IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. IV (Nov. - Dec.2016), PP 61-69
www.iosrjournals.org

Attenuation of Monosodium Glutamate-Induced Hepatic and Testicular Toxicity in Albino Rats by Annona Muricata Linn. (Annonaceae) Leaf Extract

[1]Eman Mohammed Mohammed Abd-Ella[*], [2]Abd-Elkarim Mohammed Abd-Lateif Mohammed

[*1]*Lecturer of histology and cytology, zoology department, Faculty of Science, Fayoum University, Egypt.*
[2]*Assistant professor of histology and cytology, zoology department, Faculty of Science, Fayoum University, Egypt.*

Abstract:
Introduction and aim of the work: *The protective effects of ANNONA MURICATA LINN leaf extract (Graviola) against orally administered Monosodium glutamate (MSG) for 4 weeks induced hepatic and testicular toxicity on male albino rats were examined.*
Methods: *The rats were divided into 4 groups • Group 1: Served as negative control: The rats were daily orally administrated with ml of distilled water for 4 weeks. • Group 2: Served as control Graviola group: The rats were orally administrated with Graviola leaf extract dissolved in distilled water at a dose level of 100 mg/kg body weight for 4 weeks. • Group 3: Served as control mono-sodium glutamate group (MSG): The rats were orally administrated with monosodium glutamate dissolved in distilled water at a dose level of 4mg/kg b.wt, for 4 weeks. • Group 4: served as treatment group: The rats were orally administrated with monosodium glutamate dissolved in distilled water at a dose level of 4mg/kg b.wt, for 4 weeks, then followed by treatment with Graviola leaf extract dissolved in distilled water at a dose level of 100 mg/kg body weight for another 4 successive weeks. Liver and testes were examined for alterations in BCL-2 and Caspase-3 protein expression and histopathology.*
Results: *MSG up-regulated BCL-2 and Caspase-3 expression in LIVER AND TESTES where Caspase-3 expression was significantly increased ($p<0.05$) and BCL-2 expression was significantly ($P<0.05$) decreased but ANNONA MURICATA LINN leaf extract (Graviola) administration as a therapeutic treatment almost normalized the histological and immunohisto chemical alterations.*
Conclusion: *present findings confirmed the protective and therapeutic effects of ANNONA MURICATA LINN leaf extract (Graviola) on MSG induced alteration in liver and testes in male albino rats.*
Keywords: *MSG, Graviola, liver, testes, BCL-2, Caspase-3*

I. Introduction

Monosodium Glutamate (MSG) is one of the world's most extensively used food additives. Monosodium glutamate (MSG) - a sodium salt of glutamic acid - can produce a unique taste, known as fifth taste (umami) that improve the quality of food intake by stimulating chemosensory perception (19,25). According to Bojanic V, (4); this flavor enhancer is extensively used in the food industry, in restaurants and homes. It is present in a wide variety of processed foods including prepared meals, flavored chips and snacks, marinated meats, flavored tuna, soups or sauces (canned, packed), bottled soy or oriental sauces, fresh sausages, and stuffed or seasoned chicken, vegetarian burgers, some hams, luncheon chicken and turkey and sausages. Moreover, glutamate occurs naturally in various foods including poultry, cheeses, meat broths, seafood and vegetables (22).

Many previous researches investigated MSG had different pathological side effects of human and experimental animals at different organs. Seo HJ1, (39) stated the higher doses of MSG were confirmed to be neurotoxic in different experimental animals; as it destructs neurons in the hypothalamic nuclei through their changes in the hypothalamo-pituitary-adrenal axis. Moreover, Ortiz GG, (34) mentioned the excessive MSG administration may lead to damage of liver, testes and kidney. These findings denote that unbound glutamate dissociated from MSG; according to Iamsaard S, (21) may possibly act on certain receptors in the central or peripheral neurons, causing histopathological changes.

There are several reasons why medicinal plants should be subjected to scientific scrutiny. Where according to Bailey and Day, (1) many herbal remedies have recognizable therapeutic effects.

Annona muricata Linn. (Annonaceae) is commonly known as 'Soursop' or 'Graviola' has been used medicinally in many tropical African countries for an array of human ailments, especially for parasitic infections and cancer. In India, Watt, J. M., (44) illustrated, several chemical compounds have been isolated from various morphological parts (roots, stem-barks, leaves, fruits, and seeds) of this plant. Where the flowers and fruit pods

are used as remedies for catarrh, while its root-bark and leaf of the plant are used as anthelmintic and antiphlogistic agents.

