Histological, Histochemical and Ultrastructural Studies on the Kidney of Rats After Administration of Monosodium Glutamate

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الملخص

دراسات هستولوجية وهستوكيميائية ودقيقة علي كلية الفئران بعد معالجتها بجلوتاميد أحادي الصوديوم

يُظهر هذا البحث تأثير إعطاء تركيزين مختلفين [(2)و(3) مجم/جم/وزن جسم] من جلوتاميد أحادي الصوديوم لمدة 21, 45 يوم علي التوالي على التركيب النسيجي للكلى في ذكور الفئران البالغة . وقد أظهرت الفحوصات النسيجية لمجاميع الفئران التي عوملت بتركيز 2مجم/جم/وزن الجسم بداية فقدان جزئي للحافة الفرشاتية المكونة الأنيبيبات الملتفة الدانية.حدوث تحلل للطبقة الطلائية المكونة للأنابيب البولية التي ظهر فيها مجموعة من الخلايا الالتهابية . كذلك تّم ملاحظة تحلل جزئي وكُلّي لأنوية الخلايا المكونة لبعض تلك الأنابيب البولية. أما إعطاء جرعة 3مجم/جم/وزن الجسم ، فقد أظهر الفحص النسيجي ظهور تغيرات مرضية خطيرة تتمثل في تضخم الشعيرات الدموية المكونة لشعيرات الكبة وتكون حويصلات دهنية بها . وقد حدثت تشوهات في التركيب البنائي للخلايا المكونة للأنابيب البولية وحدوث نزيف داخل الفصيصات التي تكونت في بعض خلايا الكبة التي أظهرت الفحوصات أيضاً سمك في الطبقة البطانية المكونة للشعيرات الدموية في بعض الكبيبات التي انكمشت وتكونت بها حويصلات دهنية وطبقة زجاجية .

أما الدراسة الهستوكيميائية فقد أظهرت نقص في محتوى الكربوهيدرات والبروتينات وذلك بعد معاملة الفئران بجلوتاميد أحادي الصوديوم. وفيما يتعلق بالدراسات الدقيقة بواسطة الميكروسكوب الإلكتروني ، فقد أظهرت تدمير واضح في عضّيات الخلايا وتغيير في الشكل العام للأنوية المكونة لها.

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ABSTRACT

The present investigation demonstrated the effect of oral administration of two different doses of monosodium glutamate (MSG): 2 mg/g/b.w. and 3mg/g/b.w. for 21 and 45 days respectively on the histological structure of the kidney tissues of adult male albino rats. The histopathological observation of the kidney tissues after the administration of 2mg/g/b.w., showed partial loss of brush border of proximal convoluted tubules and necrotic lesions in the epithelial lining of renal tubules. Numerous inflammatory cells infiltrated in the interstitial tissues and pyknotic, karryolysed nuclei in some tubules were also observed. Severe histopathological alterations were recorded after treatment of rats with 3mg/g/b.w. of MSG. These changes included distention of glomerular capillaries with fat globules, distortion of renal architecture in most of the cells, lobulation in some glomerular tufts with haemorhogic lesions, shrunken glomeruli which invaginated by fatty globules and diffused hyaline thickening of capillary endothelium .Histochemical investigations revealed marked reduction in both carbohydrates and proteins after the treatment with MSG. Ultrastructural studies revealed destruction of cytoplasmic organelles, thickening of the basement membrane of the proximal convoluted tubules and irregularly- shaped nuclei.

Key words: Sodium glutamate, histochemistry, histopathology, Ultrastructure, kidney.

INTRODUCTION:

A common example of one of the thousands of chemicals used in our new high-tech foods is the monosodium glutamate MSG.It is the sodium salt of the amino acid , glutamic acid and a form of glutamate.It is added to the food either as a purified monosodium salt or as a component of a mixture of amino acids and small peptides resulting from the acid or enzymatic hydrolysis of proteins, (Schwartz 2004).When it is added to this food in relatively small quantities, the palatability of those foods is increased (Yamagu ,1998). This is the substantial evidence that the sensory basis for this effect is that MSG stimulates the sense of taste (Kawamura et al.,1987).

A study conducted by (Halpern, 1997) has provided a convincing evidence that the taste quality elicited by (MSG) and related substances such as inositol monophosphate is unique. That is, it is not some combination of sweet, sour, salty and bitter, the presumed other primary taste qualities(Ohguro,et al.2002).

