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Health Risks of Some Meat Additives on Male Rats

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Abstract: Natural and synthetic meat additives approved by Food and Drug Administration "FDA" are commonly used to maintain or improve safety, nutrient value, taste and texture. Although many of the 3,000 these additives enhance meat supply, others are the subject of fierce controversy. The present study investigated the hazardous effects of sodium nitrite and monosodium glutamate "MSG" used in manufacturing of meat on some hematological, serological parameters and histopathological changes in male albino rats. Manually processed meat supplemented with doses of sodium nitrite and Monosodium glutamate "MSG", below, within and over the permissible limits, was fed to male rats for 30 days. All the used doses sodium nitrite and MSG, below, within and over the permissible limits, induced significant decrease in hemoglobin content and some hematological parameters, significant increase in serum levels of aspartate aminotransferase "AST" and alanine aminotransferase "ALT", urea and creatinine as well as various pathological changes in liver, kidney, brain and tests. The severity of these changes increased from mild to severe by increasing the concentrations of both sodium nitrite and MSG. In conclusion, the obtained data indicated that prolonged use of sodium nitrite and MSG, even within the permissible limits according to EOS (2005), was unsafe for consumer because they cause anemia, liver and kidney dysfunctions, damage to brain cells in association with deleterious effect on male fertility. Consequently, it is not recommended to use these meat additives in meat even within the permissible limits.

Key words: Sodium nitrite · Monosodium glutamate (MSG) · Hematological changes · Liver and kidney functions · Histopathological changes.

INTRODUCTION

Meat preservation has become an increasingly important practice in modern food technology with the increased production of processed and convenient food [1]. Natural and synthetic food additives approved by Food and Drug Administration "FDA" are commonly used to maintain or improve safety, the nutrient value and the taste and texture of food [2]. Although many of the 3,000 these additives enhance our food supply, others are the subject of fierce controversy. The discovery that children at the age of nursery consume food containing great amounts of additives prompted the scientific community to oversee this issue.

Nitrate and nitrite have been used as food additives in many types of meat products for many years primarily to fix color, contribute to the flavor of the final product, help in the inhibition of growth and toxin production of Clostridium botulinum which causes botulism [3], effectively control rancidity by inhibiting lipid oxidation leading to excellent storage stability [4] and improves the microbiological safety of these foods and extends their safe shelf-life [5]. This offers significant benefits to consumers in terms of the availability of a variety of different foods that are safe, convenient and cost effective.

The toxic effects of nitrates and nitrites are well documented in mammalians, including impairment of reproductive function [6], hepatotoxicity and methaemogobenemia [7]. Instance, highly carcinogenic N- nitroso-compounds are produced when nitrite reacts with secondary amines and N-alkyl amides under acidic conditions in vitro [8]. An alternative to sodium nitrite for production of cured meats has not been identified despite significant research effort [9].

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Monosodium glutamate "MSG" is the sodium salt of naturally occurring non-essential amino acid L-form of glutamic acid [10]. It is one of the main popular flavor enhancers used as a food additive in various food products in modern time [11]. It is widely used in many commercial packed food, restaurant and household cooking. The unique flavor and taste of this compound has been categorized and established as a separate taste sensation UMAMI taste [12] which is recognized as the fifth basic taste, very similar to "meat aroma" or "broth aroma" so enhances food palatability and encourages flavor acceptance [13]. MSG can be added "pure" or as a "hidden ingredient" of yeast extracts or hydrolyzed proteins, both containing high percentages of glutamic acid [14]. It is a component of many proteins and peptides. When bound to proteins, glutamate is tasteless. The sweet umami taste and flavor becomes perceptible only when free glutamate dissociates from proteins during the processes of fermentation, ripening and cooking [15]. It increases the perception of sweetness and saltiness and diminishes the sourness and bitterness of food. MSG serves as an energy source for certain tissues and as a substrate for glutathione syntheses [16]. MSG is a naturally present excitatory neurotransmitter in brain, mediating fast synaptic transmission in one third of all CNS synapses.

A report from the Federation of American Societies for Experimental Biology "FASEB" (1995) identified two groups of people susceptible to high MSG doses: a group intolerant to high quantities of MSG and asthmatics.

There is no complete agreement about the safety of MSG, even though FDA includes it among the substances generally recognized as safe (GRAS) [17, 16]. Despite its taste stimulation and improved appetite enhancement, reports indicated that MSG is toxic to human and experimental animals [18] and develops severe abnormalities as short stature, small endocrine organs (pituitary gland, adrenal gland, thyroid gland, ovaries, testes and pancreas), high risk of seizures and impaired learning [19]. On the contrary, several government institutions and international organizations as JECFA, FAO and WHO, have declared that MSG is safe for human consumption [20].

