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ORIGINAL ARTICLE.

The Possible Ameliorating Effect of Intermittent Fasting on Histological and Biochemical Changes Induced by Monosodium Glutamate on Renal Cortex of Adult Male Albino Rat

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ABSTRACT

Background: Intermittent fasting (IF) is a recent dietary regimen that has beneficial health effects. The aim of this study is to evaluate the effect of intermittent fasting on the nephrotoxicity induced by monosodium glutamate (MSG). **Methods:** Thirty adult male albino rats were used; divided into three groups: group I (the control group), group II (the MSG group), and group III (the MSG group with IF regimen). Kidney function tests and serum C-reactive protein (CRP), interleukin-6 (IL-6),tumor necrosis factor alpha (TNF-α) were measured.Tissue malondialdehyde(MDA) level, and superoxide dismutase (SOD), catalase (CAT) activities were measured. Histological examination of the kidney tissues using the light and electronmicroscopes and morphometric analyses were performed.

Results: Serum urea, creatinine, and uric acid were highly elevated in group II and moderately increased in group III. Serum CRP, IL-6, TNF-α were elevated in group II and group III. Tissue MDA level was increased and SOD and CAT activities were decreased. There were distortion of kidney structure, increased collagen deposition, and enhanced apoptosis. In group III, there were improvement in the kidney function and histological structure.

Conclusions: Our data suggested that intermittent fasting partially prevented the nephrotoxicity of MSG.

Keywords: Intermittent Fasting, Monosodium Glutamate, Nephrotoxicity, Inflammation, Apoptosis.

INTRODUCTION

Kidneys are affected by many environmental toxins such as lead, mercury, and industrial chemicals[1].Additionally, the increased intake of processed food leads to consumption of high amounts of simple sugars, phosphorus, salt, and added potassium. This causes increased incidence of chronic kidney diseases [2].

Monosodium glutamate (MSG) is the sodium salt of glutamic acid. It is widely used as a flavor enhancer in food stuffs. It is responsible for a type of taste called 'umami taste'. Excessive intake of monosodium glutamate induces oxidative stress in different organs leading to health problems such as obesity, metabolic syndrome, and cardiovascular and renal diseases [3]. In industrialized countries, the mean daily intake of MSG is estimated to be about 0.3–1.0 g[4] .

Monosodium glutamate (MSG) causes inflammation of the liver, increased aggressiveness, and diminished locomotor activity. It also induces histological changes in the testes, ovaries, and fallopian tubes[5] . Additionally, intake of MSG is recently proved associated with the development of cancer due to the formation of the reactive oxygen species (ROS) and micronuclei (MN) [6].

Intermittent fasting is a form of dietary restriction (DR). It has several forms; the basic form is to alternate a day of normal caloric consumption with a day when caloric consumption is markedly reduced, time-restricted feeding (TRF) where fasting is achieved during 4 to 12 h daily , and the more recent form, the 5:2 strategy where there are only 2 days per week of caloric

restriction[7,8].Muslims and Christians have forms of intermittent fasting as a religious act. Ramadan fasting includes fasting from foods and fluids intake during daylight hours and the Daniel Fast is a partial fast that lasts for 3 weeks[9]

Intermittent fasting can prevent the dysfunction of the neurons through decreasing neuroinflammation and the oxidative stress[10] .Intermittent fasting improves metabolism and cardiovascular performance. It also guards against premature aging, diabetes mellitus, cancer, and cardiovascular diseases[11,12].

Most of the published studies on intermittent fasting focused on its effectiveness to reduce body weight and increase insulin sensitivity. Therefore, in this study, we investigated the possible protective effect of intermittent fasting against MSG affection on the renal cortex of the adult male albino rats.

METHODS

Calculation of sample size: Sample size was calculated as 30 rats; 10 rats in each group using Online Sample Size Calculator(https://www.calculator.net/sample-size-calculator.html).

Chemical and kits: Monosodium glutamate (Sigma Chemicals, St. Louis, MO, 2wsDiagnostics, USA), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase kits (Biodiagnostic Company, Dokki, Giza, Egypt) were used.

Animals: This study was performed at departments of the Medical Histology and Cell Biology, Physiology, and Medical Biochemistry & Molecular Biology, Faculty of Medicine, Zagazig University. All animal experiments comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals.

Thirty adult male albino rats weighing 180-200 g were obtained from the Animal House at the Faculty of Medicine, Zagazig University. The rats were housed in metallic cages with well-aerated covers with a 12 h light/dark cycles. Each cage contained 3-4 rats. They were housed for about 10 days before using in the experiment under standard conditions (temperature of 25°C, 50%–60% humidity, and a 12/12 h light/dark cycle) consistent with the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" published by the National Institute of Health. Food and water ad libitum were permitted.

Grouping: After 10 days of acclimatization, rats were grouped randomly into three groups: group I (control group) they were fed on a standard diet , group II(MSG group) rats in this group were given 2.5 g/kg orally of MSG dissolved in 1 cc of saline every other day dose for 30 days[13]and group III (MSG group receiving IF diet):They were received the same dose of MSG given to group II, and at the same time the rats were fed an IF diet regimen. In other words, they were deprived of food for a full day, every other day, and are fed adlibitum together with receiving the dose of MSG on the other days for 30 days [14].

