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Original Research Article

Protective Role of Pomegranate on kidney of Albino Rat Treated with Monosodium Glutamate

Rasha Rabea Salem [1], Dalia Mahmoud Biram [2], Nesrine Mostafa El homosany *[3].

[1] Department of Anatomy and Embryology, Faculty of Medicine, Alexandria University, Alexandria, Egypt. Orcid iD: https://orcid.org/0000-0002-6912-8235

[2a] Department of Anatomy and Embryology, Faculty of Medicine, Alexandria University, Alexandria, Egypt, [b] Department of Anatomy and Embryology, Faculty of Medicine, Mutah University, El Kark , Jordan. Orcid iD: https://orcid.org/0000-0001-5494-8583

[3] Department of Anatomy and Embryology, Faculty of Medicine, Alexandria University, Alexandria, Egypt. Orcid iD: https://orcid.org/0000-0003-2404-7738

ABSTRACT

Background: Sodium mono glutamate (MSG), the sodium salt of glutamic acid, is a food flavoring agent that is widely used in many countries. Pomegranate is used as a traditional medication in numerous countries, it is planted in Asian countries, Mediterranean countries and the U.S.A.

Aim of the work: The present study aimed to detect structural and functional changes in adult rat kidney tissue treated with sodium mono glutamate, and the possible protective effect of pomegranate on the kidney treated with MSG.

Materials and Methods: This study was done by using 60 adult Wistar Albino rats of both sexes were divided into three equal groups: **Group I** (control group), **Group II** (sodium mono glutamate treated group), and **Group III** (combined MSG and pomegranate treated group) Doses were given once daily for 8 weeks every day. At the end of the treatment period, blood samples collected from each rat were used for measuring the values of urea and creatinine. Also animals of the different groups were sacrificed at the end of the experiment, quickly dissected and the kidneys were removed and stained with hematoxylin and eosin (H&E) for the histological examination by light microscopy, other tissue sections were evaluated using a transmission electron microscope. Both were used to examine the effect of sodium mono glutamate on cortex of the kidneys of albino rats ,compared with control group and the combined MSG and pomegranate group.

Results: There was a major rise in blood urea level and blood creatinine level in sodium mono glutamate treated group in contrast to the control group. There was a significant reduction in blood urea level and blood creatinine level in combined sodium mono glutamate and pomegranate treated group in comparison to MSG treated group. Examination of kidney tissue of rats treated with sodium mono glutamate (Group. II) showed damaging changes of its structure. The glomerulus had markedly widened blood capillaries with thickened filtration membrane. The epithelial tubular cells had marked degenerative changes. Examination of rats kidney tissue treated with sodium mono glutamate and pomegranate (Group III) revealed improvement of the lesions in the glomeruli and renal tubules.

Conclusion: Pomegranate protected the kidneys and restricted the histological and functional alterations caused by sodium mono glutamate, and thus, there is an advantage of usage of pomegranate with sodium mono glutamate.

KEY WORDS: Pomegranate, Sodium mono glutamate (MSG), Kidney, Blood Urea.

Corresponding Author: Dr. Nesrine Mostafa El homosany, Department of Anatomy and Embryology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
E-Mail: nesrinemostafa@hotmail.com

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INTRODUCTION

Sodium mono glutamate, named E621 in the European Union [1], was used particularly by Asians as food additive [2]. Sodium mono glutamate ingestion was associated with numerous toxic effects , one of them was "CHINESE RESTAURANT SYNDROME" It included over-all weakness, anesthesia of the neck, light-headedness, flushed face, and fainting [3].

Also sodium mono glutamate consumption caused obesity: by aggregating the food palatability [4], or by acting on the hypothalamus, the body developed the tendency to energy storage. This was based on that sodium mono glutamate disturbs the hypothalamic hormonal activity specifically that of leptin [5,6].

The consumption of sodium mono glutamate caused a reduction in the size of the pituitary and testes, dropping of testosterone concentration in the blood [7].

It was stated that sodium mono glutamate had toxic effects on the nervous system with resultant brain cell injury [8], degenerating changes of the retina, endocrinal disorders, cerebrovascular stroke, schizophrenia, and depression [9].

