

## Modulating Effect of *Nigella sativa* on Renal Structural Changes by Monosodium Glutamate in Female Mice

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### ABSTRACT

There is considerable evidence, suggest that, consumption of food additives monosodium glutamate (MSG), places humans at risk and the greatest risk is faced by children. The present work aimed for explaining the risks of excessive use of recent food additives MSGasa flavor enhancer and to study the role of black seeds extract *Nigellia sativa*as a natural and safe product in modulating effect of renal structural changes in female mice. Eighty female Swiss albino mice *Musmusculus* (2 months old, 20-25 g) divided into 4 groups (20 females each). The first group was used as control one (given distilled water only without MSG and/ or NS). The second group, (given orally a dose of 8 mg MSG/kg of body weight in distilled water). The third group, (given orally a dose of 180 mg powder NS/kg of body weight suspended in distilled water )and the fourth group, (given orally a dose of 8 mg MSG and 180 mg powder NS/kg of body weight in distilled water). Mice were sacrificed at the end of 10 weeks to remove kidneys for histological and histochemical studies. Histological and histochemical investigations of kidney of MSG group showed several changes include dilation and congestion of some blood vessels, certain cells of convoluted tubules affected comprising cellular swelling, hydropic changes, necrosis, enlarged dilation of several convoluted tubules. Investigation also showed infiltration of large number of inflammatory lymphocytes and abnormality in the pattern of kidney tissues and distortion of some glomeruluithat appeared enlarged or lobulated and sometimes congested with red blood cells. Also increased intensity of collagen fibers, proteins and lipids with the general lack of glycogen granules was observed as compared with controls one. The result of this work showed the role of *Nigellia sativa* extract as a natural and safe product in modulating renal structural changes in female Swiss albino mice treated with MSG and explained the risks of excessive use of MSGas a flavor enhancer.

**Keywords:** Monosodium Glutamate, Black Seeds (*Nigellia sativa*), kidney and Mice.

### INTRODUCTION

Monosodium glutamate, also known as sodium glutamate or MSG, is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids (Ninomiya, 1998). It was classified by the U.S. Food and Drug Administration as generally recognized as safe and by the European Union as a food additive (JECFA, 1992). The glutamate of MSG confers the sameumami taste of glutamate from other foods, being chemically identical (Loliger, 2000). MSG has the HS code 29224220 and the E number E621 (Ikeda, 2002). Industrial food manufacturers market and use MSG as a flavor enhancer because it balances, blends and rounds the total perception of other tastes (Yamaguchi, 1991).

Although we have a mindset of what unhealthy food is, sometimes ignorance is pleasure, we constantly worry and monitor our fat and caloric content of what we eat; however, there may be more important aspects that we should be worried or concerned about. For example, MSG, is added to almost every fast food and take-out meal we eat. It is widely used in many commercial packed food (Maggi Noddles, Knorr Soup etc), restaurant and household cooking. The majority of people pay no attention to it simply because they are either unaware of its presence in food or are unsure of what MSG really is. MSG may have more harmful effects on the human body than simply being a food additive. Is it harmful even though it is approved by the Food and Drug Administration (FDA, 1995).

MSG has been used as a flavor enhancer for over a century. In 1908, Kikunae Ikeda, a Japanese scientist, extracted glutamic acid from the seaweed *Laminaria Japonica* and discovered its flavor-enhancing properties, thus was the birth of MSG. MSG is a naturally present excitatory neurotransmitter in brain, mediating fast synaptic transmission in one third of all CNS synapses. It is metabolized in liver. Kidney plays an important role in its elimination (Bhattacharya, *et al.*, 2011).

MSG has various harmful effects, which include triggering asthma attacks and exacerbating migraine headaches (Stevenson, 2000 and Allen *et al.* 1987). Studies have shown that oral ingestion of MSG can provoke asthma attacks in patients diagnosed with asthma, and bring about symptoms of the Chinese Restaurant Syndrome (CRS). The CRS is a collection of symptoms that include sweating, headache, flushing, and in more serious cases, swelling of the throat and chest pain (Kwok, 1968).