ANNONA MURICATA LINN (Graviola) was found to exhibit a variety of biological activities including antiviral activities and anti-inflammatory properties (28), antitumor (40) and antioxidant (41). The present study aimed to evaluate the protective effect of *ANNONA MURICATA* LINN (Graviola) leaf extract against monosodium glutamate (MSG) induced liver and testes damage in male albino rats.

II. Materials and Methods

Monosodium Glutamate: Monosodium glutamate (MSG) was obtained from El Dawlia for Medical Equipment's and Chemicals Co. Egypt. It was dissolved in distilled water.

Plant material: ***ANNONA MURICATA* LINN (Graviola) leaf powdered** obtained as capsules (natural product drug) from local pharmacy of Brazil. Each capsule contains 250mg of concentrated Graviola leaves extract and the suggested use is one vegetarian capsule 1-3 daily for adult human. The dose calculated and converted from human to rats, 100mg\kg bwt, then the vegetarian capsules of Graviola opened and dissolved in distilled water according to the calculations.

Animals and Treatments:

Animals: Tow-month old (120 - 150 g body weight) male albino rats (*Rattus rattus*) were selected from animal house of National Research Center, Giza, Egypt. The animals were housed under controlled environment conditions (12 h light/dark cycle) at a temperature of 25˚C + 10˚C and fed standard diet and water Ad libitum for the experimental period.

Experimental protocol: The rats were randomly divided into 4 groups of 12 animals each as follows:

• Group 1: Served as negative control: The rats were daily orally administrated with ml of distilled water for 4 weeks.

• Group 2: Served as control Graviola group: The rats were orally administrated with Graviola leaf extract dissolved in distilled water at a dose level of 100 mg/kg body weight for 4 weeks.

• Group 3: Served as control mono-sodium glutamate group (MSG): The rats were orally administrated with monosodium glutamate dissolved in distilled water at a dose level of 4mg/kg b.wt, for 4 weeks **(38).**

• Group 4: served as treatment group: The rats were orally administrated with monosodium glutamate dissolved in distilled water at a dose level of 4mg/kg b.wt, for 4 weeks, then followed by treatment with Graviola leaf extract dissolved in distilled water at a dose level of 100 mg/kg body weight for another 4 successive weeks.

Examinations: Rats of each group were sacrificed by cervical dislocation at the end of the experimental periods. Liver and testis of each animal were obtained and fixed in buffered neutral formalin 10% solution for 24 hrs, dehydrated through alcohols, cleared in xylene and embedded in paraffin wax. Five-micrometer thickness paraffin sections were prepared and mount on clean slides.

For histopathological studies, according to Drury, R.A.B.,(11), such as sections were stained with Ehrlich.s hematoxylin and counterstained with eosin.

For immunohistochemical studies; other sections were de-paraffinized, placed on charged slides, and used for localization of **BCL2;** (**B-cell lymphoma 2**), regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis; and **Caspase-3: The CASP3** protein is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of **Caspase-3** plays a central role in the execution-phase of cell apoptosis.

Anti- BCL2 and CASP3 antibodies respectively were employed to stain the cells in an Avidin–biotin-complex (ABC) immunoperoxidase technique. Specifically, the sections were incubated in 5% H_2O_2 (in methanol) solution for 10 min to block endogenous peroxidase activity and then incubated with primary **Anti- BCL2 and CASP3** rabbit monoclonal antibody respectively (1:50 dilution in 1% bovine serum albumin solution; Pan-T Clone SP7, Thermo Scientific, Lab Vision, Fremont, CA) for 60 min at room temperature. After rinsing with phosphate-buffered saline (PBS, pH 7.4) to remove unbound primary antibody, the samples were incubated with diaminobenzidine (DAB) chromogenic solution for 5 min at 25°C. The sections were then counterstained with haematoxylin for 15 sec. (24)

For statistical analysis, each section was counted manually at high power (X400) after identifying at low power (x100). The representative areas with the highest concentration of stained cells were detected according to the recommendation of Cohen, J.S., **(7).** About 1000 cells/slide were counted in each of five microscopic fields from well-labeled areas to determine the average of **BCL2** Labelling index. **BCL2** was expressed as number of labeled cells (positive for **BCL2**) as a percentage of the total number of cells counted in each specimen. All identifiable staining was regarded as positive. Then this method applied with **CASP3** expression.

Statistical analysis:
The obtained results of each of, **BCL2 and CASP3** were expressed as mean + Standard Error (SE). They were also statistically analyzed by using the SPSS11 computer software program (ANOVA) analyses.

