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HPLC analysis and role of the Saudi Arabian propolis in improving the pathological changes of kidney treated with monosodium glutamate

HPLC analizi ve monosodyum glutamata bağlı patolojik böbrek değişikliklerinin düzeltilmesinde Suudi Arabistan propolisinin rolü

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SUMMARY

BACKGROUND: Monosodium glutamate is commonly used in our foods and reported many physiological effects. Propolis is a natural product widely used in folk medicine due to its bioactive compounds. It is considered one of the richest sources of phenolic acids and flavonoids.

METHODS: The phenolic acids and flavonoids content of Saudi Arabian propolis was determined by HPLC analysis. Three groups of albino rats were used in the present study for histological and histochemical studies. Group 1 (control group) received 0.9% NaCL, group 2 was given monosodium glutamate (6 mg/g bw) and group 3 received monosodium glutamate (6 mg/g body weight) and propolis (50 mg/kg body weight).

RESULTS: The HPLC analysis of the Saudi Arabian propolis revealed presence of predominant phenolic acids; *trans*-cinnamic, *p*-coumaric, caffeic, ferulic, sinapic, and flavonoids; apigenin, kaempferol, quercetin, rutin. The rats administered orally with the monosodium glutamate (6 mg/g body weight) and propolis (50 mg/kg body weight) for 8 weeks showed a significant protective effect of propolis in prevention monosodium glutamate induced toxic pathological changes in kidney of the rats.

CONCLUSION: The presence of phenolic compounds in the Saudi Arabian propolis is coincided with its role in improving the histological and ultrastructural pictures of kidney treated with monosodium glutamate.

Key words: Propolis, monosodium glutamate, Phenolic compounds, HPLC, histopathology, kidney.

ÖZET

AMAÇ: Monosodyum glutamat yiyeceklerimizde sıklıkla kullanılmakta ve birçok fizyolojik etkisinin olduğu bildirilmektedir. Propolis, biyoaktif bileşiklerinden dolayı halk tıbbında yaygın olarak kullanılan doğal bir üründür. Fenolik asitlerin ve flavonoidlerin en zengin kaynaklarından biri olarak bilinmektedir.

YÖNTEM: Suudi Arabistan propolisinin fenolik asit ve flavonoid içerikleri, HPLC analizi ile saptandı. Bu çalışmada, histolojik ve histokimyasal analizler için 3 grup Albino sıçan kullanıldı. Grup 1'e (kontrol grubu) %0.9 NaCl; grup 2'ye monosodyum glutamat (6 mg/kg vücut ağırlığı) ve grup 3'e propolis (50 mg/kg vucüt ağırlığı) verildi.

BULGULAR: Suudi Arabistan propolisinin HPLC analizi, baskın olarak fenolik asitlerden *trans*-sinamik, *p*-kumarik, kafeik, ferulik, sinapik; flavonoidlerden ise, apigenin, kampferol, kuersetin, rutin varlığını ortaya koydu. Oral olarak 8 hafta boyunca monosodyum glutamat ve propolis verilen sıçanlarda, monosodyum glutamat ile oluşturulmuş toksik patolojik böbrek değişikliklerinin önlenmesinde propolisin belirgin bir koruyucu etkisi olduğu gösterildi.

SONUÇ: Suudi Arabistan propolisinde fenolik bileşiklerin bulunması, monosodyum glutamat uygulanan böbreklerin histolojik ve ultrastrüktürel görüntülerinin geliştirilmesindeki rolü ile tutarlıdır.

Anahtar kelimeler:Propolis, monosodyum glutamat, fenolik bileşikler, HPLC, histopatoloji, böbrek

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INTRODUCTION

Monosodium glutamate (MSG) is a common example of one of the chemicals used in our new foods. It is the sodium salt of the glutamic acid. 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. The body uses glutamate as nerve impulse transmitters in the brain, and that there are glutamate responsive tissues in other parts of the body [1]. Numerous studies have been conducted on the physiological role of MSG; indicated that kidney, liver, brain, and heart weight were significantly increased in weight in rats treated with MSG [2]. The rat treated with MSG showed a severe growth retardation as a consequence of the impairment of rat growth hormone (rGH) anabolic effects description of the pathological effects of MSG on organs of the rats were lacking [3].