Although, different pathological changes in liver following MSG were reported previously by several authors [21,22], still not many literatures have described detailed histological features as effect of MSG in liver tissue. On the other hand, there was shortage in the literatures that had been recorded the pathological changes occurring in various organs such as kidney, brain and testes following MSG administration [21].

Therefore, the aim of this study was to determine the alterations in hematological, serological and pathological parameters of liver and kidneys in combination with pathological changes in brain and testes of male rats that had been fed on manually processed meat supplemented with different doses of sodium nitrite and MSG, including the permissible limits recommended by EOS [23] for 30-successive days.

MATERIAL AND METHODS

Experimental Animals: The present investigation was carried out on a total number of 35 apparently healthy adult male white albino rats (one month age and 130-150 g body weight). These animals were housed for two weeks at constant environmental and nutritional conditions similar to those under which the experiment was performed for accommodation. Rats were housed in separate cages a way from any stressful stimuli and supplied with diet and water ad libtium.

Meat Additives:

Sodium Nitrite: Food grade sodium nitrite was incorporated in fresh minced beef at concentrations of 100 ppm, 125 ppm (permissible limit according to EOS [23] and 150 ppm.

Monosodium Glutamate "MSG": Food grade MSG was incorporated in fresh minced beef at concentrations of 3 ppm, 5 ppm [24]and 7 ppm.

Experimental Design: The male rats were divided randomly into 7 main groups (n=5) feeding on manually processed meat supplemented with different doses of nitrite and MSG as follows:

Group	Dose
C	Control group received manually processed kofta without any treatment.
N1	Group fed on meat supplemented with 100 ppm sodium nitrite for 30 days.
N2	Group fed on meat supplemented with 125 ppm sodium nitrite for 30 days.
N3	Group fed on meat supplemented with 150 ppm sodium nitrite for 30 days.
MSG1	Group fed on meat supplemented with MSG 3 mg/Kg for 30 days.
MSG2	Group fed on meat supplemented with MSG 5 mg/Kg for 30 days.
MSG3	Group fed on meat supplemented with MSG 7 mg/Kg for 30 days.

At the end of the experimental period, overnight fasted animals were sacrificed by cervical dislocation and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at 860 g for 20min and then quickly frozen at -20°C for biochemical analysis. Moreover, Specimens for histopathological examination were taken from different organs including liver, kidney, brain and testes. Tests were performed in triplicate.

Hemogram Parameters: Complete blood picture was performed using HA-Vet Automatic Hematology analyzer (CLINDIAG SYSTEM).

Chemical Analysis of Serum:

Aspartate Aminotransferase "AST" and Alanine Aminotransferase "ALT": were determined spectrophotometerically according to Schumann and Klauke [25].

Urea: Was determined spectrophotometerically according to Patton and Crouch [26].

Creatinin: Was determined spectrophotometerically according to Henry *et al*. [27].

Histopathological Examination: Five rats were sacrificed successfully from each group after 30 days post feeding of diet supplemented with different concentrations of nitrite and MSG. Specimens for histopathological examination were taken from different organs including liver, kidney, brain and testes. These samples were fixed in 10% buffered neutral formalin solution. Then after proper fixation, the samples were dehydrated in ascending grades of ethyl alcohol, then cleared in xylol, embedded in paraffin and finely blocking occurred. These samples were sectioned at 5µm in thickness and stained with hematoxylin and eosin (H & E) for microscopical examination [28]. According to the severity of the microscopical lesions, all specimens were examined for histological alterations and classified on a modified semi-quantitative scale of Bagheri *et al*. [29] into none (-), mild (+), moderate (++) and severe (+++). Each lesion was graded as follow: (-) normal histology, (+) with up 1/3 of the examined section exhibiting the evaluated lesion, (++) in which 1/3 to 2/3 of the inspected section revealed the estimated pathological change, while (+++) means greater than 2/3 of the assessed section showed the estimated pathological alteration.

Statistical Analysis: The data was statistically treated by one-way ANOVA using SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL and USA) and Duncan's post hoc test with $P < 0.05$ considered to be statistically significant. The results were expressed as means + SD.

RESULTS

Hematological Examination: With respect to the hematological changes of sodium nitrite and MSG treated rats, there were significant decrease in RBCs count, HGB, WBCs, MCV, MCH, MCHC, lymphocytes and monocytes in treated rats with the three dose levels as compared to those of control animals (Tables 1 and 3) revealing anemia in all sodium nitrite treated groups during all the experimental duration.

Serological Examination: Serum biochemical constituents revealed that sodium nitrite and MSG treated groups showed significant increase in the levels of AST, ALT, urea and creatinin levels (Tables 2 and4).