III. Results

Histopathological results:

The liver sections of the negative control group exhibited normal architecture where it consists of a roughly hexagonal arrangement of plates of hepatocytes radiating outward from a central vein in the centre and cytoplasm of hepatocytes was eosinophilic and granular. The cords of hepatocytes around central vein separated by sinusoids **(Figure1; A).** Light microscopic examinations demonstrated that liver tissue of the rats were orally administrated with Graviola leaf extract dissolved in distilled water at a dose level of 100 mg/kg body weight for 4 weeks; had a view like extent to the normal sections of negative control sections **(Figure1; B & C).**While examinations of the liver obtained from rats treated with MSG dissolved in distilled water at a dose level of 4mg/kg b.wt, for 4 weeks showed destruction of the normal hepatic architecture and severe pathological alterations, and many hepatocytes showed vacuolar degenerative changes in their cytoplasm. In addition, focal necrotic areas in-filtered with mononuclear leukocytes were observed to contain pyknotic and karyolitic nuclei of necrotic hepatocytes. Furthermore, central veins, portal veins and sinusoids were severely damaged; they appeared dilated and congested **(Figure1; D, E & F)**. On the other hand, the rats were orally administrated with monosodium glutamate dissolved in distilled water at a dose level of 4mg/kg b.wt, for 4 weeks, then followed by treatment with Graviola leaf extract dissolved in distilled water at a dose level of 100 mg/kg body weight for another 4 successive weeks; revealed no inflammatory changes and marked restoration of the hepatic configuration were observed **(Figure1; G & H)**.

Histological sections of testes of negative control rats (group 1) showed a thick fibrous capsule (tunica albuginea) enclosing a number of adjacent seminiferous tubules separated by interstitial cells. The germ cells (spermatogonia, primary and secondary spermatocytes, spermatides and spermatozoa) and Sertoli cells within the seminiferous tubules were normal. The seminiferous tubules appeared as rounded or oval surrounded by a thin Basal Lamina **(Figure1; I).** Testes sections of the rats treated with Graviola leaf extract at a dose level of 100 mg/kg body weight for 4 weeks (group 2); showed no histological alterations like extent to the normal sections of negative control sections **(Figure1; J).** Testes of rats treated with MSG at a dose level of 4mg/kg b.wt, for 4 weeks (group 3) displayed variable degree of histopathological alterations like blood hemorrhage and deformed and detached germ cells as well as Sertoli cells from the irregular basal lamina with pyknotic nuclei. The interstitial tissue appeared with different vacuoles and many seminiferous tubules were severely damaged and had few Sertoli cells. Spermatocytes and early spermatides were lost from most of the tubules **(Figure1; K & L).** Animals treated with MSG then Graviola leaf extract as a treatment group (group 4) showed an improvement of seminiferous tubules and an increase in the number of germ cells where there were no inflammatory changes and marked restoration of the testes configuration were observed **(Figure1; M).**

Immunohistochemical results:

As shown in **Figure2 (A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P) and table 1,** the positive cells containing Bcl-2 protein expression (brown stain) were decreased in the liver and testes sections of rats treated with MSG at a dose level of 4mg/kg b.wt, for 4 weeks (group 3) **(Figure2 C & G)** localized within hepatocytes and testicular cells **(significantly decrease P < 0.05 in values 24.10 + 1.59 and 21.60 ± 2.02; liver and testes respectively)** when compared with those of the negative control (group 1) liver and testis sections **(Figure2 A & E) in values 68.70± 4.24 and 54.30 + 2.86; liver and testes respectively**. The intensity and amount of immunostaining of liver and testis sections of Graviola treatment control at a dose level of 100 mg/kg body weight for 4 weeks (Group 2) showed a fewer increase than those of the negative control group but not significantly **(in values 72.10 ± 6.11 and 59.20 ± 2.42 liver and testes respectively) (Figure2 B & F).** In contrast, at group 4; the positive cells containing Bcl-2 protein expression (brown stain) in the sections of liver and testes highly increased from rats orally administrated with monosodium glutamate dissolved in distilled water at a dose level of 4mg/kg b.wt, for 4 weeks **(Figure2 D & H),** referred **increase significantly P < 0.05 in values 47.80 + 3.24 and 44.90 ± 4.31; liver and testes respectively;** after treatment with Graviola leave extract dissolved in distilled water at a dose level of 100 mg/kg body weight for another 4 successive weeks when compared with MSG group 3**.**

However, the positive cells containing Caspase-3 protein expression (brown stain) were increased in the liver and testes sections of rats treated with MSG (group 3) spread throughout the liver and testicular cells **(Figure2 K & O) (significantly increase P < 0.05 in values 54.50 + 2.84 and 51.90 ± 4.31; liver and testes respectively)** when compared with those of the negative control (group 1) **in values 9.20 ±1.05 and 13.40 ± 2.20; liver and testes respectively; (Figure2 I & M)**. The intensity and amount of immunostaining of Graviola control (Group 2) of Caspase-3 showed a fewer decrease than those of the negative control group but not