It was shown that fibrosis of the kidney was linked to the ingestion of sodium mono glutamate [10] and oxidative stress was the chief reason of renal toxicity [11]. Oxidative stress was caused by the overproduction or a reduced removal of (ROS) [12]. Consequently, abundant metabolism of glutamic acid by the kidney as in sodium mono glutamate consumption was a source of Reactive Oxygen Species [13,14]. Thus, sodium mono glutamate resulted in a disproportion between oxidants and antioxidant defense system; then, antioxidants could decrease the nephrotoxicity produced by sodium mono glutamate.

Pomegranate had been extremely appreciated for its valuable properties, and it had usually been planted mainly in Mediterranean countries, and some Asian countries [15]. The pomegranate had elevated levels of antioxidants [16]. It had a many polyphenols, remarkably anthocyanins, and ellagitannins [17].

Pomegranate was used as an antimutagenic agent, anti-bacterial agent and anti-atherosclerosis agent [19]. Consequently it had been used on a wide range [19]. This upraised our attention to become aware of the possible protective effect of pomegranate against the nephrotoxity produced by sodium mono glutamate.

Aim of the work: The present study aimed to detect structural and functional changes in adult rat kidney tissue treated with sodium mono glutamate, and the possible protective effect of pomegranate against MSG induced renal damage.

MATERIALS AND METHODS

Animals: The present study was done by using 60 adult albino rats of both sexes with body weight from 220-250 gm. The experiment was conducted to 8 weeks, and the animals were randomly divided into three groups, each of 20 rats , Group I received a diet containing 0.9% NaCl; Group II received diet containing MSG 6 mg/g/b.w.; and Group III received a diet containing MSG 6 mg/g/b.w [20]. followed by drinking water supplemented with pomegranate fruit extract. Doses were given once daily via a gavage for 8 weeks every day.

Assessment of Kidney Function: After the end of the experiment blood samples collected from each rat were used for the determination of urea and creatinine concentrations.

Serum Urea: Urea content of the serum samples was estimated by means of an automated analyzer, Blood Urea Analyzer.

Serum Creatinine: Creatinine content of the serum samples was estimated by means of an automated analyzer, Blood creatinine Analyzer.

Histopathological light microscopic Examination: Rats of the different groups were sacrificed, dissected and the kidneys were removed and fixed in 10% formalin. Fixed then embedded in paraffin wax and sections of were cut. Slides were stained with hematoxylin and eosin (H&E) for the histolopathological examination [21].

Transmission Electron Microscopy Studies: Small pieces (1 mm) of kidney tissues were

cut and fixed in a 3% glutaraldehyde (pH 7.4) phosphate buffer and post fixed in a 2% osmium tetroxide phosphate buffer. Following the fixation, tissues were dehydrated at increasing concentrations of ethanol. They were then embedded in araldite resin. Ultrathin sections were cut using an ultratome. Ultrathin sections were stained by uranyl acetate and lead citrate [22]. Tissue sections were evaluated using a transmission electron microscope.

Statistical analysis: The data was collected and moved into the computer. Statistical analysis was carried out by means of Statistical Package for Social Sciences (SPSS/version 21) software. Quantitative data was done using mean, standard deviation. Significance of the attained results was judged at the 5% level, where values $\leq$ 0.05 are significant.

RESULTS

Kidney function tests: There was extremely major rise in blood urea level and blood creatinine level in sodium mono glutamate treated group in contrast to the control group. There was a significant reduction in blood urea level and blood creatinine level in combined sodium mono glutamate and pomegranate treated group in comparison to MSG treated group. This is shown in table 1.

Table 1: Blood urea and creatinine levels in group I (control), group II (sodium mono glutamate) and group III (combined sodium mono glutamate and pomegranate treated groups).

Group	Group I	Group II	Group III
	N=20	N=20	N=20
Creatinine (mg/dl)	0.54 ± 0.05	1.82 ± 0.33	0.61 ± 0.18
Urea (mg/dl)	29.44 ± 1.23	40.26 ± 2.78	32.87 ± 1.44

There was a significant increase in the blood urea and serum creatinine levels in group II in comparison to the group I and group III. P value $\leq$ 0.05

The histological examination of the renal tissue stained with H&E of the control group (Gp. I) showed normal typical construction of the glomeruli, and renal tubules . The renal corpuscles showed regular Bowman's capsule , normal glomerular capillaries and normal urinary space. This is shown in figure (1).