Table 1: The effect of different doses of sodium nitrite on some hematological changes in male rats

	Lymphocytes		Monocytes							
Groups	No.	%*	No.	%*	HGB*	RBCs*	WBCs	MCV*	MCH	MCHC
Control	0.96±0.44^{a}	9.73±0.31^{a}	0.27±0.14^{a}	2.50±0.14^{a}	30.86±1.60^{a}	8.27±0.43^{a}	11.32±5.72^{a}	59.6±2.88^{a}	39.20±1.43^{a}	68.18±3.15^{a}
N1	0.83±0.07^{a}	9.07±0.43^{ab}	0.22±0.02^{a}	2.48±.13^{a}	29.12±1.47^{a}	7.76±0.24^{ab}	8.94±0.80^{a}	59.4±2.88^{a}	39.10±1.94^{a}	67.88±1.42^{a}
N2	0.81±0.06^{a}	8.96±0.24^{b}	0.21±0.02^{a}	2.42±0.15^{a}	26.96±1.22^{ab}	6.90±0.44^{bc}	8.90±0.90^{a}	55.0±1.87^{b}	37.59±0.78^{a}	65.18±1.82^{a}
N3	0.75±0.07^{a}	8.65±0.33^{b}	0.21±0.01^{a}	2.41±0.12^{a}	24.62±5.03^{b}	6.35±1.42^{c}	8.34±0.68^{a}	54.4±4.10^{b}	37.40±1.26^{a}	65.12±3.86^{a}

Table 2: The effect of different doses of sodium nitrite on some liver and kidney function tests in male rats

Groups	AST	ALT	Urea	Creatinine
Control	310.10±70.74^{a}	58.32±3.61^{b}	39.44±5.77^{b}	0.44±0.05^{a}
N1	354.68±59.48^{a}	70.52±19.67^{ab}	49.58±12.48^{ab}	0.46±0.05^{a}
N2	365.26±27.03^{a}	71.60±14.22^{ab}	56.42±10.50^{a}	0.50±0.00^{a}
N3	373.30±37.16^{a}	89.42±17.63^{a}	60.92±5.78^{a}	0.52±0.11^{a}

Table 3: The effect of different doses of monosodium glutamate (MSG) on some hematological changes in male rats

	Lymphocytes		Monocytes							
Groups	No.*	%*	No.*	%	HGB	RBCs*	WBCs*	MCV*	MCH*	MCHC*
Control	9.73±0.31a	0.96±0.44[a]	2.50±0.14[a]	0.27±0.14[a]	30.86±1.60[a]	8.27±0.43[a]	11.32±5.72[a]	59.6±2.88[a]	39.20±1.43[b]	68.18±3.15[a]
N1	9.35±0.61a	0.79±0.05[a]	2.48±0.13[a]	0.21±0.01[a]	30.06±0.10[a]	8.02±.40[a]	8.90±0.90[a]	57.80±3.70[b]	38.57±1.23[a]	67.52±1.74[a]
N2	9.16±0.40b	0.76±009[a]	2.46±0.19[a]	0.20±0.02[a]	29.16±3.36[a]	7.58±0.82[b]	7.82±1.11[ab]	55.20±2.77[b]	37.57±1.46[b]	66.34±2.38[b]
N3	8.77±0.23b	0.64±0.10b	2.35±0.18[a]	0.17±0.03[b]	27.86±1.94[a]	6.85±0.44[b]	6.94±1.03[b]	54.40±4.10[b]	37.40±1.26[b]	63.36±1.51[b]

Table 4: The effect of different doses of monosodium glutamate (MSG) on Some liver and kidney function tests in male rats

Groups	AST	ALT	Urea	Creatinine
Control	310.10±70.74[a]	58.32±3.61[a]	39.44±5.77[b]	0.44±0.05[a]
MSG1	321.26±46.51[ab]	80.68±12.28[a]	54.44±12.50[a]	0.46±0.05[a]
MSG2	334.54±38.76[ab]	84.54±34.05[a]	56.16±3.79[a]	0.50±0.00[a]
MSG3	365.26±27.03[a]	104.44±64.87[a]	60.92±5.78[a]	0.52±0.11[a]

The values represent Mean ± SD of three experiments.

Means within a column followed by different letters are significantly different ($P < 0.05$).