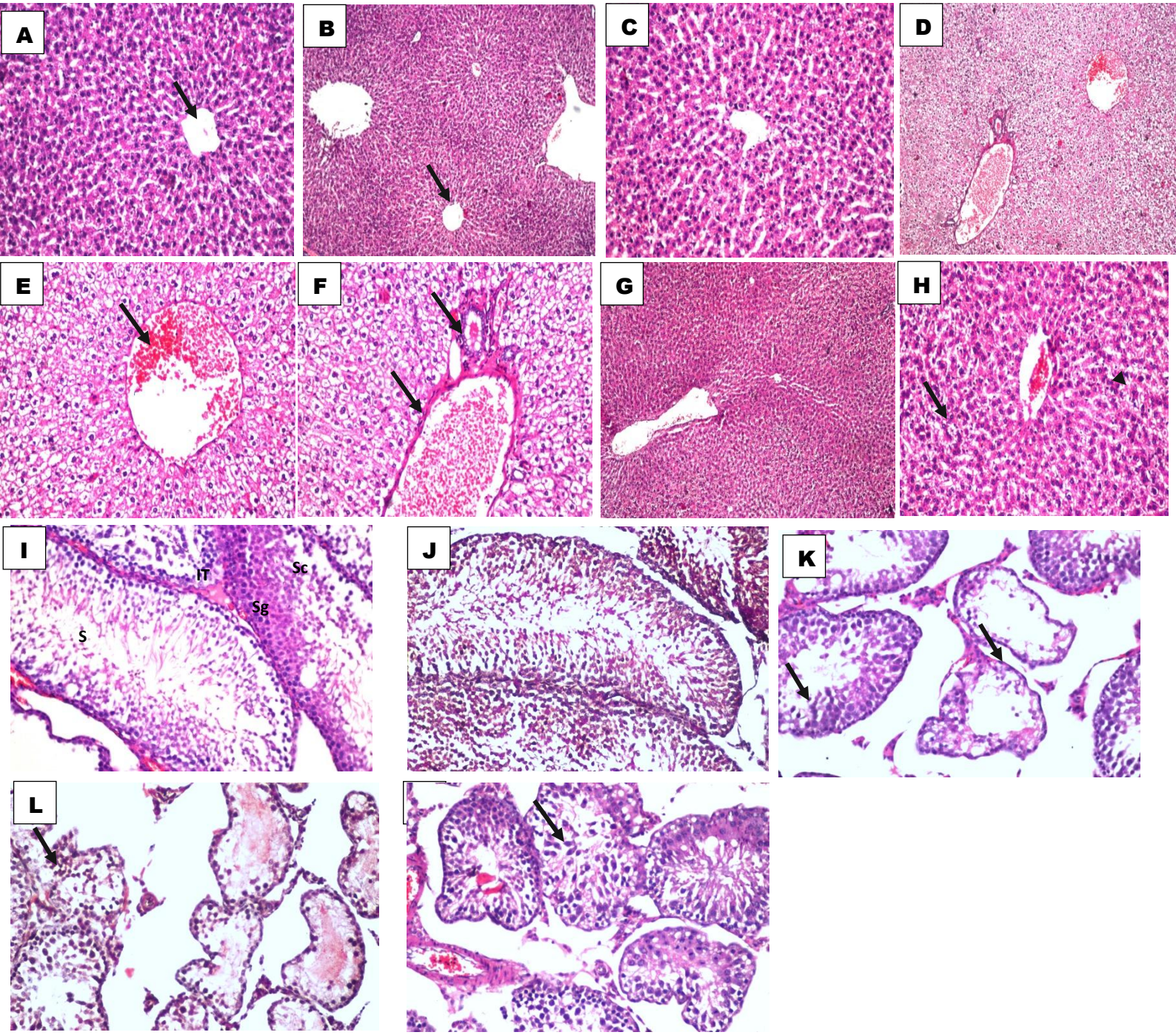

Figure [1]: (A) Normal histological features of liver tissue of the negative control group 1 showing normal liver architecture with radially arranged hepatocytes with well demarcated, regularly sized nuclei, intervening sinusoids, normal central vein (arrow) (H&E X200). **(B & C)** Normal histological features of liver tissue of the Graviola control group 2 showing normal liver architecture more or less like the negative control section (H&E X100, 200 respectively). **(D, E & F)** liver sections of rats administered MSG group 3 showing loss of normal architecture, degenerating hepatocytes with numerous vacuolations, nuclei of varying shapes and sizes and at varying degrees of degeneration and pyknosis, loss of sinusoidal spaces, inflammatory cells scattered all over hepatic tissue and congested dilated central vein (arrow) (H & E X100,400,400 respectively). **(G & H)** liver sections of therapeutic treatment group 4, there is mild histopathological alterations with little degenerating hepatocytes (arrow) but there is no inflammation or degenerating nuclei more or less like normal tissue (head of arrow) (H & E X100,200 respectively). **(I)** Normal histological features of testis tissue of the negative control group 1 showing normal testis architecture and lobules with normal spermatogonia (Sg), Spermatocytes (Sc), Sperm (S) and interstitial cells (IT) (H&E X400). **(J)** Normal histological features of tests tissue of the Graviola control group 2 showing normal architecture more or less like the negative control section (H&E X400). **(K & L)** testis sections of rats administered MSG group 3 showing loss of normal architecture, degenerated lobules and detached