It was reported that when MSG is added to the food in small quantities, the palatability of those foods is increased [4]. The substantial evidence of the sensory basis for this effect is that MSG stimulates the sense of taste [5]. 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 [6, 7].

During intestinal absorption, a large amount 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, this elevation results in an increase hepatic metabolism of glutamate, leading to release of glucose, lactate, glutamine, and other amino acids, into systemic circulation [8].

MSG reported many physiological effects. It causes an enlargement of the liver and an increase of serum albumin and decrease in serum globulin [9], alters the activity and sensitivity of rat hypothalamo- pituitary- adrenocortical axis [10] and changes several endocrine functions in neonatally treated rats [11]. The injection of MSG (4 mg/g bw) 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 [12, 13].

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias [14]. Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing. However; there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis [15]. There is growing interest toward natural antioxidants from natural sources [16]. Epidemiological and in vitro studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [17-19].

Propolis or bee glue is a resinous product, collected by honey bees from plant exudates and has gained popularity as a food and alternative medicine. It is created from resins, balsams and tree saps. It is used by the bees to varnish the hive interior, seal cracks and cement things together. Propolis is the substance responsible for neutralizing any bacteria, fungi or virus that enters the hive. It is containing approximately 55% resinous compounds and balms, 30% beeswax, 10% aromatic essential oils, 5% bee pollen and about 150 compounds [20]. It had been shown to have broad biological activities which are principally attributed to the presence of flavonoids (quercetin, galangin, rutin, chrysin, apigenin, kaempferol) and phenolic acids (*trans*-cinnamic, p-coumaric, caffeic, ferulic, sinapic) [21-24]. Propolis has been reported to be an important antioxidant. Recently, propolis had been reported as a powerful scavenger of reactive oxygen species (ROS) [25, 26]. In addition, propolis was reported to have, antimicrobial [27], anti-inflammatory [28] properties. Propolis has a neuroprotective effects, but these effects partly mediated via antioxidant effects [29].

MATERIALS AND METHODS

Propolis sample

Propolis from Al-Shafa region situated 20 km from Taif, Saudi Arabia with different dominant floras was used. The sample was collected from different sites inside the beehive at March 2010; and was kept in the dark in a freezer.

General procedure

TLC was performed on silica gel 60 F254-coated aluminum sheets (Merck, Darmstadt, Germany). Plates were visualized by spraying with 1% w/v aluminum chloride.

EI-MS analysis was carried out on JEOL JMS 600 Hz mass spectrometer (Japan).

HPLC analysis was carried on L-6200A intelligent pump and L-4000 UV detector (Merck, Germany).

HPLC analysis

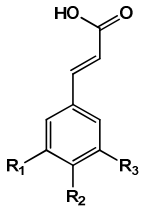
One g of Propolis was ground and extracted with 70% ethanol at room temperature with moderate shaking. The extract was filtered and evaporated till dryness.

The residue was re-dissolved in 1 ml methanol and filtered. The filtrate was used for quantitative determination of the total phenolic acids and flavonoids using HPLC (30 µl was injected). This was performed on a nucleosile 100-5 C-18 column using water (A) and methanol (B) as solvents. The following gradient was employed: 10% B for 6 min, 15-40% B within 35 min, then isocratic elution at 50% B for 10 min. The flow rate was 1 ml/min and the detection wavelength set to 320 nm. Caffeic acid and quercetin (Fluka, Germany) in a concentration of 1 µg/100 µl were used as a reference compounds for phenolic acids and flavonoids respectively. The quantity of total phenolic acids and flavonoids were estimated on the bases of their area with respective to the area of references (0.3 µg each) as external standards.

Extraction and phytochemical investigation

Thirty g of grounded propolis was extracted by maceration with 70% ethyl alcohol at room temperature to yield 2.9 g dry propolis extract. The phytochemical and chromatographic screening of the extract revealed the presence of phenolic acids and flavonoids.