Table 5: Lesions scores of various histopathological changes in different organs of rats fed manually processed kofta supplemented with various concentrations of sodium nitrite for 30- successive days

	Treatment		
	Sodium Nitrite		
Lesion score	Lower than permissible	Permissible	Higher than permissible
Liver			
Congestion of hepatic blood vessels	-	+	++
Perivascular hemorrhage	+	+	++
Leukocytic infiltration	+	++	++
Disturbance in hepatic cords	-	-	-
Degenerative changes	+	++	+++
Edematous fluid in hepatic parenchyma	-	-	-
Nuclear changes	-	+	++
Necrosis of hepatic cells	-	+	++
Hyperplasia of bile duct	+	++	++
Portal cirrhosis	+	++	++
Kidney			
Congestion	+	+	++
Cellular vacuolation	+	++	+++
Tubular epithelial necrosis	-	+	++
Hyaline casts	+	++	+++
Leukocytic infiltration	-	+	+
Periglomerular edema	-	+	++
Segmentation of glomerular tuft	-	+	++
Shrinkage of glomerular tuft	-	+	++
Brain			
Neural degeneration	+	+	++
Hemorrhage	-	++	+++
Encephalomalacia	-	+	++
Perivascular lymphocytic cuffing	-	-	-

Table 5: Continued

	Treatment		
	Sodium Nitrite		
Lesion score	Lower than permissible	Permissible	Higher than permissible
Testes			
Degeneration of lining epithelial cells of SNT	+	++	+++
Vacuolation in the SNT lumen	-	+	++
Destruction of basement membrane of SNT	-	+	++
Calcification of degenerated cells	-	-	-
Accumulation of edematous fluid either in SNT or in the interstitial tissue		-	+ ++

(–): normal histology,
(+): up to 1/3 of the examined section exhibiting the evaluated lesion,
(++): up to 1/3 to 2/3 of the inspected section revealed the estimated pathological change,
(+++): greater than 2/3 of the assessed section showed the estimated pathological alteration

Table 6: Lesions scores of various histopathological changes of different organs of rats fed manually processed kofta supplemented with various concentrations of MSG for 30- successive days

	Treatment		
	MSG		
Lesion score	Lower than permissible	Permissible	Higher than permissible
liver			
Congestion of hepatic blood vessels	-	+	++
Perivascular hemorrhage	+	+	++
Leukocytic infiltration	+	++	++
Disturbance in hepatic cords	+	+	+
Degenerative changes	+	++	+++
Nuclear changes	-	+	++
Edematous fluid in hepatic parenchyma	+	+	+
Necrosis of hepatic cells	-	+	++
Hyperplasia of bile duct	+	+	+
Portal cirrhosis	-	-	+
Kidney			
Congestion	+	+	++
Cellular vacuolation	-	++	+++
Tubular epithelial necrosis	-	-	++
Hyaline casts	+	++	++
Leukocytic infiltration	-	+	+
Periglomerular edema	-	-	-
Shrinkage of glomerular tuft	+	++	++
Segmentation of glomerular tuft	-	+	+
Brain			
Neural degeneration	+	++	++
Hemorrhage	+	++	++
Encephalomalacia	+	++	+++
Perivascular lymphocytic cuffing	+	+	++
Testes			
Vacuolation in the SNT lumen	+	+	++
Destruction of basement membrane of SNT	-	+	++
Calcification of degenerated cells	-	-	+
Accumulation of edematous fluid either in SNT or in the interstitial tissue		-	+ ++

(-): normal histology,
(+): up to 1/3 of the examined section exhibiting the evaluated lesion,
(++): up to 1/3 to 2/3 of the inspected section revealed the estimated pathological change,
(+++): greater than 2/3 of the assessed section showed the estimated pathological alteration

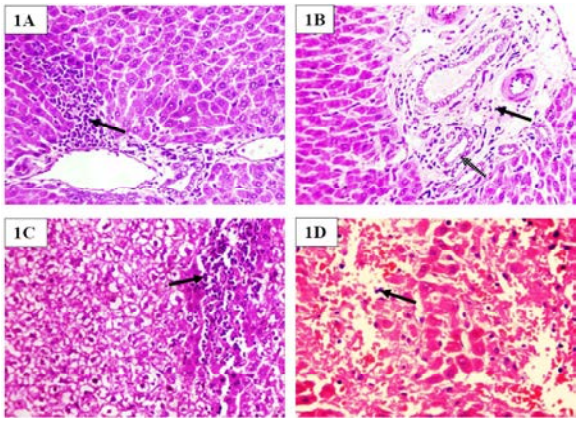

Fig. 1: Liver of rats fed diet supplemented with different concentrations of sodium nitrite for 30-successive days,(A) At a level lower than the permissible one showed few periportal mononuclear leukocytic cellular infiltration (black arrow),(B) At a level lower than the permissible one showed portal fibrosis infiltrated with leukocytes (black arrow) in combination with newly formed bile ductules (spotted arrow),(C) At the permissible level showed focal area of coagulative necrosis in the hepaticparenchyma with leukocytic infiltration (black arrow). Notice also diffuse hydropic degeneration of hepatocytes,(D) At a level higher than the permissible one showed multiple areas of lytic necrosis characterized by disappearance of hepatocytes and replaced by erythrocytes and few mononuclear leukocytic cells mainly lymphocytes (black arrow). H&E stain x400.