Glutamate is absorbed from the gut by an active transport system specific for amino acids. This process is saturable, can be competitively inhibited, and is dependent on sodium ion concentration (Schultz et al., 1970). During intestinal absorption, a large proportion of glutamic acid is transaminated and consequently alanine levels in portal blood are elevated. If large amounts of glutamate are ingested, portal glutamate levels will increase (Stegink, 1984). This elevation results in an increase hepatic metabolism of glutamate, leading to release of glucose, lactate, glutamine, and other amino acids, into systemic circulation (Stegink, 1984).

Numerous studies have been conducted on the physiological role of MSG. Berry et al., (1974) found that it caused an enlargement of the liver and an increase of serum albumin and decrease in serum globulin. Machol, et al., (1999) found that it changed several endocrine functions in neonatally treated rats. Larsen et al.,(1994) found that it altered the activity and sensitivity of rat hypothalamo- pituitary- adrenocortical axis . Osfor-Mmh, et al., (1997) indicated that kidney ,liver ,brain ,and heart weight were significantly increased in rats treated with MSG. Miskowiak et el., (1999) found that injection of monosodium glutamate (4mg/g b.w.) to the rats resulted in a decrease of the number of Graafian follicles and lowered the thickness of endometrial controls. MSG induced alterations in metabolic rate of glucose utilization and decreased antioxidant defenses(Diniz-Ys,et al., 2004).

There uses have become controversial because of some reports of adverse reaction in people who have eaten foods that contained MSG. Studies conducted by Schwartz (2004) have shown that the body uses glutamate as a nerve impulse transmitters in the brain, and that there are glutamate responsive tissues in other parts of the body. The MSG treated rats showed a severe growth retardation as a consequence of the impairment of r GH anabolic effects (Sakai et el., 2004). Description of the pathological effects of MSG on organs of the rats were lacking. So, the present study aims to highlight on the histopathological alterations of the kidneys of albino rats treated with MSG.

MATERIAL AND METHODS:

Sixty adult male albino rats (Rattus norvigicus) weighing 100±10g. were used in the present study. They were obtained from Helwan laboratory farms for the Egyptian Organization for Vaccine and Biologic Preparations. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard diet and water available ad libitum.

The rats were equally divided into three groups and orally treated as follows:

Group(1): The control group in which rats were administrated with 0.9% NaCL day after day for 45 days.

Group(2): The experimental group in which rats were administrated orally with the therapeutic dose of monosodium glutamate (2mg/gm body weight) dissolved in 0.9% NaCL day after day for 21 days.

Group (3): The treatment group in which rats were administrated orally with the therapeutic dose of monosodium glutamate (3mg/gm body weight) dissolved in 0.9% NaCl day after day for 45 days.

Histological and Histochemical Preparations:

The kidneys from the control and experimental groups were rapidly excised after the previously mentioned duration, cut into small pieces and dropped in Bouins fluid in which they were kept for appropriate time. After fixation, they were subjected to the normal procedure for paraffin embedding. Sections were cut at the thickness of 5 microns and stained with haematoxyline-eosin(Drury and Wallington,1976). Periodic Acid Schiff technique (PAS)(Hotchkiss,1948), was used for the studying of general

carbohydrate and bromophenol blue technique) was used for the demonstrating of total proteins(Mazia et al.,1953).

Transmission Electron Microscopy Studies:

Small pieces (1 mm) of treated tissues were cut and fixed in 3% glutaraldehyde (PH7.4) in phosphate buffer and post fixed in 2% osmium tetroxide in phosphate buffer. Following fixation, tissues were dehydrated at increasing concentrations of ethanol. They were then embedded in araldyte resin. ultrathin sections were cut using an ultratome. Ultrathin sections were stained by uranyl acetate saturated in 70% ethanol, and lead citrate ***(Reynolds,1963).***Tissue sections were evaluated using a JEOL transmission electron microscope JEM-1200. Ex, Japan**.**

RESULTS:

Histological Observations:

Group I : kidney sections of control rats showed normal histological structures of the glomruli, and renal tubules in the cortical (Fig.1) and medullary portions(Fig.2).

1. Histopathological Observations:

Group II : Rats treated with a dose of 2 mg/gm b.w. of MSG for 21 days showed variable pathological changes in glomeruli and some parts of the urinary tubules. Such changes exhibited an existence of hyperemia with swelling in the lining epithelium of the glomerulus associated with a priglomerular focal area. Also, they were represented by dramatic renal injury with tubular cell swelling, loss of brush border of proximal convoluted tubules as well as necrotic lesions (Fig.3).Dilatation and hyperemia in the intertubular cortical blood vessels were seen clearly in figure (4).