TLC screening using $CHCl_3$ - MeOH –H_2O (7.5: 2.5: 0.5 v/v) as a solving system of extract indicated that they are richest of phenolic compounds ($AlCl_3$ detection) and revealed several spots, eight of them were major. They were isolated by a preparative TLC on silica gel G_{60} F_{254}-coated glass sheets (Merck, Germany). Further purification of the isolated compounds was achieved by HPLC. Their EI-MS spectrum were agreed with published data for phenolic acids; *trans*-cinnamic, *p*-coumaric, caffeic, ferulic, sinapic and for flavonoids; apigenin, kaempferol, quercetin, and rutin [20, 30, 31] (Figs. 1a & 1b). Furthermore, co-chromatography (TLC & HPLC) with reference compounds (Fluka, Germany) was performed.



Compounds	R_1	R_2	R_3
Trans-Cinnamic acid	H	H	H
p-Coumaric	H	OH	H
Caffeic acid	H	OH	OH
Feruloic acid	H	OH	OCH_3
Sinapic acid	OCH_3	OH	OCH_3

Fig. 1a: Chemical structure of the isolated phenolic acids

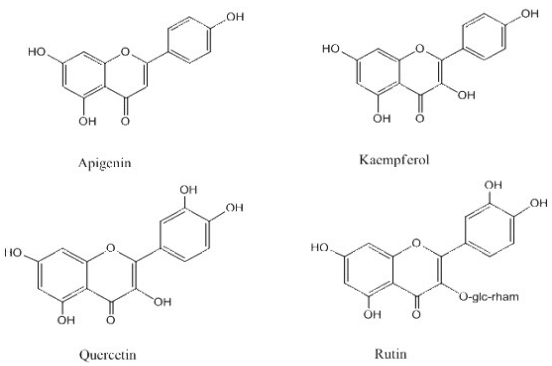


Fig. 1b: Chemical structure of the isolated flavonoids

Animal Groups

Sixty adult male albino rats weighing 250 ± 10 g were used in the present study. Animals are obtained from Jeddah. Animals are caged in three groups given feed and water ad libitum. The rats were kept in the laboratory for 8 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 (control group); in which rats were administered with 0.9% NaCL for 8 weeks, Group 2; in which rats were administered orally with the therapeutic dose of MSG (6 mg/g bw) dissolved in 0.9% NaCl for 8 weeks, Group 3; in which rats were administered orally with the MSG

(6 mg/g body weight) dissolved in 0.9% NaCl and propolis (50 mg/kg body weight) for 8 weeks.

Histological and histochemical preparations

The kidney 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 [32].

Transmission electron microscopy studies

Small pieces (1 mm) of treated tissues were cut and fixed in 3% glutaraldehyde (pH 7.4) 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 araldite resin. Ultrathin sections were cut using an ultratome. Ultrathin sections were stained by uranyl acetate saturated in 70% ethanol, and lead citrate [32]. Tissue sections were evaluated using a JEOL transmission electron microscope JEM-1200. Ex, Japan.

RESULTS

Quantitative determination of phenolic acids and flavonoids

Ethanolic extract of propolis considered one of the richest sources of phenolic acids and flavonoids. It was investigated by HPLC and revealed a series of phenolic compounds. For this reason, the quantity of major phenolic acids and flavonoids were estimated on the bases of their area with respective to the area of references; caffeic acid and quercetin (0.3 μg each) as external standards. The results of HPLC analysis of Saudi Arabian propolis sample revealed that, it contains a high amount of *trans*-cinnamic acid and rutin (table 1). The presence of predominant phenolic compounds is coincided with role of the Saudi Arabian propolis in improving the histological and ultrastructural pictures of kidney treated with MSG.

Histological observations

Light microscopic observations

Control group: The Kidney of control rats had normal renal structure of both cortex and medulla. The cortex (Fig. 2) showed a normal structure of; renal glomeruli. The proximal convoluted tubules are lined with typical thick cubic epithelium. The distal convoluted tubules show considerably lower cubic epithelium. The tubules have a relatively regular distinct lumen; the glomerular capsule is lined with a flat epithelium. The medulla (Fig. 3) showed collecting tubules lined with the relatively low simple cubic epithelium. The thick descending and ascending parts of Henle's loops are lined with simple cubical epithelium with small caliber, and a small amount of interstitial tissue can be seen normally in the cross-sections.