Body weight: The body weight of each rat was measured before and at the end of the experiment.Weight gain was calculated.

Collection of the blood samples: Just before the sacrifice of the rats, blood samples were collected in heparinized tubes from the retro-orbital vein using a capillary tube for measurement of serum CRP, IL-6, and TNF – α and kidney function tests.

I.Measurement of serum CRP, IL-6, and TNF – α: Serum CRP was measured using a DuoSet ELISA kit (R&D Systems, Inc., Minneapolis, Minnesota, USA) according to manufacturer's instruction[15].Serum IL-6 was determined by ELISA, using reagent kits of Genzyme Corporation (Cambridge, MA) [16]and serum TNF-α was measured using solid-phase Enzyme Linked ImmunoSorbent Assay (ELISA) using rat TNF-α kits [17].

II. Kidney function tests: Serum urea, uric acid, creatinine were measured using(Spinreact, Spain)[18].

Tissue Sampling: All rats were anesthetized with ether inhalation and humanly sacrificed. Thekidneys were carefully dissected for the following tests:

I. Determination of MDA level and CAT and SOD activities: Right kidneys were used for biochemical analysis of MDA, SOD, and CAT. Parts of the frozen renal tissue samples from all groups were homogenized with a power homogenizer. Homogenates were centrifuged at 4,000 rpm for 15 min at 4°C. The supernatant was used for analysis of MDA according to the method of Odukoya et al. [19] ,and catalase and superoxide dismutase activities according to the methods of Weydert and Cullen[20] .The kits used were colorimetric kits (Biodiagnostics, Giza, Egypt).

II.Light microscopic and immunohistochemical study: Three of the left kidneys from each group were fixed in Bouin and two of the left kidneys were fixed in 10% formalin. Then, all specimens were dehydrated with ascending grades of ethanol (70, 90, and 100%). Dehydration was then followed by clearing the samples in two changes of xylene. Samples were then impregnated with two changes of molten paraffin wax, then embedded and blocked out to paraffin blocks. The tissue sections (4-µm thickness) were cut using a microtome and mounted on a glass slide. Sections

fixed in Bouinwere stained with H&E and Masson trichrome[21] while those fixed in 10% formalin were used for immunohistochemical staining of caspase-3 using streptavidin–biotin complex immune-peroxidase system according to Ramos-Vara et al[22].

III.Transmission electron microscopic study: Small pieces of the cortex of the other half of leftkidneys from all animalgroups were rapidly fixed in 10% glutraldehyde solution.Preparation of the specimensand photographing of the ultrathin sections were performed in the Electron Microscope Research Units of Faculty of Agriculture, Mansoura Universityand Faculty of Science, Zagazig University [23].

Morphometric analysis: The diameter of the glomeruli,renal tubules, area percentage of collagen fibers, and the area percentage of immune reaction to caspase 3 were measured within ten non-overlapping fields at total magnification X 400 using Fiji image J image analysis software (National Institute of Health; NIH, Bethesda, MD, USA [24].

Statistical analysis:

Data were presented as mean ± standard deviation (SD). Data were analyzed using One-way analysis of variance test (one-way ANOVA) followed by Tukey's post hoc multiple comparisons test for comparative analysis among the groups using SPSS 19 software . Values for $p \leq 0{\cdot}05$ were considered statistically significant[25].

RESULTS:

Body weight:

The final body weight of group II was significantly higher than that of the group I. The final body weight of group III was significantly higher than that of the group I. The weight gains of group II and group III was significantly higher than that of the group I. There was a significant decrease of weight gain in group III when compared with group II(Table 1).

Serum CRP, IL-6 and TNF – α:

Serum CRP,IL-6, TNF – α were highly increased in group II, and moderately increased in group III. There was a significant decline in their levels in group III when compared with group II (Table 2).

Kidney function test:

Serum levels of urea, uric acid and creatinine were significantly increased in group II, and moderately increased in group III when compared with group I. There was a significant decline in its level in group III when compared with group II(Table 3).

MDA level and SOD and CAT activity:

Tissue levels of MDA were highly increased in group II, and moderately increased in group III when compared with group I. There was a significant decline in its level in group III when compared with group II. Tissue levels of SOD and CAT activities were significantly reduced in group II, and moderately decreased in group III when compared with group I. There was a significant increase in their levels in group III when compared with group II (Table 4).

Histological Results:

I.H&E:

H&E sections showed that the renal cortex consists of renal glomeruli surrounded by Bowman's space, renal tubules, and blood vessels. They were separated by the renal interstitial tissue (Fig. 1.A). Sections of group II revealed distorted histological architecture of the cortex. The glomeruli were shrunken. Renal tubules showed degenerative changes with extruded cell debris in their lumens. Some nuclei are small darkly stained apoptotic. There were foci of mononuclear inflammatory cells and congested blood vessels(Fig. 1.B, C). Sections of group III showed some degenerated renal tubules with extruded cell debris in their lumens,and few inflammatory cells.Some nuclei are small darkly stained (Fig. 1.D).