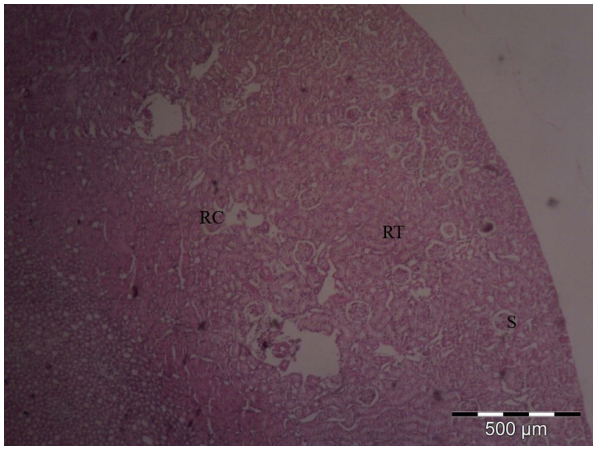

Fig. 1: A photomicrograph of rat's kidney of control group (gp. I), showing normal renal corpuscle(RC) , urinary space (S) is well-preserved, and, normal renal tubules (RT),

The histological examination of the renal tissue of sodium monoglutamate treated group (Gp. II) showed distorted construction and degenerative changes in the renal corpuscles. Some corpuscles showed atrophy with widening of urinary space, others were without urinary space , others seemed normal . Necrosis was shown in the renal tubules. The tubules were with markedly dilated lumina. This is shown in figure (2).

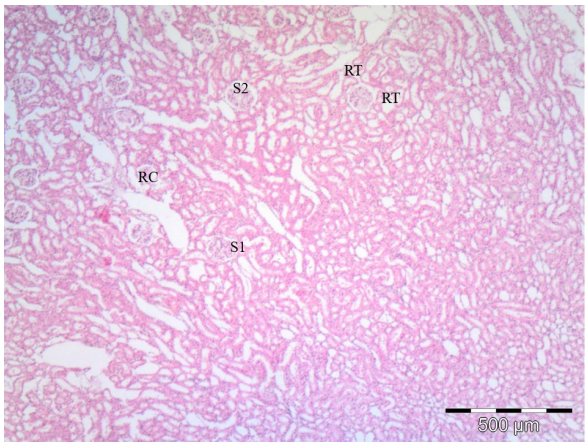

Fig. 2: A photomicrograph of rat's kidney of sodium mono glutamate treated group (gp. II), showing abnormal renal corpuscle (RC), some of them with narrow urinary space(S1), others with dilated urinary space (S2), others were with normal urinary space. Markedly degenerated renal tubules (RT) with manifest dilated lumina.

The histological examination of the renal tissue of combined sodium mono glutamate and pomegranate treated groups (Gp. III) , showed marked improvement. Most renal corpuscles had normal urinary space, restored the normal construction. The renal tubules also restored the normal construction. This is shown in figure (3).

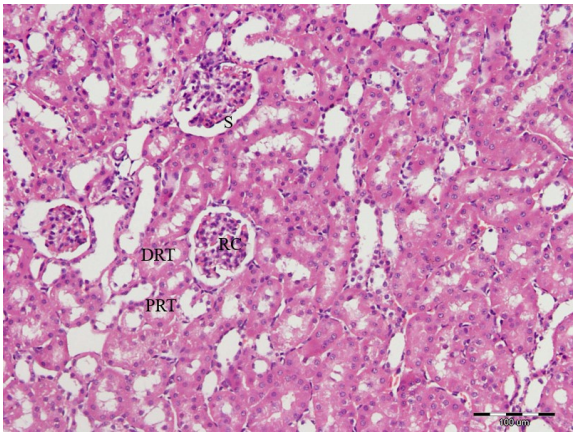

Fig. 3: A photomicrograph of rat's kidney of combined protected group (Gp. III) showing marked improvement of the renal construction, normal renal corpuscle (RC), normal proximal tubule (PRT) and normal distal tubule(DRT).

EM Examination of control rats' kidney tissue of control group (Gp. I) exposed normal glomerular structure, blood capillaries had thin filtration membrane. The podocytes had long foot processes. The Renal corpuscles had narrow urinary space. The epithelial cells of the renal tubules rested on a normal thickness basememt membrane. Proximal tubular cells showed round basal nuclei, and their cytoplasm showed numerous elongated mitochondria, few lysosomes, and few lipid droplets. The distal tubular epithelial cells had round apical nuclei, the cytoplasm had basal infoldings bounding elongated mitochondria and narrow lumen. And their cytoplasm had numerous normal mitochondria. This is shown in figures (4), and (5).