Table 1: Concentration of phenolic acids and flavonoids in propolis sample mg/g, ± SD

Compound	Concentration mg/g, ± SD
trans-Cinnamic acid	1.12 ± 0.04
p-Coumaric acid	0.85 ± 0.03
Caffeic acid	0.61 ± 0.04
Ferulic acid	0.73 ± 0.07
Sinapic acid	0.53 ± 0.05
Apigenin	0.325 ± 0.004
Kaempferol	0.227 ± 0.006
Quercetin	0.465 ± 0. 004
Rutin	1.003 ± 0.001

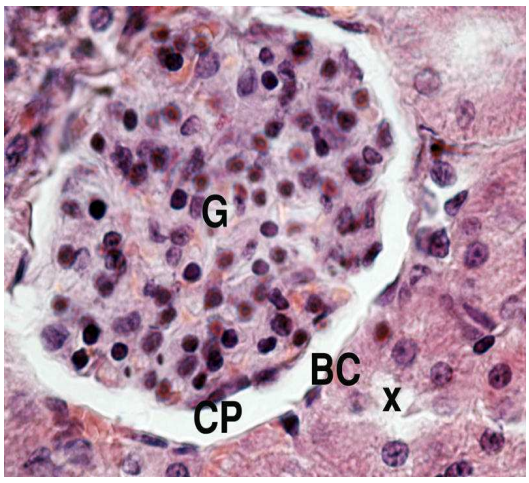


Fig. 2: Light photomicrography of Kidney (cortical part) of a control rat. The renal glomeruli (G) show glomeruli has flat epithelium lining the glomerular capsule (BC) with distinct capsular space (CP), the proximal (X) are lined with typical thick cubic epithelium .H&E, x1000

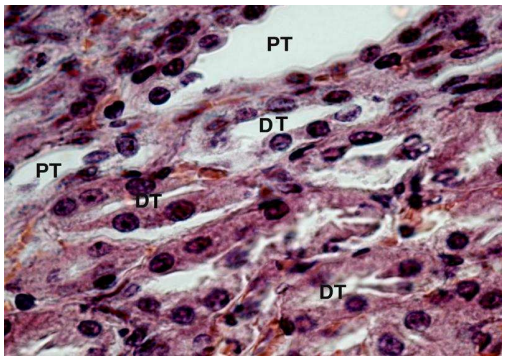


Fig. 3: Light photomicrography of Kidney (medullary part) of a control rat. The proximal convoluted tubules (PT) are lined with typical thick cubic epithelium with large diameter and distal convoluted tubules (DT) are lined with the relatively low simple cubic epithelium with smaller diameter. H&E, x1000.

Group 2: Light microscopic examination in the kidney of rats which administered MSG showed many areas of tubular damages ranged from mild to severe in the kidney were observed in all treatment animals. Showing a vascular glomeruli are enlarged, tightly filling the glomerular capsular space, with flat epithelium lining the Bowman's capsule (Fig. 4). There were renal damages appeared as hypertrophy and degeneration of epithelia of renal tubules with distinct of mononuclear cells infiltration. A few renal tubules showed single epithelial cells desquamated to their lumen (Fig. 5).

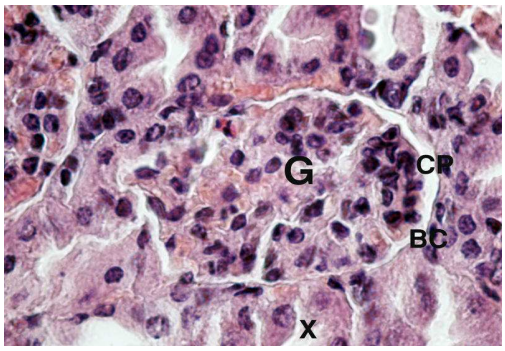


Fig. 4: Light photomicrography of Kidney of rat after eight weeks of exposure to MSG, showing a vascular glomeruli (G) are enlarged, tightly filling the glomerular capsular space (CP),with flat epithelium lining the Bowman's capsule (BC) Some cells of the proximal (X) and distal (D) convoluted tubular epithelium show features of oedema. Capillaries are filled with blood cells; some tubules contain single desquamated cells. H&E, x1000