The diameter of renal glomeruli was significantly decreased in group II when compared to group I .It was significantly increased in group III when compared to group II ,but still significantly lower than group I(Table 5, Fig., 2A). The diameter of renal tubules was significantly increased in group II when compared to group I. It was significantly in group III increased when compared to group II, but still significantly higher than group I(Table 5, Fig., 2B).

II. Masson trichrome stain:

Staining for collagen fibers in kidney sections using Masson trichome stain showed that group I had a minimal amount of collagen fibers in the interstitial tissue of the kidney (Fig.3 A). In group II, there was excessive deposition of collagen fibers (Fig.3 B). In group III, there was a high deposition of the collagen fibers (Fig.3 C). The area percentage of collagen fibers was increased in group II when compared to group I. It was significantly decreased in group III when compared to group II, but still significantly higher than group I (Table 6, Fig., 3 D).

III. Caspase-3 immuno stain":

Sections stained with caspase -3 immuno stain showed that group I had a very weak reaction to caspase-3 (Fig.3 A). In group II, there was very strong immune reaction to caspase-3 (Fig.3 B). In group III, there was a moderate reaction to caspase -3 (Fig.3 C). The area percentage of immune reaction to caspase 3 was increased in group II when compared to group I. It was significantly decreased in group III when compared to group II,

but still significantly higher than group I (Table 7, Fig., 4 D)

IV. Electron Microscope results

Electron micrographs of cells of the renal corpuscle in group I showed the podocytes with euchromatic nucleus and pedicles resting on the glomerular basement membrane(Fig., 5A). Group II showed heterochromatic nucleus of the podocytes and atrophied pedicles of podocytes (Fig., 5B).Group III showing euchromatic nucleus of the podocytes and partially regenerated pedicles(Fig., 5C).The thickness of glomerular basement membrane was significantly decreased in group II when compared to group I. It was significantly increased in group III when compared to group II, but still significantly higher than group I (Table 8, Fig. 6).

Electron micrographs of proximal convoluted tubules in group I showed euchromatic nuclei with prominent nucleolus.The apical cell surface contained microvilli. The basal surface showed basal in foldings with numerous mitochondria(Fig., 7A).Group II showed degenerated cells with heterochromatic nuclei and few small electron dense mitochondria in the cytoplasm.There were loss of basal infoldingsand microvilli(Fig., 7B).Group III showing euchromatic nuclei and few small electron dense mitochondria in the cytoplasm.There was loss of microvilli and basal in folding .Some cells showed few large vacuoles in the cytoplasm(Fig.,7 C)

V. Grading of the histological and immunohistochemical results:

Grading of the results obtained from the histological and immunohistochemical examinations was shown in Table 9.

Table 1: Statistical analysis of Initial body weight (g) and Final body weight (g):

	Group I	Group II	Group III	F	P
Initial body weight (g)	182.1±8.9	183.8±8.2	183.5±10.2	0.09	0.9^{ns}
Final body weight (g)	208.4±4.4	284.1^{a}±33.9	250.4^{ab}±10.9	33.4	<0.001**
Weight gain (g)	32.4±5.12	107.3^{a}±33.7	73.9^{ab}±10.7	33.19	<0.001**

ns: non-significant
a: Significant difference from group I.
b: Significant difference from group II
**: highly significant one-way ANOVA test

Table 2: The level of serum CRP, IL-6 and TNF-α:

	Group I	Group II	Group III	F	P
Serum CRP	1.2±0.15	4.6^{a}±0.6	2.04^{ab}±0.4	168.19	<0.001**
Serum IL-6 (pg/ml)	52.8±7.7	85.6^{a}±11.3	64.4^{ab}±9.6	29.5	<0.001**
Serum TNF-α (pg/ml)	72.3±6.2	98.7^{a}±13.2	83.4^{ab}±8.4	18.73	<0.001**

- Data represent mean ± SD
a: Significant difference from group I.
b: Significant difference from group II
**: highly significant one-way ANOVA test

Table 3: Serum urea, uric acid and creatinine:

Parameter	Group I	Group II	Group III	F	P
Serum urea (mg/dL)	44.6± 2.3	74.6± 8.1[a]	62.9±11.9[ab]	30.2	<0.001**
Serum uric acid (mg/dL)	0.95 ± 0.14	4.5± 0.72 [a]	2.1± 0.45[ab]	131.98	<0.001**
Serum creatinine (mg/dL)	0.5 ± 0.04	5.08± 0.8[a]	3.37± 0.49[ab]	159.7	<0.001**

a: significant from group I
b: significant from group II
**: highly significant one-way ANOVA test

Table 4:MDA level and SOD and CAT activity:

Parameter	Group I	Group II	Group III	F	P
Tissue MDA (nmol /g)	10.2±1.05	25.31±4[a]	19.46±4[ab]	52.6	<0.001**
Tissue SOD (U/gm tissue)	20.2±1	14.9±1.7[a]	17.3±2[ab]	26.5	<0.001**
Tissue CAT (U /gm)	1.2±0.1	0.8 ±0.1[a]	1 ±0.13[ab]	26.6	<0.001**

a: significant from group I
b: significant from group II
**: highly significant one-way ANOVA test

Table 5: The diameter of glomeruli and renal tubules:

	Group I	Group II	Group III	F	P
Diameter of the glomeruli (um)	83.5±7.5	56.1[a]±8.3	70.7[ab]±10.6	21.4	<0.001**
Diameter of the tubules (um)	60.6±9.1	87.4 [a]±11.5	73.3[ab]±8.8	16.6	<0.001**

a: Significant difference from group I.
b: Significant difference from group II
**: highly significant one-way ANOVA test

Table 6 : Statistical analysis of area percentage of the collagen fibers

Group / Parameter	Group I	Group II	Group III	F	P
Area percentage of the collagen fibers	1.4±0.4	16.95[a]± 2.8	8[ab]±1.6	172.07	<0.001**

a: significant from group I
b: significant from group II
**: highly significant one-way ANOVA test

Table 7 : Statistical analysis of area percentage of immune reaction to caspase 3.

Group / Parameter	Group I	Group II	Group III	F	P
Area percentage of immune reaction to Caspase 3	0.75±0.16	15.7 [a]±2.2	5.6[ab]±0.8	306.7	<0.001**

a: significant from group I
b: significant from group II
**: highly significant one-way ANOVA test

Table 8:Statistical analysis of the thickness of glomerular basement membrane.

Group / Parameter	Group I	Group II	Group III	F	P
The thickness of glomerular basement membrane (nm)	260.1±46.13	143.14[a]±23.43	183.33[ab]±41.44	21.6	<0.001**

a: significant from group I
b: significant from group II
**: highly significant one-way ANOVA test

Table 9: Grading of the histological and immunohistochemical results.

	Group I	Group II	Group III
Diameter of glomeruli	Normal	↓↓	↓
Diameter of tubules	Normal	↑↑	↑
Tubular degeneration	_	+++	++
Interstitial fibrosis	_	+++	++
Inflammatory cells	_	++	_
Apoptosis	_	+++	++
Thickness of the basement membrane	Normal	↓↓	↓