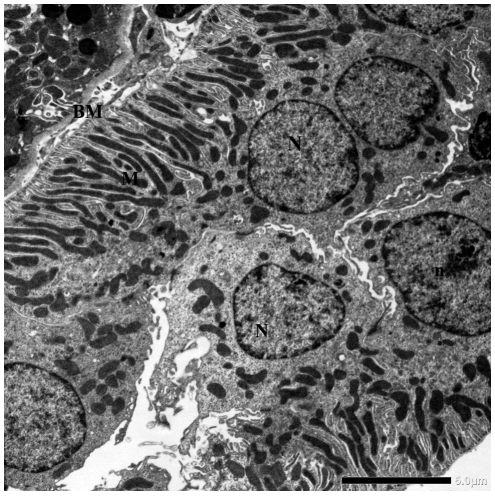

Fig. 4: An electron photomicrograph of control group rat¼Æs kidney (Gp. I) showing distal tubular cells resting on basement membrane (BM) having basal foldings containing many arranged mitochondria (M). Euchromatic nuclei (N) with noticeable nucleoli (n) was also shown

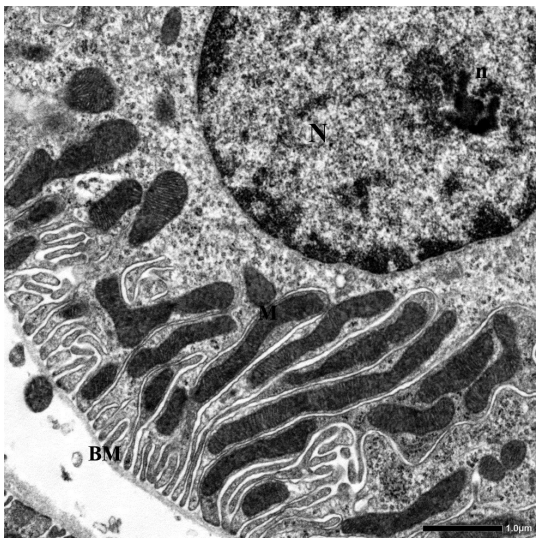

Fig. 5: An electron photomicrograph of control group rat¼s kidney (Gp. I) showing tubular cells resting on a well defined basement membrane (BM) having basal foldings containning many arranged mitochondria (M). Euchromatic nucleus (N) with noticeable nucleolus (n) was also shown.

EM examination of kidney tissue of rats treated with sodium mono glutamate (Gp. II) showed damaging changes of its structure. The glomerulus had markedly widened blood capillaries with thickened filtration membrane. Podocytes had dark nuclei and light cytoplasm. Dilatation of the urinary space was seen in some renal corpuscles. The epithelial tubular cells nuclei had irregular envelope and clumped chromatin, the cells also had a thick basement membrane, The cytoplasm had different sized bizarre mitochondria, and many large lysosomes. This is shown in figures (6), (7), and (8).

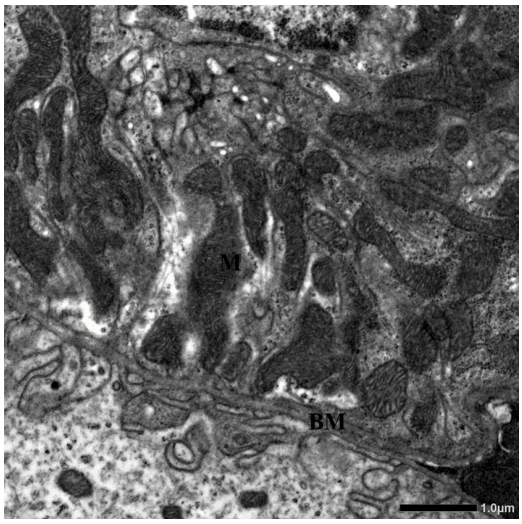

Fig. 6: An electron photomicrograph of rat¼s kidney of sodium mono glutamate group (Gp. II) showing tubular cell having different shaped and sized mitochondria (M) (some small, and other large and dark), and thick basement membrane (BM).