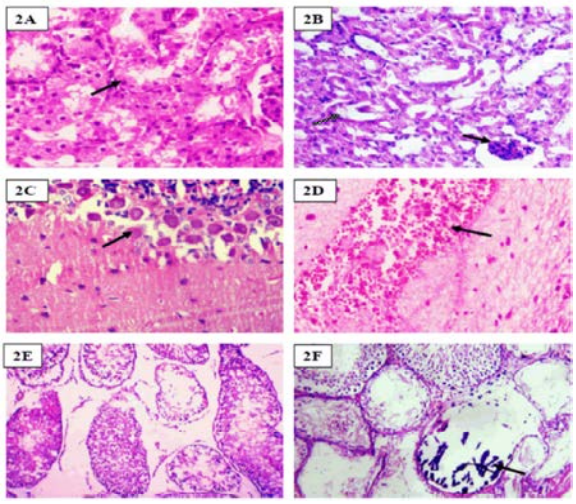

Fig. 2: Pathological alterations in various organs (kidney, brain and testes) of rats fed diet supplemented with different concentrations of sodium nitrite for 30-successive days,(A) At the permissible level, the kidney showed degeneration of the lining epithelium of renal tubules in association with pyknotic nucleus of the renal tubules epithelium (black arrow),(B) At a level higher than the permissible one, the kidney showed necrosis of the lining epithelium of renal tubules (spotted arrow) with the presence of eosinophilic hyaline casts in the lumen of some tubules, note also shrinkage of the glomerular tuft (black arrow),(C) At a level lower than the permissible one, the brain showed neural degeneration with swollen, rounded cell bodies and indistinct or eccentric pyknotic nucleus (black arrow),(D) At a level higher than the permissible one, the brain showed focal area of encephalomalacia in cerebellum admixed with erythrocytes (black arrow),(E) At a level lower than the permissible one, the testes showed degeneration of some seminiferous tubules. Note also vacuolation in the lumen some tubules.(F) At a level higher than the permissible one, the testes showed severe degeneration of spermatogenic cells of some seminiferous tubules in combination with calcification of the degenerated cells in some tubules (black arrow). H&E stain x400.

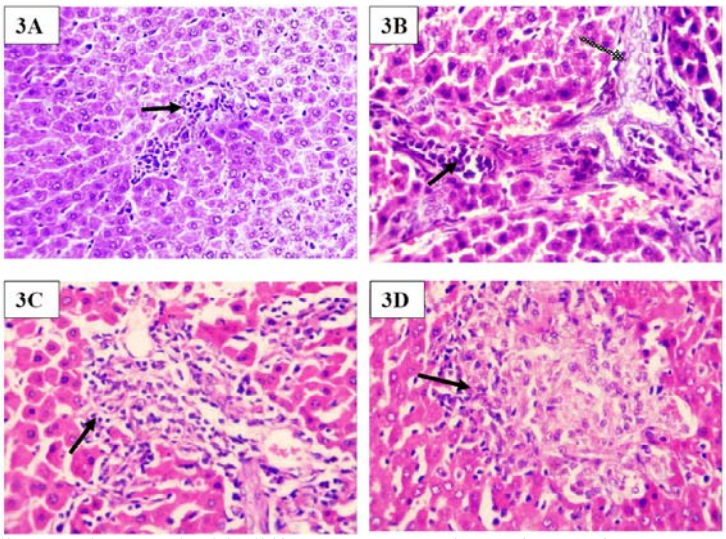

Fig. 3: Liver of rats fed diet supplemented with different concentrations of MSG for 30-successivedays,(A) At a level lower than the permissible one showed few perivascular mononuclear lukocytic cellular infiltration (black arrow), note also degeneration of hepatocytes with activation of vonkupffer cells.(B) At a level lower than the permissible one showed mild hyperplasia of the billiary epithelium (spotted arrow) with mononuclear leukocytic cellular infiltration in the portal area (black arrow),(C) At the permissible level showed portal and periportal leucocytic cellular infiltration mainly lymphocytes and few macrophage (black arrow), notice also pyknosis and karyolysis of hepatic cell nuclei,(D) At a level higher than the permissible one showed focal area of coagulative necrosis in the hepatic parenchyma (black arrow) with pyknosis and karyolysis of hepatic cell nuclei. H&E stain x400