Not only is MSG found to induce asthma and migraine attacks, but is also linked to diseases such as obesity, Type 2 diabetes and Alzheimer's disease (Nagata *et al.* 2006 and Scher and Scher, 1992). Moreover, MSG has been shown to stimulate appetite in humans (Schiffman, 1998). The elderly are more susceptible to over stimulation of the brain caused by MSG and risks degeneration of nerve cells in the brain leading to Alzheimers (Schiffman, 2000).

In spite of the harmful effects of MSG, the FDA approves of MSG in our food products based on its "naturally occurring" ingredient. Because glutamate is also found in nature, MSG is a safe food additive. Many manufacturers rename the monosodium glutamate ingredient to neutral terms such as, malt extract, corn syrup, cornstarch, or hydrolyzed "anything". There were many unreliable reports of adverse reactions to MSG in the 1980s and 1990s, however research has produced mixed results. It is unclear how much of an effect MSG has on allergies and asthma, however reactions attributed to MSG include headache, flushing, sweating, nausea, numbness, tingling or burning in or around the mouth, weakness, rapid heart beat, chest pain and shortness of breath ( Williams and Woessner, 2009).

*Nigella sativa* (NS), or also known as black cumin has been used for centuries in medicinal and culinary purposes throughout the Middle East, India, and Northern Africa. It is an annual flowering plant with pale blue flowers that belongs to the Ranunculaceae family. The plant has a fruit which contains angular black seeds, and the seeds are considered to be the most valuable part contributing beneficial health effects. NS as a natural remedy has been documented to possess numerous therapeutic values, including diabetes, tumour, hypercholesterolemia, hypertension, inflammation, and gastrointestinal disorders (Tas,ar, *et al.*, 2012; Terzi, *et al.*, 2010; Kocyigit, *et al.*, 2009; Meddah *et al.*, 2009; Nagi and Almakki 2009; Ghannadi, *et al.*, 2005). Studies have revealed various therapeutic values of NS such as anticancer, antioxidant, antibacterial, antifungal, antiparasitic and antiasthmatic (Aljabre, *et al.*,

2005; Randhawa, *et al.*, 2005; Boskabady and Shirmohammadi, 2002; Badary and Gamal El-Din 2001; Burits and Bucar, 2000 and Morsi 2000). NS may also inhibit renal calculi and improve poultry quality (Islam, *et al.*, 2011 and Hadjzadeh, *et al.*, 2007). NS contains 36–38% fixed oils, proteins, alkaloids, saponin, and 0.4–2.5% essential oil (Ali and Blunden G. 2003) High-performance liquid chromatography (HPLC) analysis of NS essential oil revealed that the main active ingredients were thymoquinone, dithymoquinone, thymohydroquinone, and thymol (Ghosheh, *et al.*, 1999). Among the compounds identified, thymoquinone (TQ) is the most abundant, which makes up 30–48% of the total compounds. This quinone constituent is the most potent and pharmacologically active compound in NS. The present work aimed to study the role of black seed extract *Nigella sativa* as a natural and safe product in modulating the effect of renal structural changes in female mice treated with recent food additives namely (MSG) and for explaining the risks of excessive use of MSG as a flavor enhancer to the public without awareness.

## MATERIALS AND METHODS

### Monosodium glutamate (MSG):

The molecular formula for MSG is  $C_5H_8NNaO_4$  (Lölinger, 2000). MSG was obtained from the local market in Al-Hassa, Saudi Arabia. MSG was dissolved in distilled water and given orally by gavage to mice at a dose of 8 mg MSG/kg of body weight (Malik & Ahluwalia, 1994 and Kawatra & Ahluwalia, 2004).

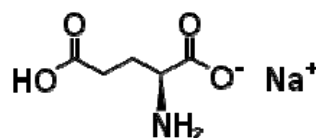


Fig. 1: Chemical formula of MSG

### *Nigella sativa* (NS)

NS is a herbal plant which belongs to Ranunculaceae family. It is also known as black cumin or habatussauda, and has a rich historical and religious background. The seeds of NS, which have a pungent bitter taste, are used in confectionery and liquors. The seed is the source of the active ingredients of this plant and has been used in Islamic medicine for its healing powers (Goreja, 2003).