Swelling in the lining epithelium of the renal tubules with narrow lumen and the presence of inflammatory cellular infiltration, mainly composed of lymphocytes and monocytes in interstitial tissues were noticed in figure (5). Moreover, figure (6) exibited vascular degeneration in the epithelial cells lining the renal tubules at the cortical zone with pyknotic and karyolysed nuclei .The pathogenic observations in figure (7,9) showed hyperemic capillaries, focal hemorrhage between the degenerated renal tubules at the

corticomedullary portion as well as Karyolysis in the epithelial lining of the urinary tubules with an infiltration of chronic inflammatory cells that shown which tend to concentrate around tubules (Fig.8).

Group III: The histopathological changes in rats received a dose of 3 mg/gm/b.w of MSG for 45 days in alternate days manifested more intensive deterioration and damage than those observed in group II. Figures (9,10) revealed distension of glomerular capillaries with fat globules .There was an increase in the incidence of marked severe vascular degenerative changes in the lining epithelial cells of the renal tubules at the cortical portion and distortion in the renal architecture (fig.11). On the other hand, figure (12) showed shrunken of many glomeruli invaginated by fatty globules, diffused hyaline and thickening of capillary endothelium. In addition, there was a focal mononuclear leukocytes inflammatory cells that infiltrate between the tubules at the corticomedullary portion (Fig.13).

With the progress of time, more degenerative changes took place in the renal tubular epithelium cell debris in their lumens (Fig. 14). Also, lobulations in some glomerular tufts with haemorrhagic lesions were seen in figure (15). Necrosis of other tubules lining cells , focal mononuclear leucocytes , inflammatory cells infiltrating renal tubules , focal haemorrhagic area in between the renal tubules and chronic inflammation replaced urinary tubules were evident in all rats treated with MSG in this group (Figs. 16, 17).

The most severe changes were in the proximal convoluted tubules which consisted of cytoplasmic vacuolation of tubular epithelial cells with swelling and detachment of them. With the progress of time, interstitial edema, more extensive necrosis of tubular epithelial cells, and dilated tubules with accumulation of eosinophilic homogenous material in tubular lumina (Fig.17).

Histochemicals Observations:

1.General carbohydrates:

A considerable amount of carbohydrates in the cytoplasm of kidney cells of control rats was noticed by PAS-technique, which gave a red or magenta colour (Fig 18). The nuclei, however, appeared entirely PAS-negative staining, indicating absolute lack of carbohydrates.

Treating rats with 2 mg/gm/b.w of MSG for 21 days, caused a decrease of total carbohydrates in the kidney cells (Fig.19). A marked reduction of total

carbohydrates was observed after 45 days of treatment with 3 mg/gm/b.w MSG (Fig. 20).

2.Total proteins:

Total proteins were demonstrated in epithelial cells lining the renal tubules of control rats as deeply blue stained diffuse granules homogenously through both the cytoplasm and nuclei (Fig 21). Their nuclear envelopes and nucleoli as well as some chromatin elements were also positively stained (Fig 21).

Treatment of rats with 2 mg/gm/b.w MSG for 21 days induced a slight decrease in the protein content of the urinary tubules (Fig 22). More reduction in proteins was manifested in the cells treated for 45 days, where the proteinic granules were clearly reduced in amount and stainability (Fig 23).

3.Ultrastructural Observations:

The cells of the proximal convoluted tubules of control rats showed long rod-like mitochondria oriented parallel to the cell axis. They have long microvilli forming the brush border appeared and large rounded nuclei (Fig. 24) .

The Ultrastructural examinations revealed changes of the cells of the renal tubules after administration of 2 mg/gm /b.w of MSG. These comprised intracellular vacuolations , destruction of cytoplasmic organelles, splitting and thickening of basement membrane (Fig. 25). Additionally, other cells of proximal tubules showed irregularly- shaped of nuclei with loose chromatin materials and invagination of the nuclei membrane (Fig.26).

DISCUSSION :

The present study indicated that MSG induced marked histopathological alterations in the kidney tissues of rats such as tissue impairment ,swelling of the lining epithelium of glomeruli, injured brush border of proximal convoluted tubules, necrotic lesions of the urinary tubules and focal hemorrhage between the degenerative renal tubules. Similar results, have been reported by others (Aughey et al., 1984; kjellstrom, 1986; Mitsumari et al., 1998); Inkielewicz and Krechniak (2003).

The mechanism of swelling starts as a decrease in O2 levels which causes a drop in aerobic respiration. To maintain ATP levels, the cells must rely more on glycolysis. Glycolysis leads to lactic acid builds up, which causes the intracellular pH to drop. An acidic environment in the cell causes dysfunction of the Na^+/K^+ ATPas and consequent cell swelling due to an influx of Na^+ and H_2o.Persistent ischemia can lead to ca^{++} influx mitochondrial and lysosomal damage, and membrane damage (Lieberthat et al., 1998).