of germ cells from the irregular basal degenerated interstitial tissue (arrow) and deformed germ cells with pyknotic nuclei (P); degenerated and disappearing germ cells (H&E X400, 400 respectively). **(M)** testis section of therapeutic treatment group 4, showing increase of sperm (S) and improved interstitial tissue (it), nuclei and sperms more or less like normal tissue (arrow) (H&E X400).

significantly **(in values 8.40 + 0.97 and 12.90 ± 1.61; liver and testes respectively); (Figure2 J & N).** In contrast, **at the treatment group 4** the positive cells containing Caspase-3 protein expression (brown stain) in the sections of liver and testes from rats orally administrated with monosodium glutamate referred **decrease significantly P < 0.05 in values 19.20 + 2.00 and 26.60 ± 2.02; liver and testes respectively;** after treatment with Graviola leave extract for another 4 successive weeks as a treatment when compared with MSG group 3 **(Figure2 L & P).**

Table 1. Average optical density (AOD) of Bcl-2, and caspase-3 positive cells in the liver and testes:

Organ	**parameter**	**Negative control G1** Mean + SE	**Graviola control G2** Mean + SE	**MSG control G3** Mean + SE	**treatment group G4** Mean + SE
Liver	**BCL2**	68.70± 4.24	72.10 ± 6.11	24.10 + 1.59 [a]	47.80 + 3.24[b]
	Caspase 3	9.20 ±1.05	8.40 + 0.97	54.50 + 2.84 [a]	19.20 + 2.00[b]
Testes	**BCL2**	54.30 + 2.86	59.20 ± 2.42	21.60 ± 2.02[a]	44.90 ± 4.31[b]
	Caspase 3	13.40 ± 2.20	12.90 ± 1.61	51.90 ± 4.31[a]	26.60 ± 2.02[b]

The values are presented as means ± standard deviation (n = 6). Compared with control group, P < 0.05,
a significant difference with the group 1
b significant difference with the group 2.
$LSD_{0.05 - 0.01}$ (1.04 - 1.39)