The seed oil of NS was found to be rich in polyphenols and tocopherols (Meziti, *et al.*, 2012 and Al-Naqeeb, *et al.*, 2009). The seeds contain 36–38% fixed oils, 0.4–2.5% essential (volatile) oil, proteins, alkaloids, and saponins (Ali and Blunden, 2003). The fixed oil is composed mainly of fatty acids, namely, linoleic, oleic, palmitic and stearic acids (Nergizand and Otles, 1993). Thymoquinone (TQ) is the most pharmacologically active ingredient found abundantly in the black seeds, together with its derivatives such as dithymoquinone, thymohydroquinone, and thymol (Ghosheh, *et al.*, 1999). NS seeds were obtained from the local market in Al-Hassa, Saudi Arabia. Seeds were crushed by the mill mixer Braun and its extract was prepared as a suspension using distilled water and given orally by gavage to mice at a dose of 180 mg powder NS/kg of body weight (Al-Jishi & Abuo Hozafa, 2003).

### Animals:

Eighty female Swiss albino mice *Mus musculus* (2 months old, 20–25 g) were obtained from an inbred strain in the College of Veterinary Medicine, King Faisal University, Al-Hassa, Saudi Arabia. Mice were housed separately in stainless steel

cages containing hard wood chips, five animals /cage. Mice were housed at room temperature (20-22C°). Animals in all groups were given a basal diet composed of 60 % of ground corn meal, 15% ground beans, 10% wheat bran, 10% corn oil, 3% casein, 1% mineral mixture, and 1% vitamin mixture (Nelson and Halberg,1986). Water was given *ad libitum*.

### **Experimental Design:**

Animals were divided into 4 groups (20 females each).The first group was used as control one, given distilled water orally by gavage without MSG and/ or NS (daily for 10 weeks). The second Group, MSG group, given orally by gavage a dose of 8 mg MSG/kg of body weight dissolved in distilled water (daily for 10 weeks). The third group 3, NS group (given orally by gavage a dose of 180 mg powder NS/kg of body weight suspended in distilled water (daily for 10 weeks). The fourth group , MSG and NS group, given orally by gavage a dose of 8 mg MSG and 180 mg powder NS/kg of body weight suspended in distilled water(daily for 10 weeks).

Mice were sacrificed at the end of 10 weeks to remove kidneys for histological and histochemical studies. Kidneys were fixed rapidly in fixatives followed by processing in the routine technique of paraffin embedding and blocking. Paraffin sections of 5 µm thick were prepared by microtomy and then routinely stained with Eherlich'shematoxylin and counterstained with eosin for studying general structures (Smith & Bruton, 1978), Masson's Trichrome Stain for Collagen fibers (Bancroft & Stevens, 1990), The periodic acid-Schiff Sain (PAS) for glycogen (Pearse, 1985), Mercuric Bromophenol Blue stain for total Protein (Pearse, 1985), and Sudan Black B stain for general lipid (Pfüller and Franz, 1977), and examined on light microscope for the histological studies.

## **RESULTS**

Histological examination of kidney control mice stained with hematoxylin and eosin staines (H&E) is normal as showing in (Fig. 2), while kidney sections of MSG group mice showing different abnormalities (Figs. 3-8). The abnormalities of kidney sections are summarized as congestionof some blood vessels (Figs.3,4&6), lymphocytes infiltrations (Fig. 5), dilations in the proximal and distal convoluted tubules (Fig. 7), abnormality in the pattern of kidney tissues and distortion of some glomeruloi structures which appeared enlarged or lobulated and sometimes congested with red blood cells (Fig. 8). Also examination of kidney sections of control NS extract group stained with H&E as shown in (Fig. 9), revealed the same appearance of control group. Whereas histological examination of kidneys of MSG treated with NS extract showed remarked improvement in their structure and appeared more or less as control groups (Fig. 10).