In the present investigation, many renal tubules of the rat kidneys showed marked degenerative lesions under the effect of MSG. This is justifiable since the renal tubules are particularly sensitive to toxic influences, in part because they have high oxygen consumption and vulnerable enzyme systems, and in part because they have complicated transport mechanisms that may be used for transport of toxins and may be damaged by such toxins. Also the tubules come in contact with toxic chemicals during their excretion and elimination by the kidneys (Tisher and Brenner, 1989). Such degenerative changes were markedly pronounced in the proximal convoluted tubules. These findings reinforce those of Koechel et al., (1984) and Damjanov (1996), who found that many chemicals had a direct nephrotoxic action and exerted their effects principally on the proximal convoluted tubules.

The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of the cells (Shimizu et al., 1996). Renal medullary necrosis occurs as a primary manifestation of renal disease. The mechanism of which is poorly understood, but it seems to involve a vascular change. Also, prostaglandin synthetase is found in the kidney , primarily in the medulla , and inhibition of this enzyme resulted in decreased production of prostaglandin E2 (PGE2) and loss of its vasodilatory effect on juxtamedullary arterioles. (Date and Shastry, 1982).

De Camargo and Merzel,(1980) observed that mice fed with 10 and 500 ppm NaF for 3 months had shrunken kidneys, degeneration of tubular cells, and dilatation in the convoluted tubules. Similar changes were seen in the present investigation.

One possible mechanism for the tubular lesions was the direct toxic effect on the cell function (Alden and Frith, 1992). Damage to the brush border and leakage of alkaline phosphates (ALP) and gammaglutamyle transferase (GGT) enzymes , which are associated with the brush border of the renal tubules, as a result of toxin binding to the brush border and considered as an early marker of toxic tubular insult (Edelstein, et al., (1995); Davies et al.,(1995); Porter , (1994); Fadel ,and Larsen, (1994).

Other possible mechanisms for the tubular lesions may involve reactive intermediates or oxidative stress, or both (Alden and Frith 1992). Biologically reactive intermediates are electron-deficient compounds (electrophiles) that bind to cellular electron-rich compounds, such as proteins and lipids (Goldstein and Schnellmann, 1995). Mixed-function oxidases catalyze the formation of there toxic metabolites. Reactive intermediates bind covalently to critical cellular macromolecules and interfere with normal biologic activity. Oxidative stress is induced by increasing production of reactive oxygen specie (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radicals (Goldstein, et al., 1995). ROS can induce lipid peroxidation, inactivate cellular enzymes, depolymerize polysaccharides, and induce deoxyribonucleic acid breaks and chromosome breakage.

Superoxide dismutase (SOD) is a naturally occurring intracellular enzyme that catalyzes the breakdown of superoxide radicals (McCord, 1993) . Ischemia leads not only to an increase in superoxide production, but also, to a rapid depletion of SOD (singh et al.; 1993 and Davies et al.; 1995).

The detection of Lymphocyte inflammatory cells in the present study indicated the production of chronic inflammatory disease under the effect of MSG. This result agreed with Ashry et al., (1990) who demonstrated chronic active cells accompanied by inflammatory cells in the hepatocytes after administration of codeine.

The results showed that treated rats with MSG caused a depletion of carbohydrates in the cytoplasm of renal tubules. This result was in correspondence with other studies reported by Sakr et al.; (2003) due to the treatment of gibberellin to the rats, and Abdeen et al.,(1994) and Sakr et al.,(2002a) duo to the use of a variety of animals under different pathological conditions.

Disturbances in carbohydrate metabolism were also observed in a variety of animals under the effect of different insecticides and were suggested to be achieved through modifying the activities of the enzymes of glycolytic pathway. TCA cycle, glucogenesis and the oxidation of phosphorylation (Kacew and Singhal, 1973 and Shakoori et al., 1988).

The present study also revealed that treatment rats with MSG induced marked decrease in protein contents of the studied cells. This result consistent with other studies reported by Mehadevaswami et al., (2001) due to the use of different compounds.

The reduction of protein contents observed in this study may be attributed partially to the decrease of hepatic protein synthesis due to the hyperactivity of hydrolytic enzymes (Sivaprasada et al., 1983). Moreover, the decrease

occurred in proteins is likely to be a consequence of the damage produced by MSG in the rough endoplasmic reticulum and Golgi apparatus as reported by (Elewa et al., (1999), El-Beih et al., (1987)and Berlin (1967). In addition, Palla et al., (1987), postulated that in many kidney diseases, the permeability of the glomerular capillaries is increased leading to increased levels of excreted proteins. They added that any lesions produced in the kidney tubules will eventually cause dysfunction in the transport mechanism to and from the renal epithelium.