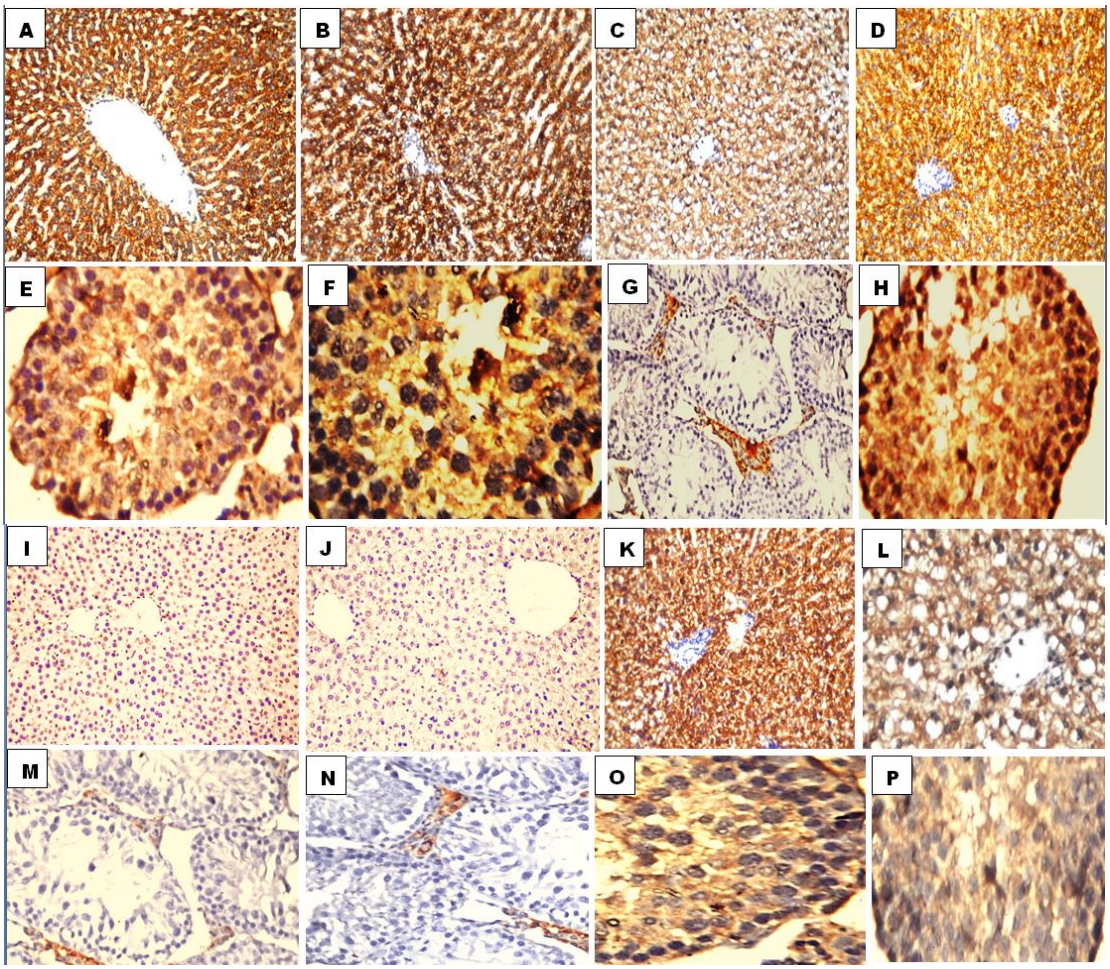

Figure [2]: (A): Liver tissue of the negative control group 1 with Cytoplasm of hepatocytes stained positive for Bcl-2 protein indicating the presence of Bcl-2 protein (Immunoperoxidase, X100). **(B)** Normal features of liver tissue of the Graviola control group 2 showing high positivity of immunostaining BCL-2 (Immunoperoxidase, X100). **(C)** Liver section of rats administered MSG group 3 showing week to mild diffuse of immunostaining BCL-2 (Immunoperoxidase, X100). **(D)** Liver section of therapeutic treatment group 4, showing a high diffuse of immunostaining BCL-2 (Immunoperoxidase, X100). **(E):** Testis tissue of the negative control group 1 with high positive staining for Bcl-2 protein indicating the presence of Bcl-2 protein (Immunoperoxidase, X400). **(F)** Normal features of testis tissue of the Graviola control group 2 showing high positivity of immunostaining BCL-2 (Immunoperoxidase, X400). **(G)** Testis section of rats administered MSG group 3 showing week diffuse of immunostaining BCL-2 (Immunoperoxidase, X200). **(H)** Testis section of therapeutic treatment group 4, showing a high diffuse of immunostaining BCL-2 (Immunoperoxidase, X400). **(I):** liver tissue of the negative control group 1 with a weak diffuse of immunostaining Caspase-3 (Immunoperoxidase, X200). **(J)** Normal features of liver tissue of the Graviola control group 2 showing week to mild diffuse of immunostaining Caspase-3 (Immunoperoxidase, X200). **(K)** Liver section of rats administered MSG group 3 showing a high diffuse of immunostaining Caspase-3 (Immunoperoxidase, X100). **(L)** Liver section of therapeutic treatment group 4, showing a week of immunostaining Caspase-3 (Immunoperoxidase, X400). **(M):** Testis tissue of the negative control group 1 with a weak diffuse of immunostaining Caspase-3 (Immunoperoxidase, X200). **(N)** Normal features of testisr tissue of the Graviola control group 2 showing week to mild diffuse of immunostaining Caspase-3 (Immunoperoxidase, X200). **(O)** Testis section of rats administered MSG group 3 showing a high diffuse of immunostaining Caspase-3 (Immunoperoxidase, X400). **(P)** Testis section of therapeutic treatment group 4, showing a week of immunostaining Caspase-3 (Immunoperoxidase, X400).