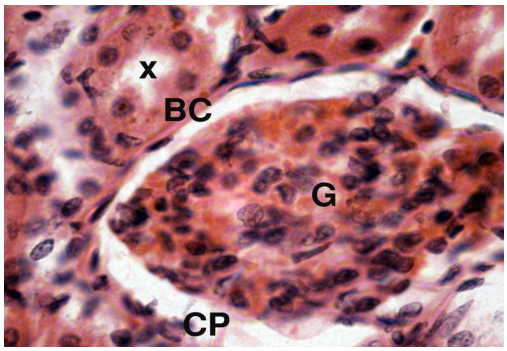


Fig. 6: Light photomicrography of Kidney of rat after eight weeks of exposure to MSG and propolis showing an decreased in the vasculature of the renal glomeruli (G),appearance of the glomerular capsular space (CP). Decrease the oedema of both the proximal (X), and distal (D) convoluted tubular epithelium. Lack of the fibroses in the Bowman's capsule (BC). H&E, x1000.

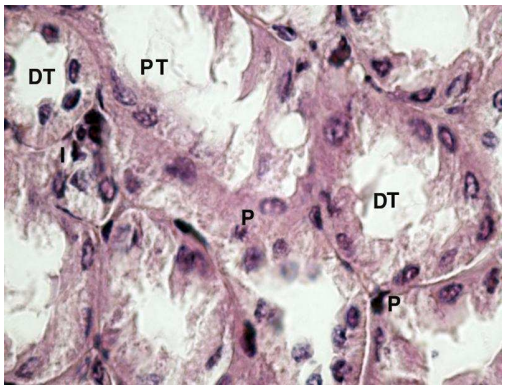


Fig. 5: Light photomicrography of Kidney (medullary part) of rat after eight weeks of exposure to MSG, showing a swelling in the lining epithelium of the proximal convoluted tubules (PT) and lining epithelium of distal (DT) convoluted tubules. Note that there were picknotic nuclei (P) and fragmented nuclei. H&E, x1000

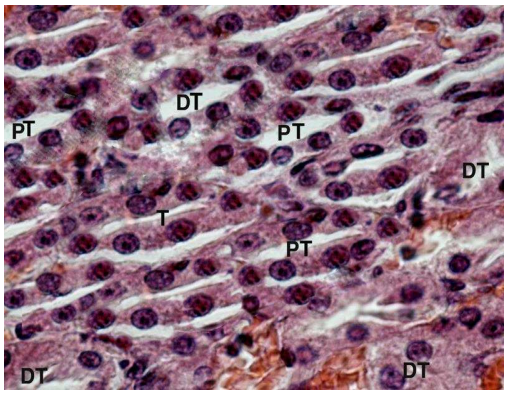


Fig. 7: Light photomicrography of Kidney (medullary part) of rat after eight weeks of exposure to MSG and propolis showing an decreased in congestion of epithelium of the cells of proximal (PT) and distal (DT) convoluted tubules are. H&E, x1000.

Group 3: Light microscopic examination in the kidney of rats which administered propolis and MSG showed decreased in the vasculature of the renal glomeruli, appearance of the glomerular capsular space (Fig, 6). Decrease the oedema of both the proximal and distal convoluted tubular epithelium (Fig. 7).

Histochemical observations

The light microscopic observations of rats of the control group revealed kidney tissues should positive PAS reaction in the cells cytoplasm, (Fig. 8) with PAS positive reaction in the brush borders of the proximal convoluted tubules, while the glomeruli were intensely positive to PAS reaction.

The PAS reaction of the kidney tissues of the rats administered MSG showed a marked reduction in PAS reaction in kidney tissues particularly in degenerative and necrotic areas. The reduction in PAS reaction was more intensive in the renal tubules and glomeruli (Fig. 9).

The PAS reaction of kidney tissues of the rats administered MSG in combination with propolis for eight weeks appeared to have a moderate increased in intensity of PAS positive reaction in the kidney tissues but not reach to the normal level (Fig. 10).