The electron microscopic examination revealed destruction in the cytoplasmic organelles including lysosomes, endoplasmic reticulum, mitochondria and Golgi apparatus. These alterations could be due to the cytotoxic effects of MSG. Thomas (1988) stated that Golgi apparatus is responsible for the packaging of hydrolytic enzymes involved in the formation of secretary products. The destruction of Golgi apparatus leads to destruction of lysosomes and this consequently leads to an increase in secretion of hydrolytic enzymes which may be responsible for the lysis of cytoplasmic organelles.

Finally, the present study reveals toxic effects of MSG on the kidney during the use of this drug. Therefore , more researches must be done on other organs of the body to highlight its effects on these organs.

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cells (H & E x 400) **.**

Figure(9): Kidney section showing dilatation of the inter tubular blood vessels and vascular lumen impacted by haemolysed blood (H & E x 400)**.**

Figure(10): Kidney section showing glomerular capillaries distended with fat globules; deep stained affinity in mesangial cells as well as degenerative changes of the renal tubules (H & E x 400) **.**

Figs. (11-17) sections of the kidney of rat after administration of 3mg/ gm / b.w. for 45 days .

Figure(11): Kidney section showing distortion of renal architecture and narrowing of renal lumens lined by degenerative cells (H & E x 400) **.**

Figure(12): Kidney section illustrating Shrunken glomeruli invaginated by fatty globules, diffused hyaline thickening of capillary endothelium; degenerative (a) and necrotic (b) lesions in proximal convoluted tubules (H & E x 400) **.**

Figure(13): Kidney section showing focal mononuclear leukocyte inflammatory cells infiltrate between tubules (H & E x 400)**.**

Figure(14): Kidney section of showing detached cell debris in the renal tissue and lymphocytic cell infiltration in interstitial (H & E x 400) **.**

Figure(15): Kidney section showing lobulations in some glomerular tufts with haemorrhagic lesions (H & E x 100)**.**

Figure(16): Kidney section illustrating necrosis of tubular lining cells and focal mononuclear leukocyte inflammatory cells infiltrating renal tubules (H & E x 400)**.**

Figure(17): Kidney section showing focal hemorrhagic area in between the renal tubules and acute tubular necrosis (H & E x 400)**.**

Figure(18): Kidney section of control rat showing positive PAS reaction in the renal tubules (PAS x 400)

Figure(19): kidney section of rat after administration of 2.0 mg/gm/b.w of MSG for 21 days showing decrease of total carbohydrates in most of the cells (PAS x 400).

Figure(20): Kidney section of rat after administration of 3.0 mg/gm/b.w with MSG for 45 days showing more reduction of total carbohydrates (PAS x 300)

Figure(21): Kidney section of a control rat showing normal contents of proteins after staining with bromophenol blue (x400).