IV. Discussion

Extensive research were carried out on different types of animals including human to clear the side pathological effects of MSG where there were doubts toward MSG gained popularity in first half of twentieth century as taste enhancer but at the same time doubts were raised about MSG as a causative agent of Chinese restaurant syndrome. Where, Marcia, C., (27) observed that according to the national institute of health, male infertility involved approximately 40% of infertile human couple and the environmental causes represent one of the major factors affecting male fertility. Moore, K.L., (30) mentioned that monosodium glutamate (MSG) is known to affect the structure and function of male reproductive system and showed to be toxic to the liver and testis of human and experimental animals.

In addition, Olney JW., (32), observed that infant mice on account of poorly developed blood brain barrier showed neurological lesion even when MSG was given in lower dose.

The present results showed that treating rats with MSG revealed a histological alterations in both the liver and testes organs like destruction of the normal hepatic architecture and vacuolar degenerative, pyknotic and karyolitic nuclei of necrotic hepatocytes. As well as testes displayed variable degree of histopathological alterations like blood hemorrhage and deformed and detached germ cells as well as Sertoli cells from the irregular basal lamina with pyknotic nuclei.

This results are in agreement with Ortiz G, (34); who reported that elevation of SGOT, SGPT with degenerative changes in hepatocyte after a single high dose intraperitoneal injection of MSG in rats. Also, Eweka AO, (13); noted presence of hemolysis RBCS in central vein and hemorrhagic necrosis in centrilobular and disruption of architecture of liver and evidence of hepatocyte degradation and hypertrophy as a response of oral MSG in adult Wister rats.

MSG-treatment caused reduction of testes and epididymis weight, sperm count and increase in sperm abnormalities as observed by Das R, (10). In addition Mohamed IK. (29); demonstrated that treating rats with MSG at short-term exhibited slight to moderate damaged seminiferous tubules, including cytoplasmic vacuolization of spermatogonia and loss of late spermatids. Long–term treatment caused severe damage of germ cells.

According to Walker R, (42); the circulating of MSG was dissociated in sodium (Na+) and L-glutamate. The L-glutamate crosses the mesothelial peritoneal cells and arrives at the bloodstream by means of a transport system using ATP. A part of the L-glutamate in the cell conjugates, in order to be eliminated, and another part is transformed into glutamine. When this occurs, the cells try to repair some of the damages by using enzymes that are present in the smooth endoplasmic reticulum but the cell is not able to completely remove the excess glutamine. According to Gill S, (15); glutamate receptors are present in different tissues; the hypothalamus, spleen, thymus, liver, kidneys, endocrine system, ovaries, etc. Probably, for this reason, it is possible to observe vesicular degeneration and necrosis at liver and testes tissues.

Collison KS, (8); explained that the effects observed in the liver could have occurred because this organ is involved in the metabolism of glutamate or as in another study it may be due particularly in the liver exacerbation of trans-fat induced fatty liver disease in mice by a mechanism that includes increased central adiposity and alterations in both hepatic and white adipose tissue gene expression. In addition, according to Farombi EO, (14); MSG has been reported to increase oxidative stress and amelioration of the hepatotoxic or nephrotoxic effects by the administration of radical scavengers such as vitamin E or C.

This study also showed different histological alterations in testes of animals treated by MSG and this investigations are in accordance to the histological studies that were carried out on the testes of different animals treated with MSG, Das R, and Mohamed IK., (10, 29) found that the MSG induced histological changes in the testes of neonatal mice showed that both the germinal epithelium and Leydig cells were affected. These histological changes may be due to either local effect of the chemical or indirectly caused by imbalance in gonadotrophic hormones as O.Atallah (31) suggested. Balasubramanian A., (2) explained the congestion of

blood vessels as being due to the inhibition of prostaglandins synthesis, since these compounds are known to be involved in the regulation of testicular blood flow. The vacuolation and exfoliation might be a sign of testicular toxicity and cell degeneration (12). Current work revealed pyknosis of cell nuclei may indicate the loss of functional efficiency of the cells. This is in agreement with results have been demonstrated by Ortiz G, (34); on male rats treated with MSG.

Hu J, (18); proved the presence of functional glutamate transporters and receptors in testes of rats. Therefore, testes are considered as a target organ for MSG. So, one of the mechanisms may be a direct effect of MSG via glutamate receptors and transporters on the epithelial cells of the seminiferous tubules. Also, Giovambattista A, (16); stipulate that there are neurotoxin effects of MSG on the function of hypothalamus–pituitary–gonadal system. Where according to Witorsch RJ. (45), the effects of such toxicants on male reproduction may be anatomical or only functional, depending on whether they produce structural changes in the reproductive system, or merely affect the functions of the reproductive organs.