Transmission electron microscopic observations

The kidney of the rats of control group showed endothelial cells in the glomerular capillaries richly fenestrated with large pores which appeared to lack any trace of a closing diaphragm. The proximal renal tubular epithelium of control rat is characterized by a dense brush border, basal or central nucleus, apical endocytic vesicles, occasional lysosomes and elongate or round mitochondria. Cisternal profiles of rough ER located between the mitochondria (Fig.11).

The podocytes were observed to lie in the urinary space and they remained in contact with the basement membrane by extensions of their cytoplasm known as foot processes. The endothelial cells in the glomerlular capillaries were very richly fenestrated with large pores which appeared to lack any trace of a closing diaphragm (Fig. 12).

The MSG induced a toxic ultrastructural changed in the kidney of rats which exposed to MSG alone which observed as, enlargement of the glumeruli with narrowing of the capillary lumen, swelling of the capillary endothelium, and loss of the fenesrtae. damage of the renal tubules were noted as focal loss of the brush border of the epithelial lining of proximal renal tubules, disturbance of the nuclear membrane, chromatin condensation, swelling of several mitochondria with regression of their crestae. The protein synthesis rough endoplasmic reticulum was degenerated. Increased number of lysosomes and cell death were also noticed (Fig. 13).

There were displayed large number of cytoplasmic vacuoles and numerous. The brush border was markedly disorganized and frequently destroyed (Figs. 14, 15).

In group 3, co-administration of propolis practically prevented most of the ultrastructural pathological toxic effects of MSG on the kidney tissues. In the kidney, many of ultrastructural pathological changes caused by the toxic effect MSG on the kidney tissues were noticed to be decreased in the kidney tissues of rats received propolis in combination with $CdCl_2$ for eight weeks (Fig. 16).

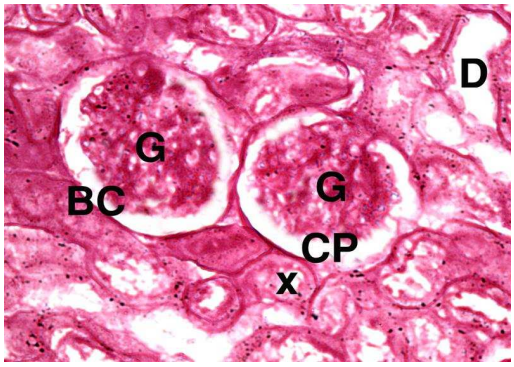


Fig. 8: Light photomicrography of Kidney (cortical part) of a control rat. The strong positive reaction of PAS in renal glomeruli (G), flat epithelium lining the glomerular capsule (BC) with distinct capsular space (CP), the proximal (X) and with distal (D) convoluted tubules can be seen. Periodic acid-Schiff's X 400.

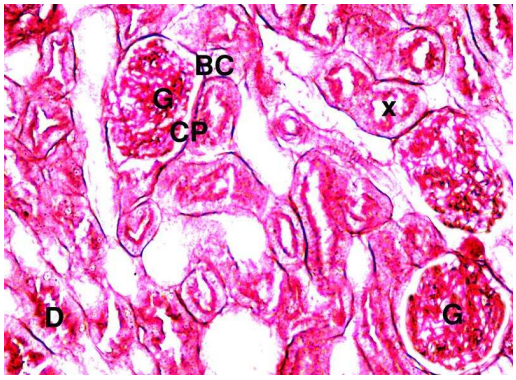


Fig. 9: Light photomicrography of Kidney of rat after eight weeks of exposure to MSG, showing an decreased in the PAS positive reaction of renal glomeruli (G), Bowman's capsule (BC), and proximal convoluted tubules (X) and distal convoluted tubules (D). Periodic acid-Schiff's X 400.

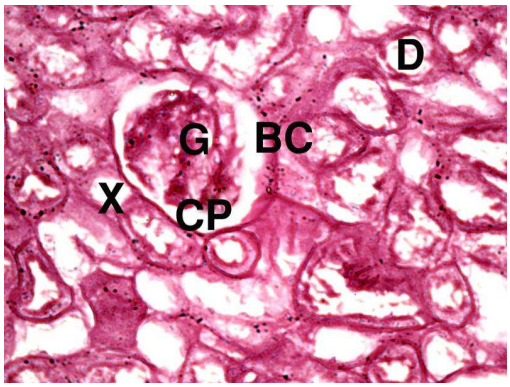


Fig. 10: Light photomicrography of Kidney of rat after eight weeks of exposure to MSG and propolis showed marked increase in PAS positive reaction in renal glomeruli (G),Bowman's capsule (BC), and both proximal (X) and distal convoluted tubules(D). Periodic acid-Schiff's X 400.