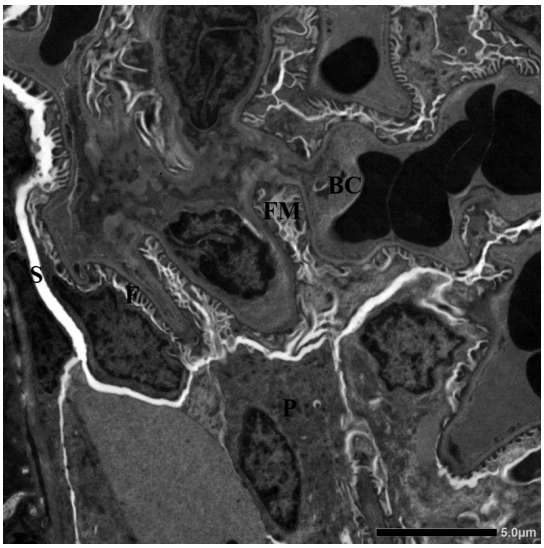

Fig. 7: An electron photomicrograph of rat¼s kidney of sodium mono glutamate group (Gp. II) showing glomerulus having noticeable widened blood capillaries (BC) and thickened filtration membrane (FM). (F) foot processes, Podocytes (P) had dark nucleus and light cytoplasm. Dilated urinary space (S).

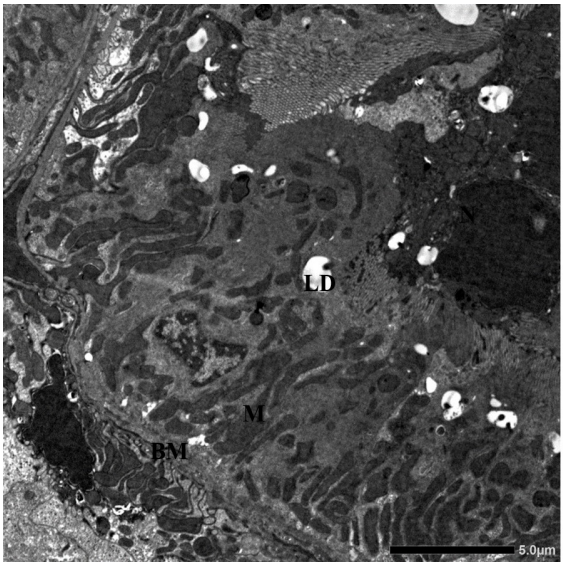

Fig. 8: An electron photomicrograph of rat¼s kidney of sodium mono glutamate group (Gp. II) showing tubular epithelial cell with dark nucleus (N), different shaped and sized mitochondria (M), big lipid droplets (LD), and thick basement membrane (BM).

EM examination of rats kidney tissue treated with sodium mono glutamate and pomegranate (Gp. III) revealed improvement of the lesions in the glomeruli and renal tubules. The renal corpuscles had narrow urinary space. Podocytes with dark nuclei and long foot processes. The tubular epithelial cells had nuclei with regular envelope. They rested on thin basement membrane. The cytoplasm had numerous different sized mitochondria, few lipid droplets, and few lysosomes. This is shown in figures (9), (10), and (11).

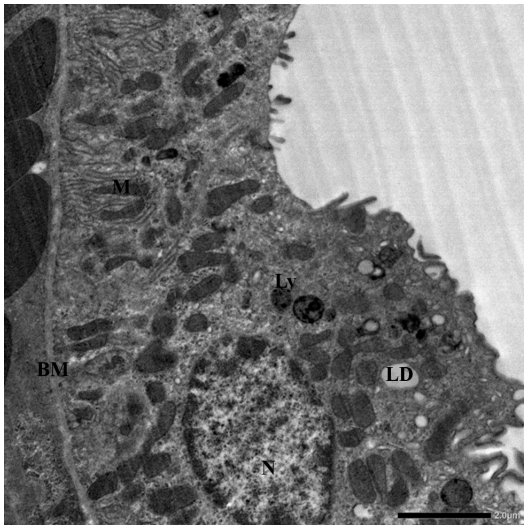

Fig. 9: An electron photomicrograph of rat¼s kidney of combined group (Gp. III) showing tubular cell having thin regular distinct basement membrane (BM), round euchromatic nucleus (N) , numerous mitochondria (M), lysosomes (Ly) and different sized lipid droplets (LD).