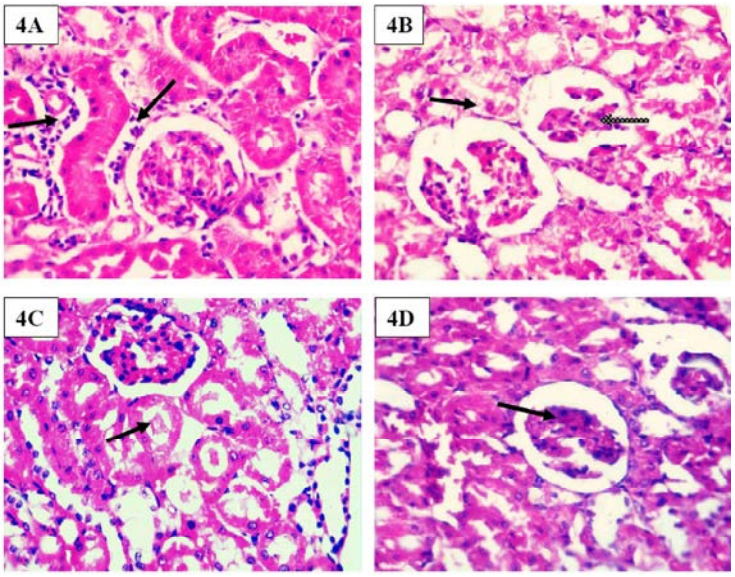

Fig. 4: Kidney of rats fed diet supplemented with different concentrations of MSG for 30-successivedays,(A) At a level lower than the permissible one showed periglomerular edema admixed with few mononuclear leukocytic cellular infiltration, note also inter-tubular mononuclear leukocytic cellular infiltration (black arrow),(B) At the permissible level showed degeneration of the lining epithelium of some proximal and distal convoluted tubules as well as disintegration of the glomerular tuft (spotted arrow).(C) At a level higher than the permissible one showed necrosis of the lining epithelium of renal tubules as well as presence of hyaline casts in the tubular lumen (black arrow),(D) At a level higher than the permissible one showed shrinkage of the glomerular tuft with necrosis of its endothelial lining (black arrow). H&E stain x400

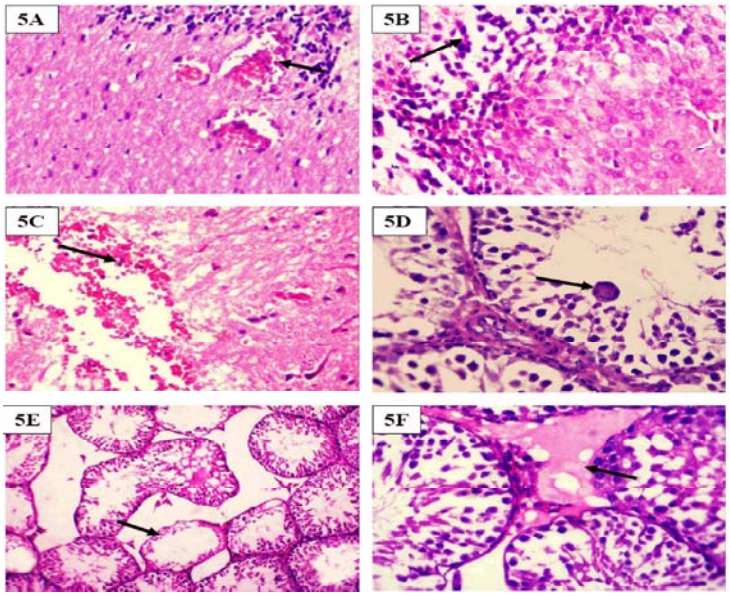

Fig. 5: Brain and testes of rats fed diet supplemented with different concentrations of MSG for 30-successive days,(A) Brain of rat fed diet supplemented with MSG at a level lower than the permissible one for 30 days showed perivascular hemorrhage (black arrow),(B) Brain of rat fed diet supplemented with the permissible level of MSG for 30 days showed showing focal areas of encephalomalacia in cerebellum admixed with spaces infiltrated by few numbers of glial cells and lymphocytes (black arrow),(C) Brain of rat fed diet supplemented with MSG at a level higher than the permissible level for 30 days showed focal areas of encephalomalacia in cerebellum and mixed with erythrocytes and mononuclear leukocytes (black arrow),(D) Testes of rat fed diet supplemented with MSG at a level lower than the permissible level for 30 days showed sperm giant cell in the lumen of some seminiferous tubules (black arrow),(E) Testes of rat fed diet supplemented with the permissible level of MSG for 30 days showed degeneration of spermatogenic cells of some seminiferous tubules (black arrow). Note also vacuolation in the lumen some tubules.(F) Testes of rat fed diet supplemented with MSG at a level higher than the permissible one for30 days showed vacuolation of the lining cells of some seminiferous tubules in combination with accumulation of edematous fluid in the interstitial cells (black arrow). H&E stain x400.

Histopathological Examination:

Histopathological Changes in Rats Fed Diet Containing Different Concentrations of Sodium Nitrite and MSG: Variable pathological alterations in liver, kidney, brain and testes were observed in treated animals. The severity of the pathological changes was increased by increasing the concentrations of sodium nitrite and MSG. Pathological lesion scores in various organs of rats fed diet supplemented with different concentrations of sodium nitrite and MSG for 30 successive days were summarized in Tables (5 and 6) and explained in figure legends.