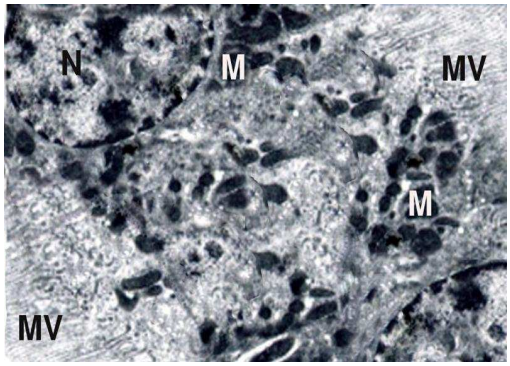


Fig. 11: Transmission electron microscopic picture of a section of kidney of control rat showing, the cells of the proximal convoluted tubule with euchromatic nuclei(N) and prominent nucleolus (N),many mitochondria (M) having normal crestae. The brush border of the cells has normal microvilli (Mv).TEM mag. =8000X

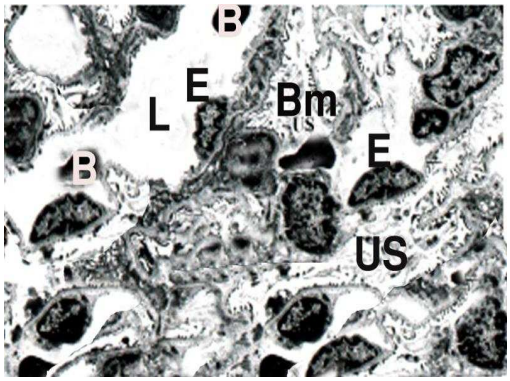


Fig. 12: Ultramicrograph of a section of kidney of rat Glomerular structure in the control group showing the urinary space (US), basement membrane (BM) with capillary lumen (L) lined by endothelial cells (E) and blood cell (B). TEM mag. =4000X

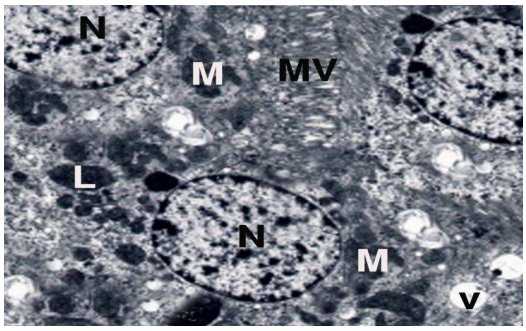


Fig. 13: Ultramicrograph of a section of kidney of rat exposed to MSG for 8 weeks, showing cell of proximal convoluted tubule with rounded heterochromatic nucleus (N), many swollen mitochondria (M), disorganized apical microvilli (MV). The protoplasm appeared to be contains numerous vacuoles (V) and many lysosomes (L). TEM mag. =6000X.

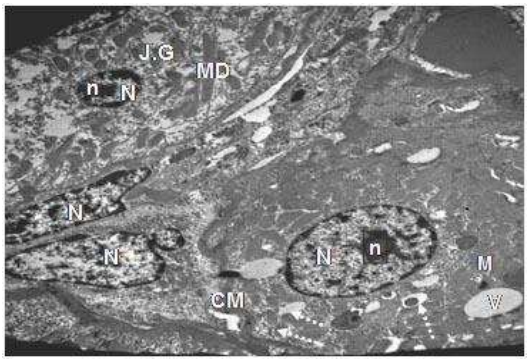


Fig. 14: Ultramicrograph of a section through joxtaglomerular complex of kidney of rat exposed to MSG for 8 weeks, showing (1) Macula densa (Md) with elongated nucleus (N), prominent nucleoli(n) and cytoplasm contains many mitochondria(M), vacuoles (V) and lysosomes (broken arrows). (2) Joxtaglomerular cell (J.G) has rounded heterochromatic nucleus (N), nucleolus (n), many mitochondria(M), and pale, vacuolated (V) protoplasm. The basal lamina (CM) of the seen cells is thick and enfolded. TEM mag. =6000X.