Histological examination of kidney control mice stained with Masson's Trichrome Stain (MT) technique showed the presence of normal thin layer of collagen fibers of (MT) positive materials in the parietal and visceral walls of the Bowman's capsule, capillaries of the glomeruli, the basement membrane of the proximal and distal convoluted tubules (Fig. 11). While examination of kidney sections of MSG group mice showedincreased intensity of collagen fibers represented as increase in the MT positive material in the mesangial cell, matrix of the glomeruli and the basement membranes of the proximal and distal convoluted tubules appear thicker (Figs. 12-17). Examination of the kidney of control NS extract group showed the presence of normal collagen fibers of MT positive materials that appear more or less as control (Fig. 18).Whereas examination of kidney sections of MSG treated with NS extract showed

remarked decrease in the collagen fibers and appeared more or less as control groups (Fig.19).

The histochemical examination of kidney sections of control group mice stained with Periodic acid Schiff's Stain (PAS) technique showed the presence of polysaccharides in the form of PAS positive materials in the parietal and visceral walls of the Bowman's capsule, capillaries of the glomeruli, basement membrane of the proximal and distal convoluted tubules and the brush border of the proximal convoluted tubules (Figs. 20 and 21). Light microscopy of the kidney sections of MSG group mice showed a decrease in the PAS positive material in the mesangial cell and matrix of the glomeruli (Figs. 22 and 23). Also histochemical examination of kidney sections of control NS extract group stained with PAS (Figs. 24 and 25) revealed the same appearance of control group and showed strong PAS positive materials especially inside glomeruli. Examination of kidneys of MSG group mice treated with NS extract indicated that the polysaccharides of kidneys appeared more or less as control (Figs. 26 and 27).

The histochemical examination of kidney sections of control group mice stained with Mercuric Bromophenol Blue Stain (MBB) showed the presence of moderate content of protein in the form of MBB positive materials all kidney tissue (Fig. 28). Light microscopy of the kidney sections of MSG group mice showed an increase in protein contents in the form of strong MBB positive material in all contents of tissue as compared with the control one (Fig. 29). Also histochemical examination of kidney sections of control NS extract group (Fig. 30) revealed the same appearance of control group (moderate content of protein). Examination of kidneys of MSG group mice treated with NS extract indicated that the protein content of kidney tissues appeared more or less as control (Fig. 31).

The histochemical examination of kidney sections of control group mice stained with Sudan Black B Stain (SBB) showed the presence of moderate content of lipids in the form of SBB positive materials (Fig. 32). Light microscopy of the kidney sections of MSG group mice showed an increase in lipid contents in the form of strong SBB positive material in all contents of kidney tissue as compared with the control one (Fig. 33). Also histochemical examination of kidney sections of control NS extract group (Fig. 30) revealed the moderate contents of lipid as the same appearance of control group. Examination of kidneys of MSG group mice treated with NS extract indicated that the lipid content of kidney tissues appeared more or less as control (Fig. 35).

## DISCUSSION

Our results showed that oral administration of NS extract for 10 weeks ameliorates the renal structural changes in mice treated with MSG. NS extract has been shown to improve renal structural changes (Figs. 10,19,26,27,31 &35). The NS extract acts as an essential trigger for kidney to revert to their normal metabolic homeostasis i.e., NS extract possesses anti-renal protective effect for oral administration of MSG.

Earlier studies, which have also been confirmed on more recent reports on the adverse effects of MSG laid emphasis on its effects on the hypothalamus-pituitary axis of the brain, leading to its neuro-excitatory/neuroendocrine effects and induction of obesity (Feldman and Weidenfeld, 2005; Hee *et al.*, 2010). More recent studies have examined other metabolic and toxic effects of MSG, with a number of the reports showing that the induction of oxidative stress in different tissues of

experimental animals after administration of chronic doses of MSG (Onyema, *et al.*, 2006; Farombi and Onyema, 2006; Diniz *et al.*, 2004; Singh *et al.*, 2003). MSG in foods acts through our taste buds on the tongue giving us the “umami” taste sensation, which means delicious in Japanese. This “umami” taste is termed the fifth taste sense of our basic tastes, and is described as meaty, brothy, and savory. From the tongue, this signal is relayed up to the cerebral cortex in the brain telling us that what we’re eating is delicious. Ingested glutamate is absorbed through the intestines, where it is transaminated and subsequently, metabolized by the liver leading to the release of glucose, glutamine, lactate, and other amino acids into the blood circulation. Glutamate is not considered to be an essential amino acid since we are able to produce it ourselves, but constant excess of glutamate from oral ingestion could lead to other problems. In MSG group mice, several immunomodulatory factors and inflammatory responses can contribute kidney injury. Recently, several reports have at least partially elucidated the cellular and molecular mechanisms underlying this inflammatory response (Cohn and Roth, 1996).