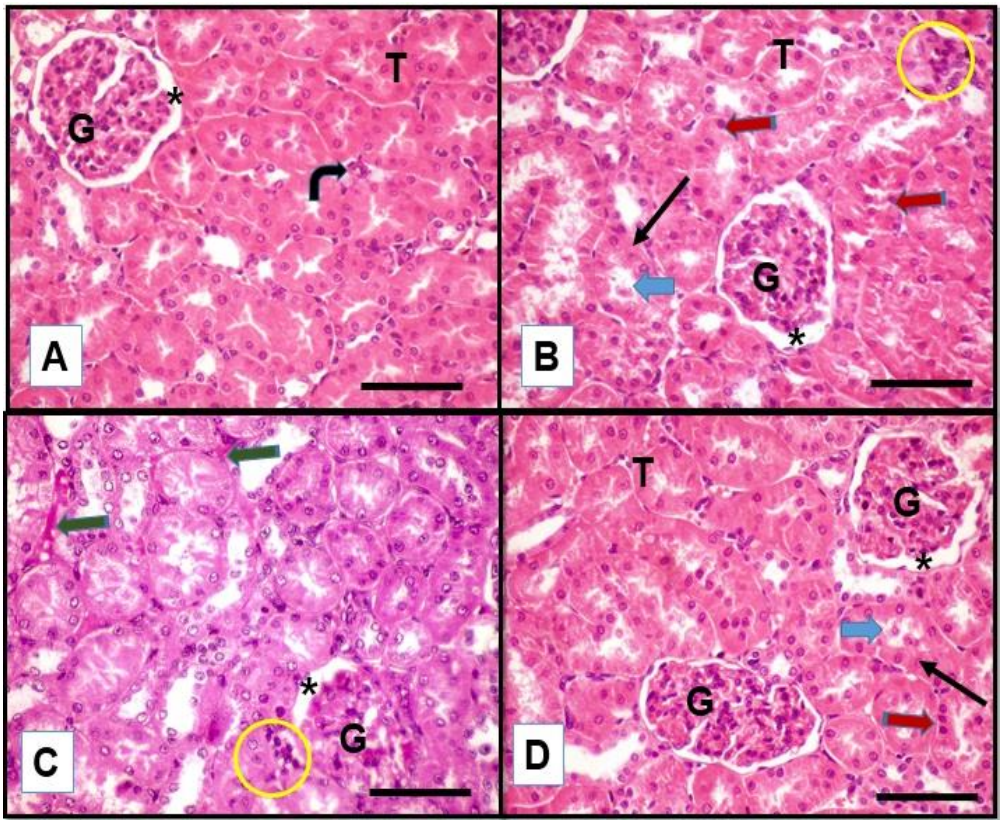

Figure (1): Photomicrographs of sections stained by H&E.A: Group I showed glomeruli (G) surrounded with Bowman's space (*), renal tubules (T), and peritublar blood capillaries (Curved arrow); B: Group II: showed shrunken glomeruli(G) Renal tubules are degenerated (Black arrow) with cell debris (Blue arrow) in their lumens. Some nuclei are dark stained nuclei (Red arrows).there are few inflammatory cells (Yellow circle).C: Group II: showed shrunken glomeruli(G), congested blood vessels (Green arrows), and few inflammatory cells (Yellow circle)D: Group III showed shrunken glomeruli(G), degenerated renal tubules (Black arrows) with cell debris (Blue arrow) in their lumens. Some nuclei are dark stained nuclei (Red arrow).[Asterix: Bowman's space; T: renal tubules](H&E X 400, scale bar= 50 µm).

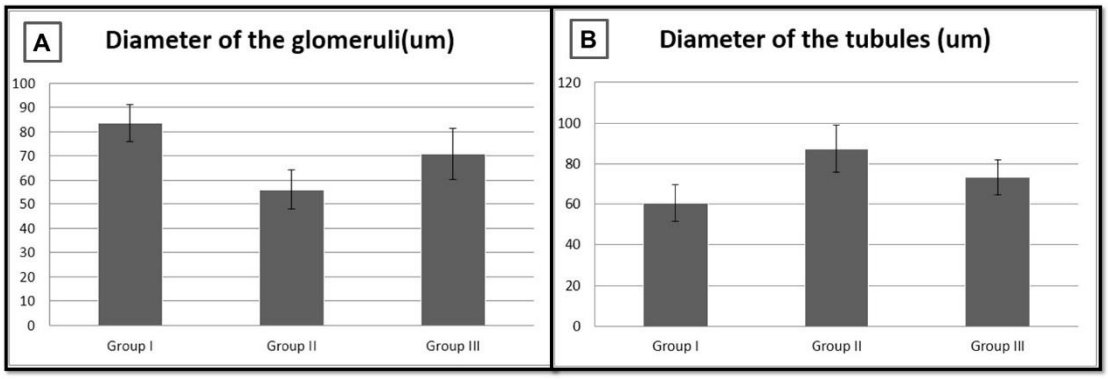

Figure (2):A: Bar chart showing the diameter of the renal glomeruli. B: Bar chart showing the diameter of the renal tubules.

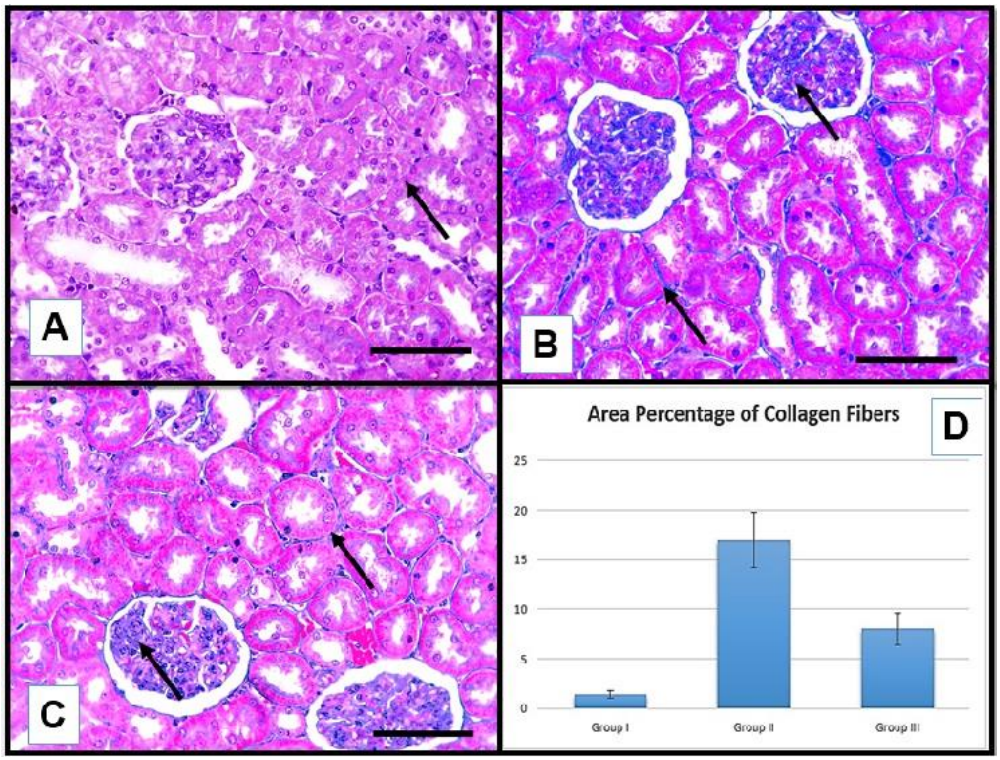

Figure (3): Photomicrographs of sections stained by Masson trichrome A: Group I showing minimal collagen fibers in the interstitial tissue (arrow). B: Group II showing an excess amount of collagen fibers in the interstitial tissue and in glomeruli (arrows). C: Group III showing a moderate amount of collagen fibers in the interstitial tissueand in glomeruli(Masson trichrome X 400, scale bar= 50 µm). D: Bar chart showing the area percentage of collagen fibers in different groups.