DISCUSSION

Nitrate and nitrite have been used as meat additives in many types of meat products for many years primarily to fix color, contribute to the flavor of the final product. On the other side, the reaction of nitrite with haemoglobin to form methaemoglobin can reduce oxygen transport in the blood and a theoretical possibility of the potential reaction with secondary amines under the acidic conditions of the stomach to form carcinogenic N-nitrosamines and free radicals in foods or in humans in vivo [30].Such products may increase lipid peroxidation, which can be harmful to different organs including liver and kidney [31].

In the present study, sodium nitrite treated rats showed significant decrease in RBCs count, HGB, WBCs, MCV, MCH, MCHC, lymphocytes and monocytes. Such results agree with those of Smith [32], Reutov *et al*. [33], Vlaskina *et al*. [34] and Eman - Helal and Mervat Abdel- Rahman [35].

The hematological analysis of treated rats revealed anemia that could be attributed increased fragility of erythrocytes due to metabolic impairment [36] or due to slowing down or obstructing the

NAD and NADP reduction processes in erythrocytes by nitrite ions resulting in disturbing their cell respiration, lysis of RBCs and breakdown of HGB to bilirubin in the liver [37] or due to hyperpolarization of erythrocyte membranes and increase in membrane rigidity as result of RBCs oxidation by sodium nitrite [38].

Additionally, nitrates and nitrites trigger destruction of vitamin A and B-group vitamins [39], thereby promoting anemia. They additionally enhance free-radical reactions that may affect the shape and permeability of a cellular membrane of erythrocytes. Oxidation of heme iron, occurring during methemoglobin synthesis from HGB under the influence of nitrites, is a source of reactive oxygen species that initiate the chain of oxidative processes in RBCs. The oxidative stress results in enhanced peroxidation of lipids and aggregation of proteins of erythrocyte membrane. These processes affect changes in the permeability of the cellular membrane and diminish erythrocyte deformability, leading in this way to enhanced degradation of erythrocytes [40]and earlier removal of erythrocytes from circulation, thus shortening their life cycle [41].

The significant decrease in HGB concentration in blood of rats administered sodium nitrite has been shown to be a result of nitrites induced disturbances in heme biosynthesis, transformation of HGB into ethemoglobin [42]. Adverse changes in values of the erythrocytic system markers, occurring under the influence of sodium nitrite, may be possibly through disorders of erythrocyte synthesis, enhanced hemolysis and water shifting to the extracellular compartment [43].

Biochemical analysis could help to identify the target organs of toxicity and the general health status. It may also provide an early warning signal in stressed organism [44]. The serological results in relation to liver function of sodium nitrite treated groups in the present study agree with those of Atef *et al*. [36] and Hanaa-Hassan *et al*. [45].

The increase in the activity of AST and ALT enzymes in the serum of NaNO2-treated rats could be attributed to the formation of over 300 cytotoxic N-nitrosocompounds, in the acidic environment of the stomach as a result of combination of sodium nitrite with secondary amines in the food or the body, causing severe hepatic and renal necrosis [46]or may be due to anemia and methaemoglobinemia which induced hypoxic injury to centrilobular hepatocytes that consequently cause enzyme leakage [47]. Also, elevated ALT can be used to diagnose myocardial infarction, arrhythmias and severe angina of heart.

Moreover, the serological results in relation to kidney function of sodium nitrite treated groups in the present study agree with those of Atef *et al*. [36], Til *et al*. [48] and Hanaa-Hassan *et al*. [45].

Furthermore, in response to NaNO2 treatment, urea and creatinine increased in the serum, suggesting an impairment of kidney functions. These effects could also be attributed to the cytotoxic effect of N-nitrosocompounds in renal tubular cells and the changes in the threshold of tubular reabsorption, renal blood flow and glomerular filtration rate [49].

The changes in the liver and kidneys function parameters could be attributed to damage in the liver and kidneys that proved by the results of the histopathological alterations demonstrated in these organs. Furthermore, several pathological changes were demonstrated in the brain tissue of treated rats with different doses of sodium nitrite. These changes could be attributed to the role of nitrite in the induction of oxidative stress in brain of rat resulting in inhibition of neuron activities as it produced some neurobiochemical alterations in the brain tissues such as inhibition of Acetylcholine esterase (AChE) and elevation of lactate dehydrogenase (LDH) activity [50]. These enzymes have been used as standard biomarkers of toxic stress [51]. AChE is responsible to promote hydrolysis of the neurotransmitter "acetylcholine" released at the nerve endings to mediate transmission of the neural impulse across the synapse [52]. Interestingly, LDH is involved in energy production and the elevation of LDH activity might play a role in brain damage after treatment with NaNO2 [53]. This elevation could be attributed to a generalized increase in membrane activity due to the increase of one of isoenzymes of LDH [54]. Furthermore, degeneration and necrosis of some of the spermatogenic cells in association with other pathological changes were detected in the testicular tissues of rats have a diet supplemented with variable concentrations of sodium nitrite. These findings could be due to defect in the function of Sertoli cell as reported previously by Grant and Butler [55]. Additionally, production of reactive nitrogen species by nitrate plays an important role in its carcinogenic effect through its reaction with body tissues and triggering lipid peroxidation, DNA lesions, enzyme inactivation and damage of different organs [56] thatcould clarify the detection of different pathological changes in various examined organs in the present work.