Histological examination of kidney tissues of MSG group showed several changes represented in congestion in several structures of kidney tissues in between renal tubules and glomeruli (Figs.3,4 &7), lymphocytes infiltrations (Fig. 5), renal cell necrosis, dilation in renal tubules (Figs. 7&8), distortion of glomeruli (Figs 12 &13), hypertrophy of Renal artery wall (Figs.16&17). Same observations were seen in the study of (Ortiz *et al.*, 2006 and Shimizu, *et al.*, 1971). Also (Inuwa, *et al.*, 2011) showed that MSG has hepatotoxicity and nephrotoxicity tendencies especially when consumed at higher concentrations. Alterations in the integrity of cellular and subcellular membranes have been proposed to play critical roles in the pathogenesis of cellular injury in various tissues, including the kidney as shown previously (Trump *et al.*, 1976; Jennings and Reimer, 1981; Farber *et al.*, 1981 and Farber, 1982). Humes and Weinberg, 1982; Humes and Weinberg, 1983 explained the resulting loss of the major intracellular cations,  $K^+$  and  $Mg^{2+}$ , and intracellular overload with the major extracellular cations,  $Na^+$  and, particularly,  $Ca^{2+}$ , have the potential to contribute to the disruption of multiple intracellular processes and, thereby, to the pathogenesis of irreversible cell injury. The infiltration of inflammatory lymphocytes and macrophages observed in the renal tissues result from MSG parallel with the opinion of Curran, (1996). They reported that the macrophages destroyed the causes of damage and injured tissues, while lymphocytes produce antitoxins and accelerate cell healing. Ariens *et al.*, (1979) explained that toxicity effects in histological preparations appeared as cellular degeneration, fat deposition and cellular necrosis.

NS decreased collagen fibers in kidney tissues of MSG and NS group mice (Figs. 19) as compared with the MSG group mice (Fig. 13). Collagen synthesis, in particular, is critical to the development of strength in a healing wound site. A healing progress, the number of proliferating fibroblasts and new vessels decreases; however, the fibroblasts progressively assume a more synthetic phenotype, and hence there is increased deposition of extra cellular matrix (ECM). Collagen synthesis by fibroblasts begins early in a healing wound. Many of the same growth factors that regulate fibroblasts proliferation also participate in stimulating ECM synthesis. Collagen synthesis, for example, is induced by a number of molecules, including growth factors and secreted by leukocytes and fibroblasts. Net collagen accumulation, however, depends not only on increased synthesis but also on diminished collagen degradation. Ultimately, the granulation tissue scaffolding evolves into a scar composed of largely inactive, spindle-shaped fibroblasts, dense collagen, fragments of elastic tissues, and other ECM components (Vinay, *et al.*, 1997). Prockop and Kivirikko (1995) explained

fibrosis or fibroplasias in two steps: (1) emigration and proliferation of fibroblasts in the site of injury, and (2) deposition of ECM by these cells. The recruitment and stimulation of fibroblasts is driven by the various growth factors include activated endothelium, but perhaps more importantly, they also include a variety of inflammatory cells. Macrophages, for example, are important cellular constituents of granulation tissue, responsible for clearing extracellular debris, fibrin, and other foreign matter at the site of injury and therefore promote fibroblast migration and proliferation. If the appropriate chemotactic stimuli are present, lymphocytes may also be present, and mast cells are increased in number; each of these can contribute directly or indirectly to fibroblast proliferation and activation.

Our results indicated that NS extract prevented the alteration in kidney pathology result from MSG which nearly return to their normal texture (Figs.10, 19, 26, 27, 31 and 35). This result agreement with previous studies Yildiz, *et al.*, (2010). They reported that, the protective effect of *Nigella sativa* against renal injury in rat kidneys. Several studies have concluded that flavonoids-especially quercetin and kaempferol- (the effective constituents of NS) have anti-inflammatory and antioxidant effects (Comalada, *et al.* 2006 and Nair, *et al.* 2006).