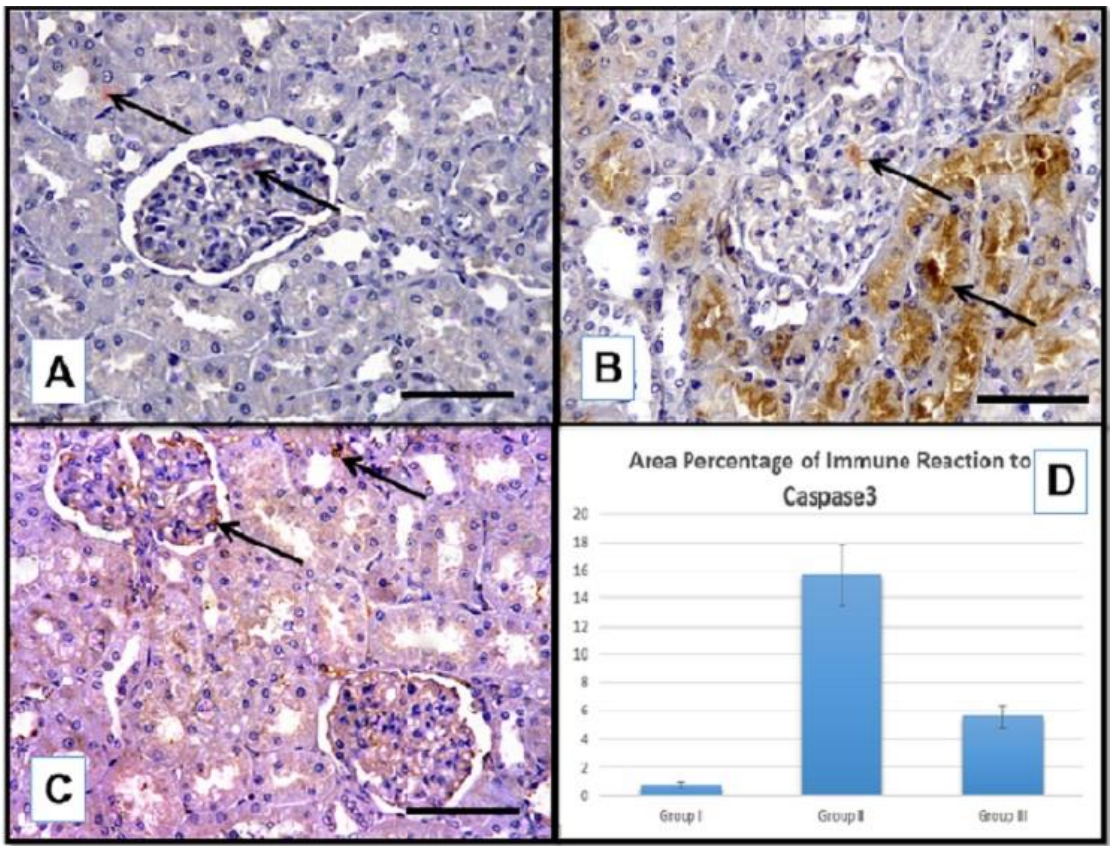

Figure (4):photomicrographs of sections stained by immunohistochemical stain to caspase 3A: Group I showing a very weak cytoplasmic reaction in the cytoplasm of the glomeruli and tubules (arrows). B: Group II showing strong cytoplasmic reaction in the glomeruli and tubules (arrows). C: Group III showed moderate cytoplasmic reaction tocaspase 3 in the glomeruli and tubules (arrows). [Immunohistochemical stain to caspase 3 X 400, scale bar= 50 µm]. D: Bar chart showing the area percentage of the immune reaction to caspase3.