Meanwhile, MSG has been recognized in first half of twentieth century as taste enhancer but at the same time doubts were raised about MSG as a causative agent of Chinese restaurant syndromewhich is characterized by headache, flushing, numbness, muscle tightness, generalized weakness and bronchoconstriction in asthmatics [57] that revealed there is no complete agreement about the safety of MSG.

Likewise, the results of the present study were nearly similar to those of reports suggesting that MSG could be toxic to erythrocytes and also cause deleterious changes in hematological and biochemical parameters [58]and [59]. However, Kolawole [60]found that treatment of adult rats with MSG (5-15 mg/kg body weight) for 28 days, showed no significant changes in HGB, RBC and WBC counts. No significant change was also observed in all the studied biochemical parameters, including indices of hepatic and renal functions. The results of this study suggest that MSG at the doses administered is not hazardous to health.

In the current study, the pathological changes in the liver parenchyma observed in MSG treated rats were nearly similar to those reported by Ortiz *et al*. [61] and Bhattacharya *et al*. [21]. MSG induced pathological alterations in the hepatic parenchyma even in the concentration at a level lower than the permissible limit. Chronic administration of MSG (4 mg/Kg and above) induced oxidative stress in experimental animals. Moreover, it produces oxygen derived free radicals [62] and causes changes in the liver parenchyma of mice around central vein, hepatocellular damage dilated sinusoids, inflammatory cells and pyknotic nuclei [21]. Previously, it was proved that hepatocytes especially in the central portion of the hepatic lobules are typically the primary site of toxic injury; as they have more surface receptors for toxins and less oxygen [63]. Additionally, the hydropic degeneration of hepatocytes could be considered as a kind of cellular defensive mechanism against injurious substances [64]. At the same time these vacuoles could preserve the biological activities of hepatocytes by collecting the injurious elements and preventing them from interfering with the biological activities of these cells [63].

In this work, different pathological changes were demonstrated in the renal tissues such as degeneration and necrosis of the lining epithelium of renal tubules as well as periglomerular and intertubular leukocytic infiltrations which are in agreement with the findings of Bopanna *et al*. [65] who described patchy tubular necrosis and interstitial infiltration. However, the alterations in the hematological and biochemical parameters of the liver and kidney of rats treated with MSG are in correlation with the hepatic and renal histopathological changes in the present study. On the other hand, it was described that MSG is metabolized in liver and kidney which play an important role in its removal that could clarify the occurrence of pathological changes in these organs.

In the present work, different doses of MSG in rats produced variable pathological changes in the brain tissues. These changes returned to the neurotoxic effect of MSG especially when implicated in high doses and contribute to development of neurodegenerative disease [66]. However, the alterations in the brain tissues produced by MSG could be also due to either increase in the free radicals generation in the body [62] or due to the ability of MSG to induce oxidative damage in the brain, liver and kidney in experimental animals [67] which considered another clarification for the deleterious effect of MSG in these organs. The excitotoxic effect of glutamate could be attributed to its ability for induction of unlimited elevation of intracellular calcium resulting in activation of various enzymes that responsible for cell death by various mechanisms [66]. These excitotoxic lesions in the lateral hypothalamus are neuronal loss and disruption of blood brain barrier [68]. Additionally, MSG is a neurotoxic agent i.e. causing damage to brain cells, retinal degeneration, many endocrine disorders, renal damage [69], addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis [18] and lesions in certain parts of the brain of neonates resulting in obesity because the blood-brain barrier in neonates is immature [70].

On the other hand, different concentration of MSG in manually processed kofta induced various pathological changes in the testicular tissues such as testicular degeneration, atrophy of the semineferous tubules as well as destruction of its basement membrane. These findings confirm the implication of MSG in cases of male infertility which in agreement with the results of Eweka and Adjene, [18]. However, these alterations may be due to the toxic effect of MSG to the testicular cells [71].

Finally, we can conclude that the prolonged use of sodium nitrite and MSG even within the permissible limits leads to anemia, liver and kidney dysfunctions, damage to brain cells in association with deleterious effect on male fertility.

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