Histochemical examination of kidneys of MSG group mice showed glycogen depletion (Figs. 22&23) with PAS reagent as compared to control group (Figs. 20&21), increase protein content in renal tissues (Fig. 29) with MBB stain and higher content of lipids with SBB stain as compared with control group. Examination of kidney's tissues of MSG group mice revealed that strong reactions with MBB and SBB stains due to higher protein (Fig. 29) and lipid contents (Fig. 33) of kidney tissues as compared to control groups (Figs. 28 and Fig. 32 respectively) which showed moderate reactions of the two stains.

Glycogen depletion and elevation of protein and lipid contents of renal tissues with MSG treated group agreed with the previous study of (Ahluwalia *et al.*, 1996; Chudhary *et al.*, 1996 and Malik & Ahluwalia, 1994), they concluded that MSG may affect carbohydrate metabolism leading to increase lipogenesis. Ahluwalia *et al.*, 1996 reported that subcutaneous administered of MSG to adult male mice significantly increased blood glucose, whereas liver glycogen and blood lactate decreased. They concluded that, MSG shifted carbohydrate metabolism towards lipogenesis and hence leads to hyperlipidemia. Chudhary *et al.*, (1996) indicated that chronic administration of MSG induced oxidative stress, and altered glucose metabolic processes in the renal tissues of rats. Thus oxidative stress and accumulation of free radicals seems to be responsible for MSG toxicity (Farombi and Onyema, 2006; Diniz *et al.*, 2005; Onyema *et al.*, 2006). They explained that, glutamate toxicity involves an imbalance in the homeostasis of cysteine, the precursor of glutathione (GSH) reduced, leading to depletion of intracellular GSH levels and reduced ability to protect against oxidative injury in the cell and, ultimately cell damage. Moreover, lipid peroxidation may eliminate the active sulfhydryl group of GSH and other enzymes. The conclusions of the present study are summarized as follows: 1) one or more constituents of NS may be responsible for modulating renal structural changes result from oral administration of MSG in mice. 2) NS extract suppressing the stress caused by MSG and converting kidney pathology to normal pattern. These findings revealed that NS may have a potential benefit in the treatment renal cytotoxic effect result from food additives MSG and plays a role in reducing the risk of MSG.