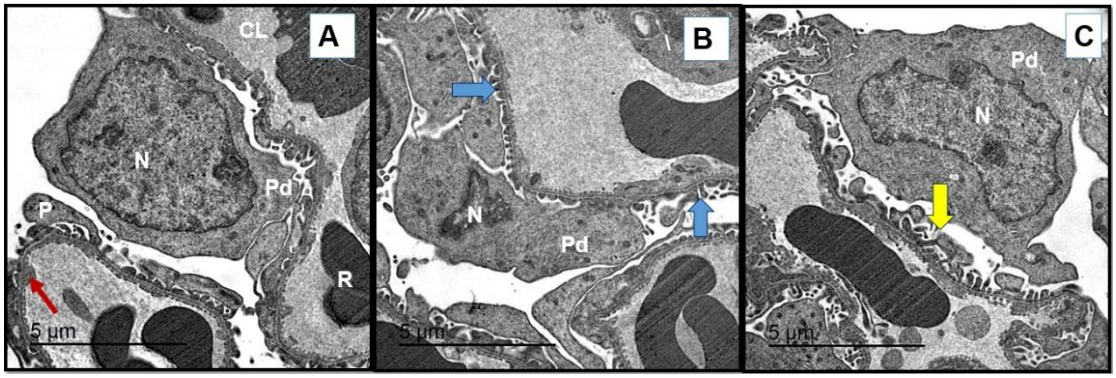

Figure (5): Electron micrographs of cells of the renal corpuscle.A: Group I showed podocytes (Pd) with euchromatic nucleus (N) and pedicles (P) resting on the glomerular basement membrane. The glomerular capillaries are lined with endothelial cells (Red arrow) and showed red blood cells in the capillary lumen (CL), B: Group II, showed heterochromatic nucleus (N) of podocytes (Pd) and atrophied pedicles of podocytes(Blue arrows) C: Group III showed euchromatic nucleus (N) of podocytes (Pd) and partially regeneratedpedicles (Yellow arrows)[TEM X1500,scale bar= 5 µm].

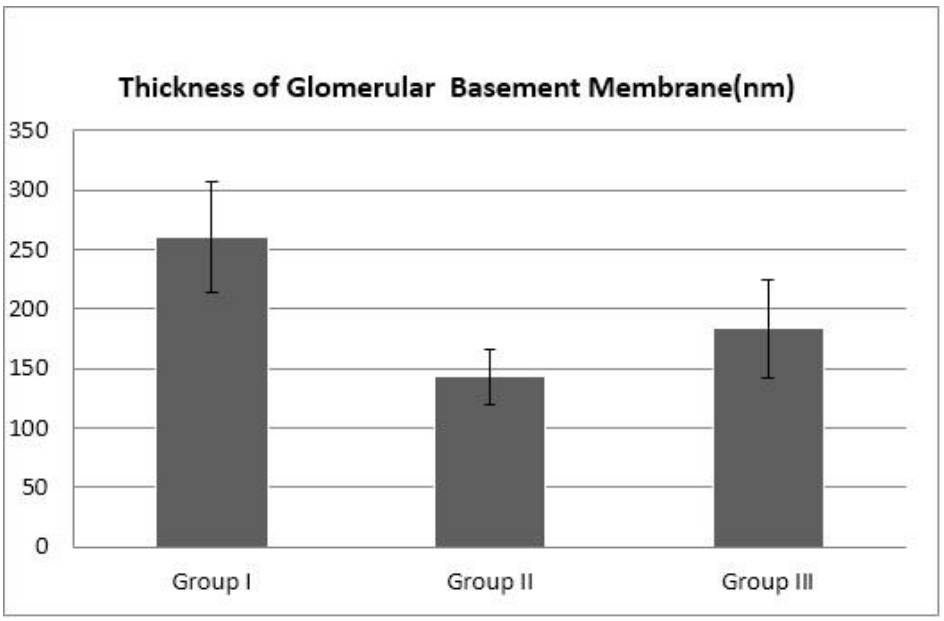

Figure (6): Bar chart showing the thickness of theglomerular basement membrane

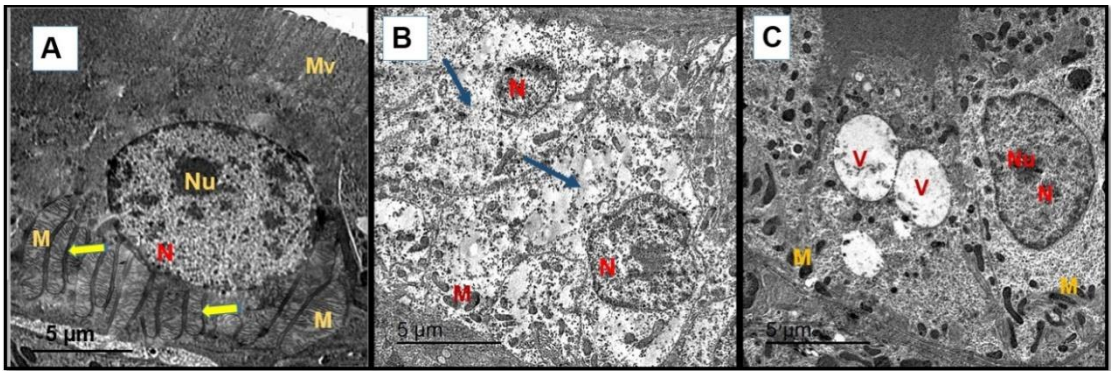

Figure (7): Electron micrographs of proximal convoluted tubule. A: Group I showed euchromatic nucleus (N) with prominent nucleolus (Nu),basal infoldings (Yellow arrows) with mitochondria(M), and apical microvilli (Mv)B: Group II showed degenerated cells (Blue arrows), small electron dense mitochondria (M),and heterochromatic nuclei(N). C: Group IIIshowed euchromatic nucleus (N) with prominent nucleolus (Nu), small electron dense mitochondria (M), and numerous vacuoles (V) [TEM X1000, scale bar= 5 µm].