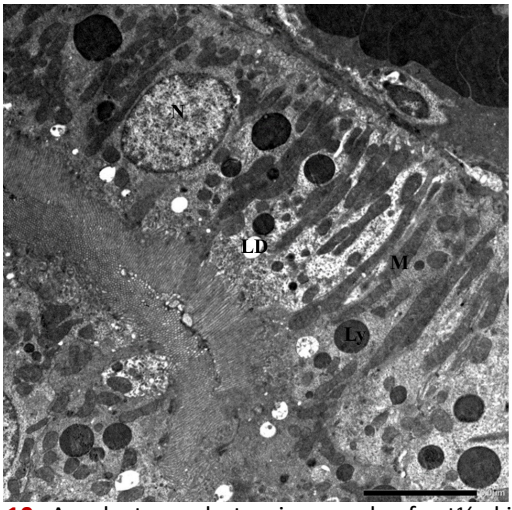

Fig. 10: An electron photomicrograph of rat¼s kidney of combined group (Gp. III) showing tubular cell having numerous organized mitochondria (M) , lysosomes (Ly) and lipid droplets (LD),and euchromatic nucleus (N).

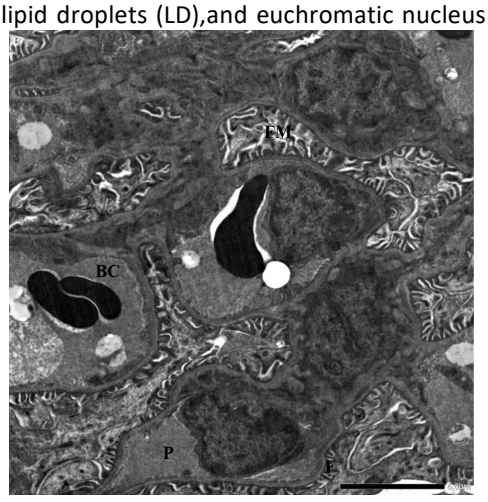

Fig. 11: An electron photomicrograph of rat kidney of combined group (Gp.III) showing glomerulus having small blood capillaries (BC) and filtration membrane (FM). Long foot processes (F) . Podocytes (P) with dark nuclei and dark cytoplasm.

DISCUSSION

In the existing study, the histological examination revealed that the kidneys in control group (Group I) and in the combined sodium mono glutamate and pomegranate group (Group III) were of normal histological construction.

While sodium mono glutamate group (Group II) showed abnormal histological construction.

Shilpi Gupta et al. [23]. described that by the influence of sodium mono glutamate, There was alteration of kidney architecture. Numerous glomeruli presented proliferation of cells of the endothelium and infiltration of inflammatory cells. The glomeruli cells were swollen with material accumulation. If widespread, there was obstruction of lumen of the capillaries of the glomeruli. The basal lamina of the capillary was thickened. The Bowman's space was increased. The alterations in glomeruli were patchy. The alterations in the kidney tubules were similarly patchy cloudy. There was edema of the cells of the tubules and constricted lumen. The deteriorated tubules presented cells detached with release of the components of the cells in the lumen of the tubules and the cytoplasm o these cells became vacuolated. In these tubules, the remaining cells were distended with somewhat dark stain of the cytoplasm. The nucleus of each cell showed either karyolysis or pyknoses .

These were considered as signs of kidney destruction and there was some points of similarity to the results of the current study in that in the present study the glomeruli had thickened filtration membrane, the basal lamina of the capillaries were thickened, the tubular epithelial cells nuclei had irregular envelope and clumped chromatin, and the tubular cells also had a thick basement membrane and other points of difference that in the current study the glomeruli had markedly widened blood capillaries, Bowman's space was increased in some glomeruli and decreased in others and the lumen of the tubules were markedly dilated .

According to Eweka [24] who worked on kidney of fully-grown Wistar rats, alteration of the kidney cortices with certain degree of cell death due to sodium mono glutamate was observed and these findings agreed with these of the current study.

In the present study, sodium mono glutamate treated rats (Group II) exhibited that the kidney lost its typical construction relative to the control rats (Group I). There were important damaging changes of its structure. The glomerulus had markedly widened blood capillaries with thickened filtration membrane. Podocytes had dark nuclei and light cytoplasm. Dilatation of the urinary space was seen in some glomeruli. The epithelial tubular cells nuclei had irregular envelope and clumped chromatin, the cells also had a thick basement membrane, The cytoplasm had different sized bizarre mitochondria, and many large lysosomes.