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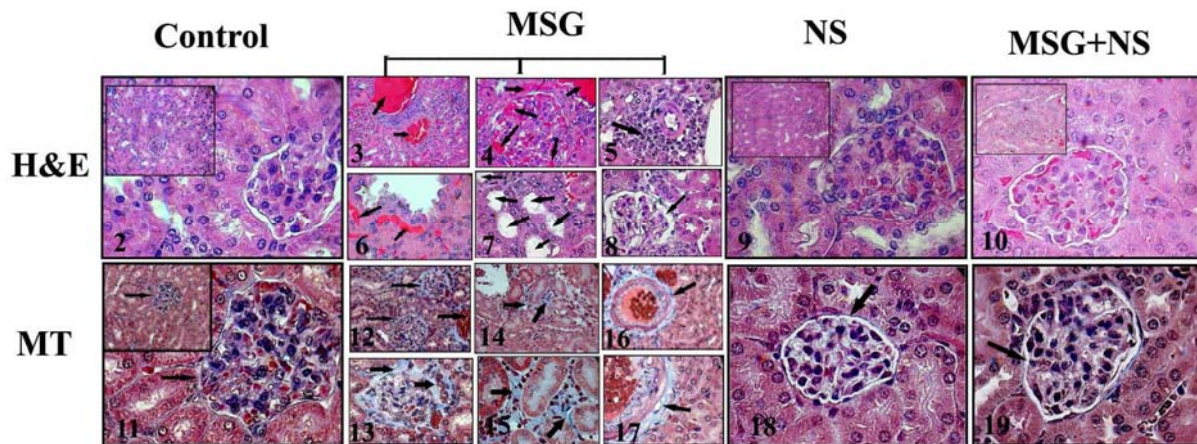
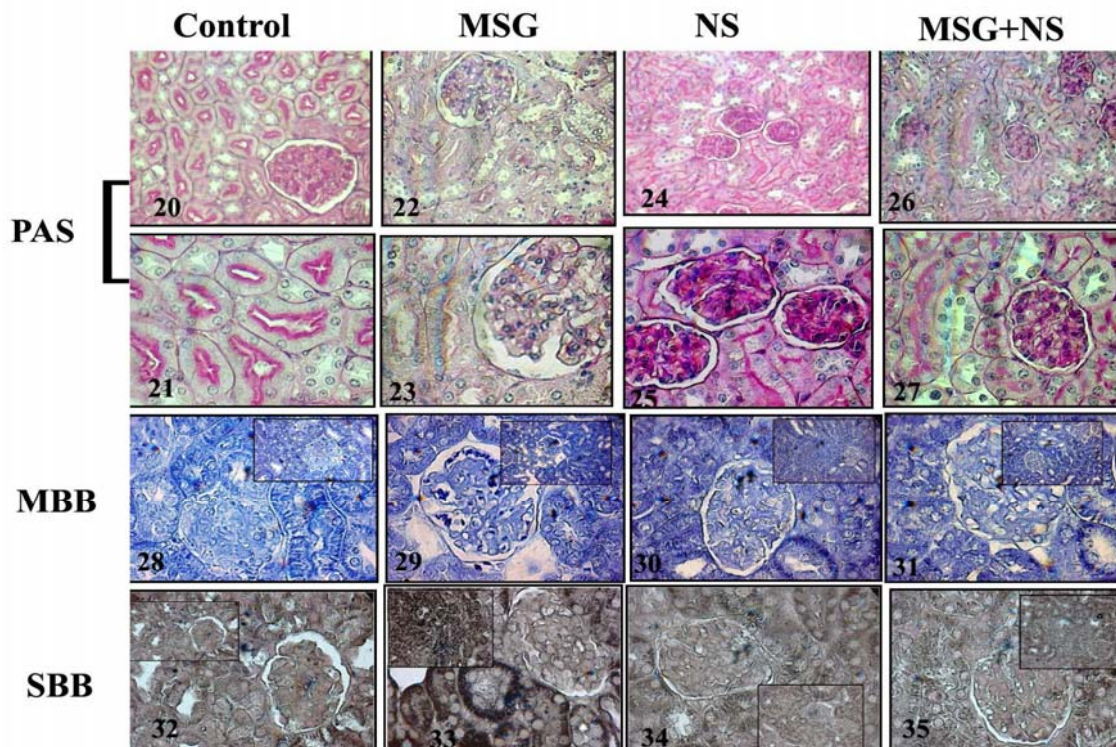


Figure 1: Histological sections of kidney tissue stained with H&E and MT. Control mice showed normal kidney structures, (Figs. 3-8 and 13-17): kidney of MSG group mice different abnormalities, as congestion (Figs. 3, 4 & 6), lymphocyte infiltrations (Fig. 5), dilations in the proximal and distal convoluted tubules (Fig. 7) and distortion of glomeruli structures (Fig. 8) increased collagen fibers (Figs. 12-17). (Figs. 9 and 18): Kidney of control mice treated with NS extract appeared as control groups and (Figs. 10 and 19): kidneys of the MSG group mice that treated with NS extract show the hepatic structure that appear more or less as control. (all figures are 150 X except figures 2, 9, 10, 11, 18 & 19 are 400 X).



b) Photographs of a section of the kidney show the histochemical changes via stains PAS, MBB and SBB, (Figs 20 and 21): Kidney of control mice showed the presence of polysaccharides in the form of PAS positive materials, protein and lipids, (Figs. 22, 23, 29 and 33) kidney of MSG group mice show decrease in the PAS positive material, decrease in protein and increase of lipids (Figs. 24, 25, 30 and 34) Kidney of control mice treated with NS extract showed the presence of normal polysaccharides, normal protein and lipids that appear as control group and (Figs. 26, 27, 31 and 35): kidneys of the MSG group mice that treated with NS extract show the polysaccharides, protein and lipid that appear more or less as control. (Figures 20, 22, 24 & 26 are 150 X whereas figures 21-35 are 400 X).