DISCUSSION

Monosodium glutamate (MSG) is a flavor enhancer that has proved adverse health effects on different organs such as the liver, cardiac muscle, gonads, and the nervous system [6].Intermittent fasting (IF) is a recent diet regimen that is primarily used to reduce body weight, however, the effect of intermittent fasting on the structure and function of the different body organs has not been fully investigated [26].In this research, we studied the effect of intermittent fasting on the nephrotoxic effect of MSG.

There was significant body weight gainin MSG treated group; this is in accordance with Abdo et al. [27].MSG induces central obesity and fatty liver. The significant increase in the body weight of rats receiving MSG was explained by the damage of the arcuate nucleus which induces increase in appetite[28,29].On the other hand, other authors as Del Carmen Contini et al.[30] detected a significant loss of body weight of MSG-treated rats.

Serum urea, uric acid, and creatinine are used as indicators of kidney function. In this study, elevated serum urea, uric acid, and creatinine were observed in group II when compared with group I. These results were similar to those found by Paul et al[31].Elevated serum uric acid is caused by the increased generation of free radicals [32]. The significant increase in creatinine was caused by the decreased renal capacity to excrete creatinine[33].

The increased level of MDA and reduced activities of SOD and CAT in the kidney tissue of group II rats agreed with Paul et al. [34]who reported similar results.Nevertheless, other researchers detected no change in the lipid peroxidation level in the kidney of rats chronically exposed to MSG[30].

To assess inflammation, we measured serum CRP, TNF- α, and Il-6. CRP is normally produced by the hepatocytes and has a role in the innate immune. Its levels are elevated in cases of tissue damage, infection, and inflammation[35]. IL-6 is a cytokine necessary for the immune responses, metabolism, and tissue repair. Levels of serum IL-6 is increased in tissue injury and infection [36].TNF- α is a cytokine produced during acute inflammation. It participates in signaling events that induces necrosis or apoptosis in the cell [37].Serum CRP,Il-6, and TNF- α were significantly increased in group II. These results are similar to those detected by Venkateshet al. [38]and Liu et al. [39].On the other hand, Caetanoet al.[40] detected lower serum TNF-α in MSG treated rats.

Regarding the histological results, group II sections showed apoptotic nuclei, shrunk glomeruli and degeneration of tubules, congestion of blood vessels and infiltration of inflammatory cells and thinning of the basement membrane. These results were in accordance with Abdel-Azizet al. [41] and Lia Longodor et al[5].The histological alterations occurred secondary to the oxidative stress and the release of free radicals and the inflammatory responses that are elicited by MSG [6].

Increased collagen deposition was observed in group II; this is agreed with Hazzaaet al. [42]who reported fibrosis in cardiac muscles treated with MSG. The fibrosis occurred because of the oxidative stress together with urolithiasis that aggravated the production of ROS[43].In group II, MSG increased the immune expression of caspase 3 indicating enhanced apoptosis in this group. In agreement with this result Abdou et al. [44]who detected a significant increase in the number of caspase-3 labeled cells in the brain and the testis of rats injected with different doses of MSG.

Concerning the findings detected in group III, the body weight was significantly decreased when compared to group II, but still higher than group I. This was similar to Li et al.[45]who detected weight loss in the fasting obese mice. They attributed that to the change of the gut microbiota that activates the formation of the beige fat.

The kidney function tests were partially improved in group III. This finding agreed with Bilenet al.[46] who detected that fasting rats showed significantly reduced levels of serum BUN and creatinine.

MDA level was reduced in fasting group in comparison with group II. This finding is similar to Nurmasitoh et al.[47] who reported that IF reduced MDA in fasting obese rats. Additionally, Ahn et al.[48]detected that intermittent fasting enhanced the expressions of SOD and catalase in the granule and polymorphic cells.

The light and electron microscopic changes were partially ameliorated in the fasting rats. These results are similar to those reported by Ebrahimet al.[49] who detected improvement of the histological changes in the cerebellar cortex in the fasting rats, and with Tikoo et al.[14]who detected that intermittent fasting resolved the histological distortion of diabetes in the kidney of rat. Inflammatory cells, serum CRP, IL-6,TNF-αwere reduced in group III in comparison with group II. This may be attributed to the role of IF in inhibiting the toll-like receptor 4(TLR-4) andnatural kappa factor- β(NF-kB) protein thus preventing the occurrence of inflammation [50].These results agreed with De Souza Marinho et al.[51]who detected that alternate day fasting reduced the inflammatory cytokines IL1ß,IL6, and NF-kB in the livers of mice and disagreed with Wang et al. [52]who concluded that intermittent fasting effectively reduced CRP and did not change level of serum IL-6 and TNF-α.

Fibrosis was diminished in group III; these results agreed with Prisco et al.[53] that detected that IF reduced fibrosis in the rat model of preclinical pulmonary arterial hypertension. On the other hand, Okoshiet al.[54] reported that fibrosis of the cardiac muscles was not improved in the fasting rats. Apoptosis was attenuated in group III. This is in accordance with Hu et al.[55] who concluded that IF decreased apoptosis in the hippocampus and Bilenet al.[46] who detected that IF decreased caspase-9 and caspase -3 mRNA expression in diabetic rats.

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