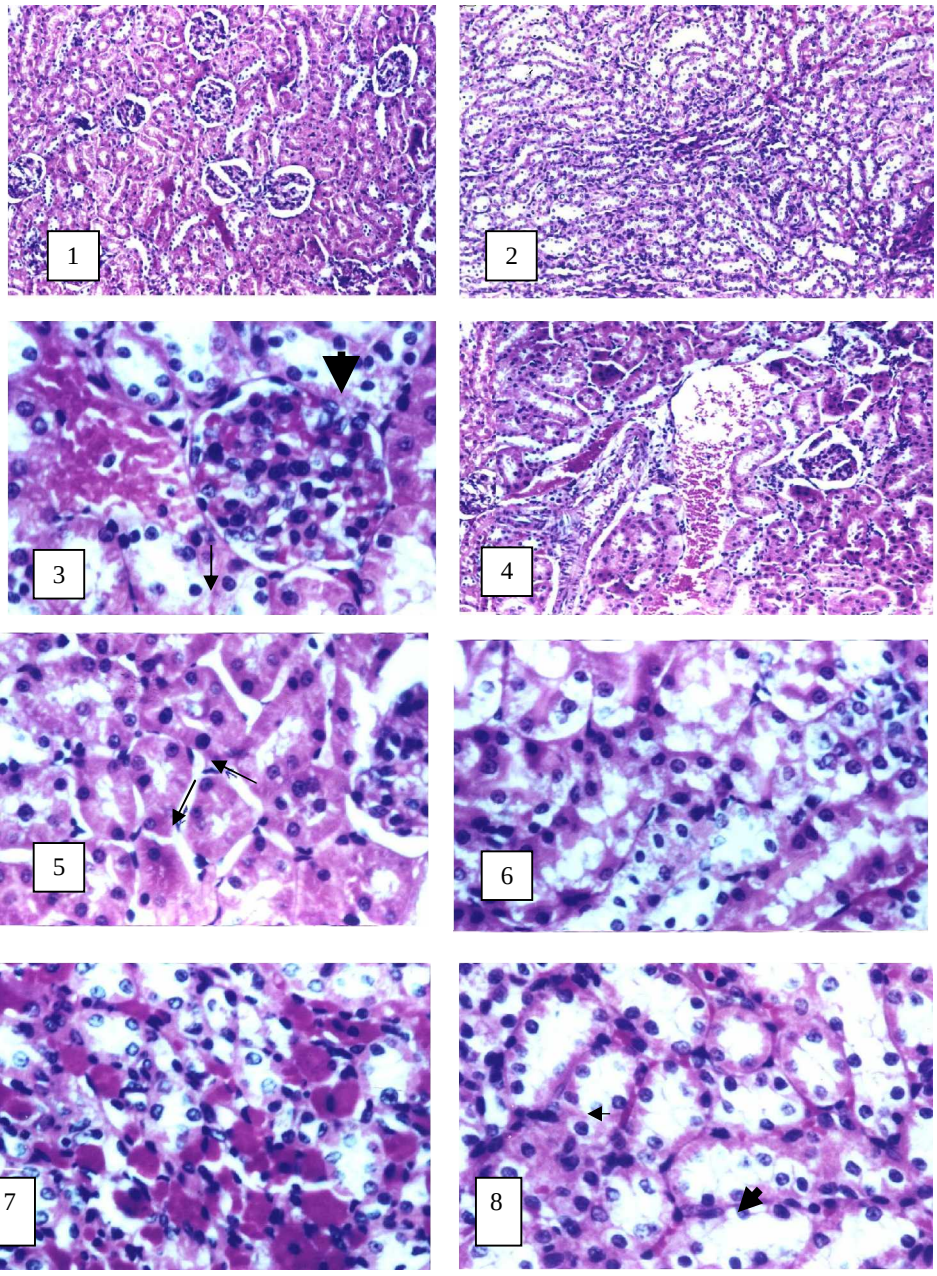
Figure(22): Kidney section of a rat after administration of 2mg/g/b.w. of MSG for 21 days showing a moderate staining affinity of bromophenol blue in the renal tubules (x400).

Figure(23): Weak blue staining affinity of bromophenol in the nuclear materials of some degenerated renal tubules of a rat kidney after administration of 3mg/g MSG for 45 days (X400).

Figure(24): An electron micrograph of some cells of a proximal convoluted tubules of a control rat kidney. The cells revealed a large spherical nucleus , a large number of mitochondria and basal enfolding , in addition to the microvilli forming the brush border (X2000).

Figure(25): Electron micrograph of proximal convoluted tubules of a kidney of rat showing clear destruction of cytoplasmic organelles , splitting and thickening of basement membram after administration of 2mg/g MSG (X3000)