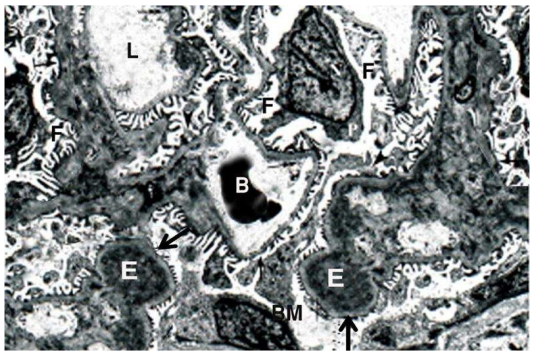


Fig. 15: Ultramicrograph of a section through glomerular structure of kidney of rat exposed to MSG for 8 weeks, showing arrow demonstrate loss of foot process disorganized foot process (F) with thickening of basement membrane (BM) filled with blood cell (B), swollen endothelial cells (E) widening capillary lumen (L). TEM mag. =6oooX.

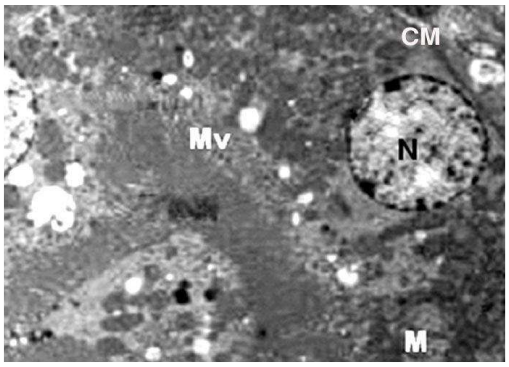


Fig. 16: Transmission electron microscopic picture of a section of kidney of rat exposed to MSG and propolis for 8 weeks displays distal convoluted tubules, with euchromatic nuclei (N) and nucleolus (n),many mitochondria (M)and dark nonvacuolated protoplasm. Intact microvilli (Mv) .and intact basement membrane(CM). TEM mag. =4000X.

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. Furthermore, there were many areas of tubular damages ranged from mild to severe in the kidney were observed in rats treated with MSG. There were also renal damages appeared as hypertrophy and degeneration of epithelia of renal tubules with distinct of mononuclear cells infiltration. A few renal tubules showed single epithelial cells desquamated to their lumen. Also some vascular glomeruli were apparently enlarged, tightly filling the Bowman's capsule with absence of the capsular spaces was observed. Moreover, hyperaemia, hydropic degeneration and necrosis of the kidney were observed. We marked a reduction in PAS reaction in kidney tissues particularly in degenerative and necrotic areas in the rats exposed to MSG alone. In addition, some Pathological changes in kidney ultrastructure; narrowing of the capillary lumen, swelling of the capillary endothelium of the glomeruli, injured brush-border microvilli and swollen mitochondria in the proximal convoluted tubular cells were observed. The effects of MSG on proximal cell ultrastructure were; focal loss of brush border, nuclear membrane damage, chromatin condensation, swelling of the mitochondria with regression of mitochondrial crestae, degranulation and disintegration of protein-synthesizing structures such as rough endoplasmic reticulum, increased number of lysosomes and ultimately cell death. These findings agreed what was published before [9-13].

Propolis has been demonstrated to play an important role in preventing the oxidative stress, apoptosis and necrosis induced by lead [33]. These results were in cooperative with the results of the present study in which there were marked reduction of the toxic effect on the kidney as mild hyperaemia in the kidney vessels, some degenerative changes in the tubular epithelium and cystic dilatation in rats exposed to propolis and MSG in the kidney tissues. There was moderate increased in intensity of PAS positive reaction in the kidney tissues of the rats exposed to MSG in combination with propolis but not reach to the normal level. The co-administration of propolis practically prevented most of the ultrastructural pathological toxic effects of MSG on the kidney tissues. These observations were mimicked what was reported before [21, 25, 26].

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