astiz M, de Alaniz M, Marra C (2012):** The oxidative damage and inflammation caused by pesticides are reverted by lipoic acid in rat brain. *Neurochem. Int.*, 61: 1231–1241.

16. **Aminuddin M, Partadiredja G, Sari D (2015):** The effects of black garlic (*Allium sativum* L.) ethanol extract on the estimated total number of Purkinje cells and motor coordination of male adolescent Wistar rats treated with monosodium glutamate. *Anat Sci Int.*, 90(2):75-81.
17. **Lau A, Tymianski M (2010):** Glutamate receptors, neurotoxicity and neurodegeneration. *Eur J Physiol.*, 460: 525–542.
18. **Liu J, Atamna H, Kuratsune H et al. (2002):** Delaying brain mitochondrial decay and aging with mitochondrial antioxidants and metabolites. *Ann N Y Acad Sci.*, 959:133–166.
19. **Elsawy H, Al-Omair M, Sedky A et al. (2017):** Protective effect of  $\alpha$ -lipoic acid against  $\alpha$ -cypermethrin-induced changes in rat cerebellum. *Journal of Chemical Neuroanatomy*, 86(): 52–58.
20. **Brufani M, Figliola R (2014):** (R)- $\alpha$ -lipoic acid oral liquid formulation: Pharmacokinetic parameters and therapeutic efficacy. *Acta Bio-Medica Atenei Parm.*, 85: 108–115.
21. **Goldsmith P (2000):** Neuroglial responses to elevated glutamate in the medial basal hypothalamus of the infant mouse. *J Nutr.*, 130:1032–1038.
22. **Daniels M, Brown D (2001):** Astrocytes regulate N-methyl-D-aspartate receptor subunit composition increasing neuronal sensitivity to excitotoxicity. *J Biol Chem.*, 276(25):22446-52.
23. **Jiang S, Lertvorachon J, Hou S et al. (2005):** Chlortetracycline and demeclocycline inhibit calpains and protect mouse neurons against glutamate toxicity and cerebral ischemia. *Journal of Biological Chemistry*, 280: 33811-33818.
24. **Eman H, Wefak A, Muna A (2020):** Protective effect of Alphilipoic acid (ALA) on Some Oxidants and Antioxidant on Heart Damage Induced by Monosodium Glutamate (MSG) in Male Rabbits. *Indian Journal of Natural Sciences*, 10: 18036-18042.
25. **Abd-Elkareem M, Abd El-Rahman M, Khalil N et al. (2021):** Antioxidant and cytoprotective effects of *Nigella sativa* L. seeds on the testis of monosodium glutamate challenged rats. *Sci Rep.*, 11(1):13519. doi:10.1038/s41598-021-92977-4.
26. **Arivazhagan P, Panneerselvam C (2002):** Effect of DL- $\alpha$ -lipoic acid on neural antioxidants in aged rats. *Pharm Res.*, 42: 219-22.
27. **Abdou H, Hassan E, Aly R (2020):** Monosodium glutamate (MSG): promoter of neurotoxicity, testicular impairment, inflammation and apoptosis in male rats. *Swedish Journal of Scientific Research*, 1(2):78-90.
28. **Haghighatdoost F, Hariri M (2019):** The effect of alpha-lipoic acid on inflammatory mediators: a systematic review and meta-analysis on randomized clinical trials. *Eur J Pharmacol.*, 849:115-123.
29. **Çakır T, Polat C, Baştürk A et al. (2015):** The effect of alpha lipoic acid on rat kidneys in methotrexate induced oxidative injury. *Eur Rev Med Pharmacol Sci.*, 19(11):2132-9.
30. **Rosa S, Quines C, Stangherlin E et al. (2016):** Diphenyl diselenide ameliorates monosodium glutamate induced anxiety-like behavior in rats by modulating hippocampal BDNF-Akt pathway and uptake of GABA and serotonin neurotransmitters. *Physiology & Behavior*, 155: 1-8.
31. **Filho M, Sousa C, Meneses L et al. (2014):** Antidepressant effect of alpha-lipoic acid: Brain-derived Neurotrophic Factor such as a new target for resistant depression. *European Neuropsychopharmacology*, 24(2): 81–82.
32. **Yusha'u Y, Muhammad U, Mustapha S et al. (2021):** Alpha-lipoic acid attenuates depressive symptoms in mice exposed to chronic unpredictable mild stress. *Journal of African Association of Physiological Sciences*, 9(1):58-68.
33. **Shao Y, Xu X, Wang N et al. (2022):** Metabolomic Biomarkers in Parkinson's Disease. In *NeuroMethods*. Humana Press. pp. 181-213. [https://doi.org/10.1007/978-1-0716-1712-0\\_8](https://doi.org/10.1007/978-1-0716-1712-0_8)
34. **Hussein U, Hassan N, Elhalwagy M et al. (2017):** Ginger and Propolis Exert Neuroprotective Effects against Monosodium Glutamate-Induced Neurotoxicity in Rats. *Molecules*, 22(11): 1928. doi: 10.3390/molecules22111928
35. **Hashem H, El-Din Safwat M, Algaidi S (2012):** The effect of monosodium glutamate on the cerebellar cortex of male albino rats and the protective role of vitamin C (histological and immunohistochemical study). *Journal of Molecular Histology*, 43: 179-186.