Figure(26): Electron micrograph of proximal convoluted tubules of a kidney of rat revealed irregular shaped of nuclei with loose chromatin materials, invagination of the nuclei membram and destruction of mitochondria after administration of 3mg/g MSG (X2000).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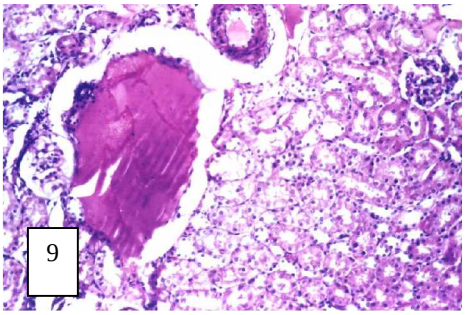
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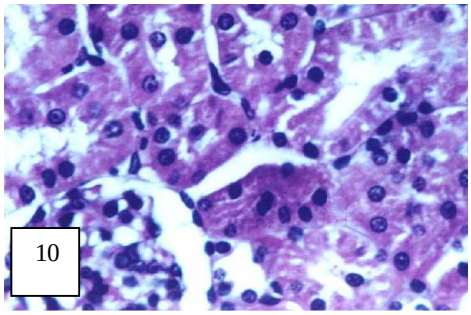
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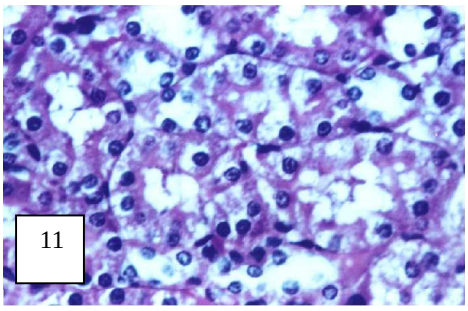
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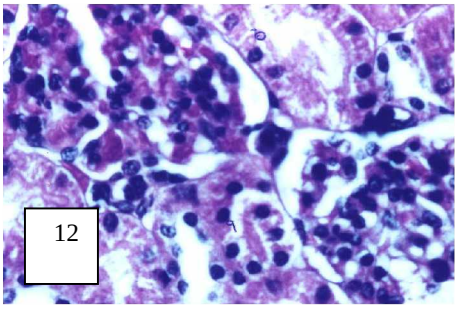
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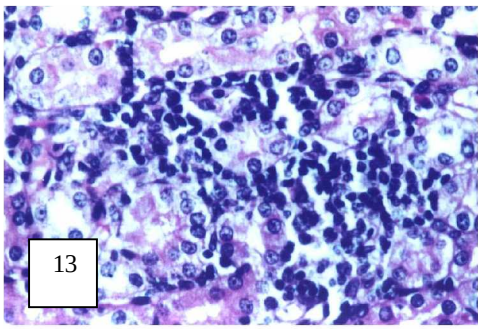
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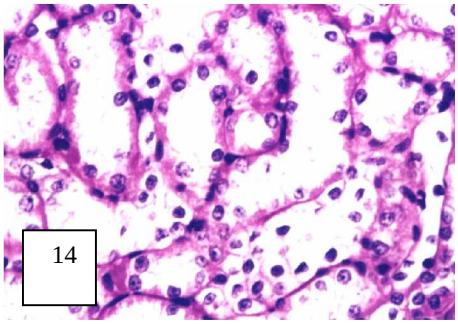
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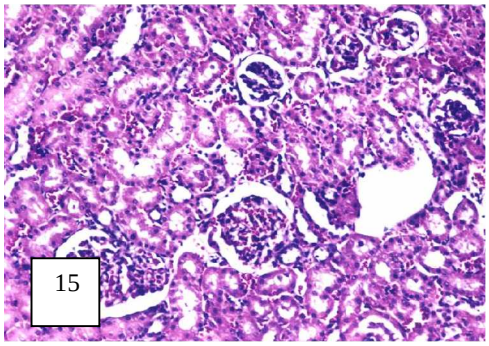
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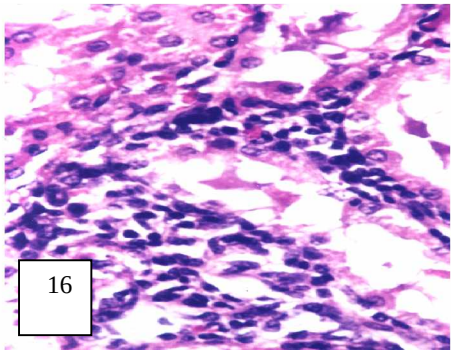