Immunohistochemical investigations in the present study, revealed that Caspase-3 protein expression was found to be significantly increased while Bcl-2 protein expression was significantly depressed in the rats treated by MSG (Group3) in both the hepatic and testicular tissues. It is well known that Bcl-2 protein is expressed in the inner mitochondrial membrane (37). Oltval ZN, (33); explained the members of the Bcl-2 protein family are known to regulate the release of apoptosis-activating factors that the ratio of Bcl-2 to Caspase-3 determines cell survival or cell death. These findings in our study are similar to those of a study on vanadium-induced DNA damage and P53 expression (46). Also, according to Rana SVS. (36), changes in the Bcl-2 expression and activation of caspase-3 induce apoptotic processes.

Wang J.M., (43); mentioned Caspase‑ 3 is the key inducer of apoptosis, and activation of Caspase‑ 3 destroy numerous cellular structures, leading to cell death. Yang J., (47); demonstrated Bcl‑ 2 which could be present in mitochondria has been shown to inhibit cytochrome c release and protect against oxidative‑ induced apoptosis, so the Bcl‑ 2 family proteins are major antagonist of apoptosis.

Dai S, (9); proved mitochondrial injury and overexpression of proapoptotic proteins are associated with the production of free radicals. Green DR., (17); mentioned lower levels of MSG-induced de-pressed the activities of antioxidant enzymes, and then free radicals accumulated in the body or organs and induced the lipid peroxidation of the membrane. It is reasonable to propose that oxidative damage could occur in mitochondria and cause the release of proapoptotic proteins into the cytosol, which resulted in cellular apoptosis.

Antioxidants have therefore been proven as a useful strategy to prevent the toxic behavior of MSG. *Annona muricata* (Graviola) leaves as a natural product is known to have potent antioxidant and anti‑ inflammatory properties (20). According to Pandya U., (35) the antioxidant effect of *Annona muricata* (Graviola) leaf extract has been considered to be mediated via its major effects on eradication of free radicals and/or via preventing lipid peroxidation and it is at least 10 times more active as an antioxidant than Vitamin E. This is so because *Annona muricata* leaves have been shown to possess antioxidant properties, due to the presence of acetogenins, which probably play the role of effective free radical scavengers (3). Where the antioxidant effect of *Annona muricata* leaf extract may referred to its ability to significantly reduce serum ROS, GSSG and MDA.

In the present study, *Annona muricata* (Graviola) leaf extract was found to decrease structural changes in the liver and testes. In addition; *Annona muricata* (Graviola) leaf extract‑ treated rats showed a significant decrease in the expression of Caspase‑ 3 immunoreactivity and significantly increased the expression of Bcl‑ 2. These finding may be explained according to the fact that *Annona muricata* (Graviola) leaf extract as a natural product reduce the changes in lysosomal enzymes activities, thereby minimizing the damage caused due to MSG (6).

In our present study, we could demonstrate apoptotic cell injury involving the activation of Caspases-3 paralleled to oxidative stress and oncotic cell death after MSG treatment (group 3). But, application of *Annona muricata* (Graviola) leaf extract (group 4) reversed all signs of injury which could be associated by the overexpression of Bcl-2. Bcl-2 overexpression has been demonstrated to protect cells against oxidative cell death by complete suppression of ROS-induced cell damage (23). In accordance with these observations, Brambrink MA, (5); demonstrated increased Bcl-2, mRNA and protein in brain tissue after Graviola preconditioning, suggesting a transcriptional process.

This is supported by La Coste A, (26) who detected that the impressive reduction of apoptotic hepatocytes as well as testicular tissues in the Graviola treated group. This is most likely facilitated via the observed up-regulation and synthesis of Bcl-2: Bcl-2 and other antiapoptotic Bcl-2 family members block cytochrome C release, either by blocking the mitochondrial permeability transition pore or by antagonism of Bax/Bak-dependent pore formation in the mitochondrial outer membrane. In addition, Zhu HC. (48); observed inhibition of caspase 3 activation and apoptosis in transient focal cerebral ischemia in rats by 3-NPA.

V. Conclusion

This study suggests that continuous consumption of MSG may result in varying degrees of liver and testes injury and *Annona muricata* (Graviola) leaf extract play an important role in the protection against monosodium glutamate (MSG)-induced hepatic and testicular toxicity, by improving the activities of BCL-2 and Cspase-3. Therefore, *Annona muricata* may be used to protect against toxic effects of MSG and other chemical agents in liver and testes. In the future, examination and further research of the protective effect of *Annona muricata* leaf extract against MSG is warranted to examine the safety profile of this widely used food additive.

Acknowledgement

The authors would like to thank all participants for their contribution in this study including animal house technicians and histology technicians.

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