## ARABIC SUMMARY

## تأثير بذور نيجلياساتيفافي تعديل الخلل التركيبي لكلى إناث الفئران البيضاء الصغيرة المعاملة بأحادي جلوتامات الصوديوم

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يهدف هذا البحث إلى دراسة تأثير بذور الحبة السوداء على كلى إناث الفئران البيضاء الصغيرة المعاملة بأحادي جلوتامات الصوديوم وذلك لمعرفة جدوى استخدام مسحوق الحبة السوداء لتلافي الآثار الناتجة عن الاستخدام المفرط لأحادي جلوتامات الصوديوم والذي يستخدم في الأغذية المختلفة دون وعي. أستخدم في هذه البحث عدد ثمانين من إناث الفئران البيضاء الصغيرة البالغة من نوع *Mus musculus* تتراوح أوزانها بين (٢٠-٢٥ جرام). حيث قسمت إلى أربع مجموعات عشرين فأر/ لكل مجموعته كالتالي : المجموعة الأولى/ الضابطة: حيث تم توفير لها الظروف الطبيعية والبيئية من حرارة ورطوبة و غذاء وأعطيت فقط ماء مقطر فقط بنفس حجم الجرعة المعطاة في المجاميع التالية. المجموعة الثانية/مجموعة أحادي جلوتامات الصوديوم: أعطيت أحادي جلوتامات الصوديوم عن طريق الفم مذابة في ماء مقطر (بجرعة ٨ مجم/كجم من وزن الجسم). المجموعة الثالثة/ مجموعة مسحوق الحبة السوداء: حيث أعطيت الحيوانات عن طريق الفم مسحوق بذور الحبة السوداء في ماء مقطر (بجرعة ١٨٠ مجم/كجم من وزن الجسم) المجموعة الرابعة / مجموعة أحادي جلوتامات الصوديوم مع مسحوق الحبة السوداء: حيث أعطيت الحيوانات عن طريق الفم أحادي جلوتامات الصوديوم مع مسحوق الحبة السوداء. وأظهرت نتائج الفحص بالمجهر الضوئي: في المجموعة الضابطة والمجموعة المعاملة بمسحوق الحبة السوداء فقد ظهر الفحص المجهرى لنسيج الكلية في إناث الفئران مماثل للتركيب النسيجي الطبيعي للكلى وذلك من حيث ترتيب الخلايا وانتظامها. أما في المجموعة المعاملة بأحادي جلوتامات الصوديوم فقد أظهر الفحص المجهرى تغيرات عديدة في القطاعات النسيجية لكلى إناث الفئران البيضاء والمعاملة بأحادي جلوتامات الصوديوم شملت اتساع واحتقان لأفرع الأوعية الدموية، تأثر بعض خلايا الأنبيبات الملتفة. كما بين الفحص المجهرى ارتشاح عدد كبير من الخلايا اللمفاوية الالتهابية ، وظهور تغير في النمط التركيبي الطبيعي وتشوه لبعض كريات ملبجي حيث ظهر بعضها متضخماً والبعض الآخر مفصصاً وظهر بعضها محتقناً بكريات الدم الحمراء. كما لوحظت زيادة كثافة الألياف الكولاجينية والبروتينات والدهون العامة مع قلة حبيبات الجليكوجين في هذه المجموعة. في المجموعة المعاملة بأحادي جلوتامات الصوديوم مع مسحوق الحبة السوداء فقد أظهر الفحص النسيجي تحسن ملحوظ في نسيج الكلية حيث ظهر تحسناً من حيث شكل وتركيب كرية ملبجي وانتظام الخلايا الطلائية المبطنة للأنبيبات الملتفة القريبة والبعيدة مع زيادة حبيبات الجليكوجين وقلة الألياف الكولاجينية أما البروتينات والدهون العامة فقد كان محتواهما متوسطاً مشابهاً إلى حد ما انسجة الكلى فى المجموعة الضابطة. وتشير نتائج هذه الدراسة إلى أن الحبة السوداء قد أدت إلى تعديل الخلل التركيبي فى نسيج الكلية الناتج عن أحادي جلوتامات الصوديوم .