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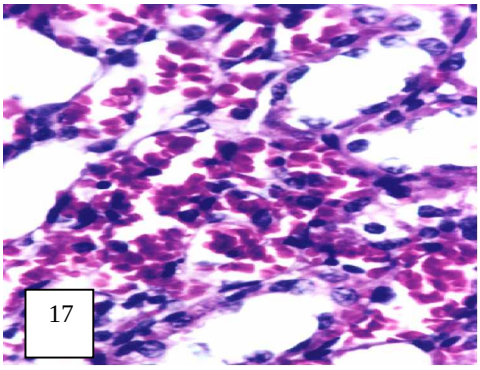
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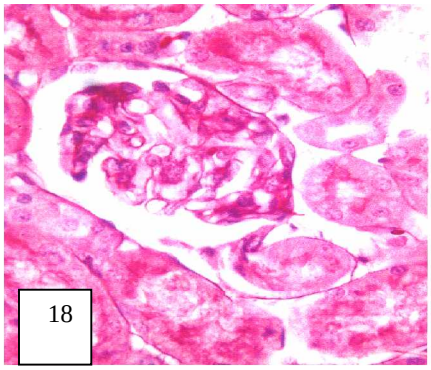
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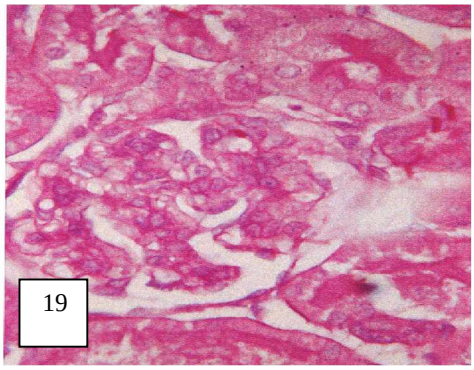
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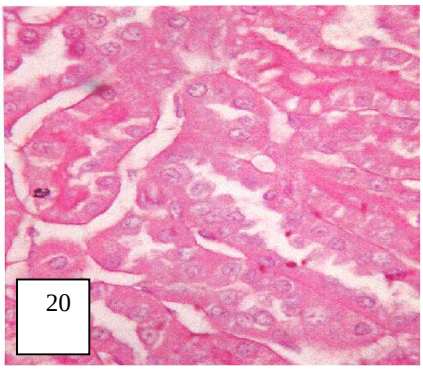
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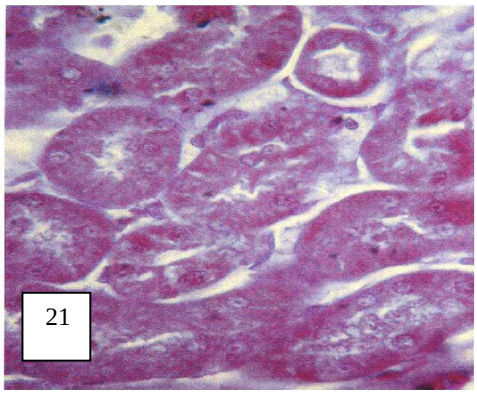
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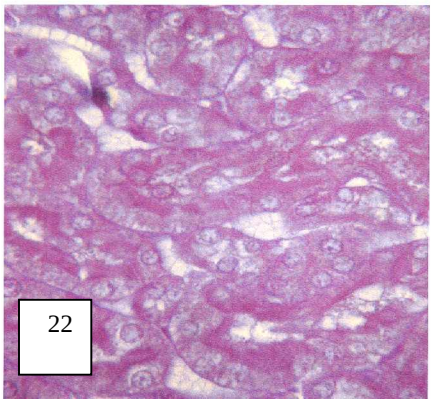
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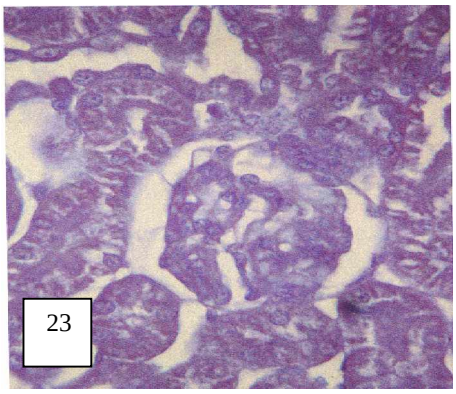
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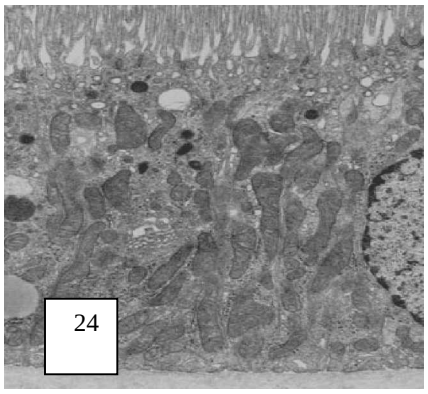
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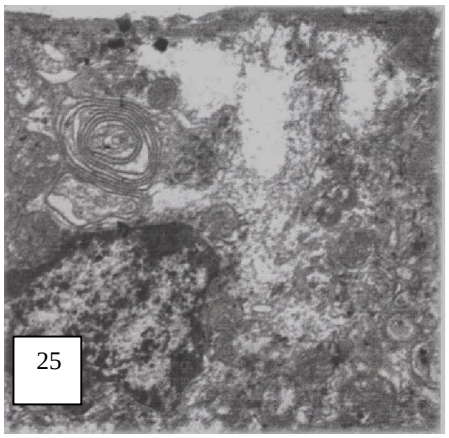
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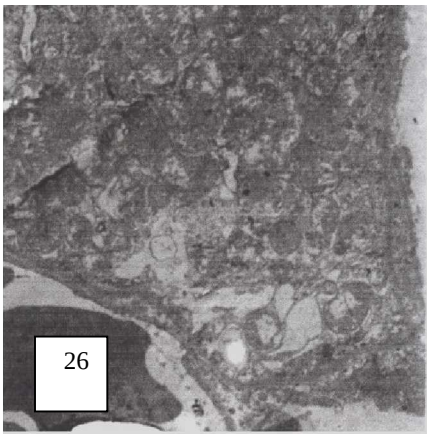
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