Another study stated that sodium mono glutamate treated female rats presented massive degenerative changes in their ovaries in the form of hypertrophy of the theca cells, degeneration in the oocytes and zonae granulosa; these changes were dose dependent [25].

Sodium mono glutamate could increase the risk of increased blood cholesterol, increased blood triglyceride level, diabetes mellitus, and obesity . Additionally, it could cause liver toxicity and neurotoxicity [26].

Eman et al. observed decrease in the level of total protein, and albumin, and major elevation in serum ALT and AST in sodium mono glutamate treated rats indicating hepatic dysfunction [27].

Helal et al. and Tawfik et al. also presented an extremely important increase in blood creatinine and urea, this was in accordance with [28,29] and this was in accordance with the present study.

In agreement with the present study, changed kidney function was described by Paul et al. following administration of sodium mono glutamate [13].

Helal et al. , Adrienne S., and Bodnár et al. reported decrease in the level of testosterone in sodium mono glutamate treated rats , and stated that this could be resultant from

interruption of the hypothalamic-pituitary-testicular axis [28,30,31].

The mechanism of action of sodium mono glutamate injury to different organs as hepatotoxicity, neurotoxicity, testicular damage , ovarian degenerative changes, and renal damage is correlated to the stimulation of oxidative stress [32].

The overproduction of reactive oxygen species (ROS) in sodium mono glutamate treated rats was considered as a chief contributor to its renal damaging properties [33]. Sodium mono glutamate caused overproduction of free radicals and the body antioxidant lines were scarce to encounter them. ROS overproduction caused alteration in protein, lipid peroxidation, and DNA destruction [34].

NO is produced by the inducible nitric oxide synthase enzyme in inflammatory conditions. The inducible nitric oxide synthase enzyme is not classically expressed in normal cells and should be induced by some cytokines and other inflammatory enzymes [35].

In the sodium mono glutamate induced pathology, low-grade inflammatory process had a vital role [36].

Additionally, certain studies found the improving effect of vitamins C, and E on sodium mono glutamate treated rat kidneys [37,13]. The mechanism by which these antioxidants apply their effect was not totally clarified. Nevertheless, these antioxidants might have a crucial role against kidney inflammation via a decrease of the action of inflammatory enzymes [38] and cytokines [39,40].

In the present study, rats administrated Pomegranate together with sodium mono glutamate (Group III) displayed improved histological construction of the glomeruli and renal tubules. The glomerulus had narrow urinary space. Podocytes with dark nuclei and long foot processes. The tubular epithelial cells had nuclei with regular envelope. They rested on thin basement membrane. The cytoplasm had numerous different sized mitochondria, few lipid droplets, and few lysosomes.

Because of all these positive results which matched with the findings of the control rats (Group I) settling the anti-oxidant properties of pomegranate.

The results of the existing study agrees with clarification of Gil, M. I. et al. who said that Pomegranate is rich in phenolic compounds that comprised its antioxidant action [41]. It exerted strong antioxidant action against lipid peroxidation [42,43].

And as oxidative stress had an significant role in the occurrence of kidney damage, polyphenols present in pomegranate were suggested to play an important role in the protection from this illness,

The current study agrees with Mohamed et al. [44] who stated that the usage of pomegranate had a protective effect on cisplatin caused nephrotoxicity. Pomegranate also caused decrease in the urea and creatinine levels in combined cisplatin and pomegranate treated animals relative to the control group.

Azab et al. described that pomegranate showed protecting effect on the kidneys from nicotine by decreasing oxidative damage and growing anti oxidative defense mechanisms and also ameliorating effect on the serum urea and creatinine [45]. And this clarifies that pomegranate could be used as protective agent against kidney dysfunction as in the current study.

Depending on the current study, sodium mono glutamate produced a severe damaging effect on the kiney, nevertheless this effect could be protected by means of natural antioxidants as pomegranate.

CONCLUSION

Pomegranate protected the kidneys and restricted the histological alterations caused by sodium mono glutamate, and thus, there is an advantage of usage of pomegranate with sodium mono glutamate.

Conflicts of Interests: None

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