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STUDIES ON NITRITES AS A CHEMICAL PRESERVATIVE IN SOME MEAT PRODUCTS

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A B S T R A C T

A total of one hundred and twenty random samples of different meat products (Beef burger, Luncheon, Canned corned beef, Sausage, Pastrami and Kofta) (20 of each) were collected from different supermarkets in Menoufia governorate. The samples were subjected to chemical determination of nitrite content (ppm) in the examined meat products. The mean values of nitrite levels (ppm) in the examined meat products as follows 94.04± 5.20 for beef burger, 98.65± 3.41 for luncheon, 42.30± 2.47 for canned corned beef, 127.15± 6.24 for sausage, 48.69± 4.68 for pastrami and 79.56± 6.25 for kofta. The minimum and maximum values of nitrite in meat products (ppm) were showed as 58-131, 68-127, 25-62, 72-172, 28-94 and 37-123 for beef burger, luncheon, canned corned beef, sausage, pastrami and kofta, respectively. 30% of beef burger samples exceed the permissible limit, 45% of luncheon samples exceed this limit as well as 25% of canned corned beef, 85% of sausage, 25% of pastrami and 15% of kofta.

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1- INTRODUCTION:

People have preserved meat naturally for thousands of years, but in recently chemical and synthetic preservatives are often used various preservatives are added to meat products to extend shelf –life and enhance food safety (1). Food preservation has become an increasingly important practice in modern food technology with the increase in the processed and convenience foods (2). Nitrite has been a valuable antibotulinal agent in cured meats and may offer some protection from other pathogens in these products as well. Nitrite's use in food has been clouded by suspicions that nitrite could react with amines in the gastric acid and form carcinogenic nitrosamines leading to various cancers (3). Preservation of meat with nitrite or nitrate has become important to humanity in controlling meat spoilage and in producing safe and palatable meat products with good keeping properties even at ambient temperature (4). Sodium nitrite reduced the growth rate and increased the lag time of Listeria monocytogens strain inoculated into slices of cooked meats stored at either 0 or 5 ˚C. (5). Sodium nitrite (50 ppm) has a role in meat products by stabilizing the pink coloration of meat and enhance the meat product flavor also, sodium nitrite has an effect on Clostridium botulinum by inhibiting toxin production (6). Nitric oxide NO plays an important role in a diverse range of physiological processes. NO reacts with the superoxide anion to generate peroxynitrite, which is a selective oxidant, and nitrating agent that interacts with numerous biological molecules, thereby damaging them (7).

Also NO is an important player in eczema, food allergy and intestinal inflammation (8). The maximum in going

level of sodium nitrite permitted in Denmark is 60 ppm for most products with some specialty products allowed to have up to 150 ppm. From 1998 to 2006, the residual nitrite in Denmark sausage and salami-type products has varied between 6-22ppm (9). In Australia nitrites (sodium or potassium salts) are allowed at a maximum level of 125 ppm in cured, dried and slow-dried cured meat and 50 ppm in sterile and canned meat (10). Nitrite remains the most effective curing agent to prevent food spoilage and bacterial contamination. Despite decades of rigorous research on its safety and efficacy as a curing agent, it is still regarded by many as a toxic undesirable food additive.

However, research within the biomedical science community has revealed enormous therapeutic benefits of nitrite that is currently being developed as novel therapies for conditions associated with nitric oxide (NO) insufficiency. Much of the same biochemistry that has been understood for decades in the meat industry has been rediscovered in human physiology (11). Nitrate itself is generally regarded nontoxic. Toxicity is usually the result of the conversion of nitrate into the more toxic nitrite. There are two major toxicological concerns regarding nitrite. First, nitrite may induce methemoglobinaemia, which can result in tissue hypoxia, and possibly death. Secondly, nitrite may interact with secondary or N-alkyl-amides to form N-nitroso carcinogens (12). There is a positive association between nitrite and nitrosamine intake and gastric cancer and between meat and processed meat, intake and gastric cancer and esophageal cancer (13), also endogenous formation of nitroso compounds are associated with colorectal cancer (14) which is the major cause of death in affluent countries (15). Nitrite is an intrinsic signaling molecule with potential therapeutic implications in mammalian ischemia/reperfusion (I/R) injury of the heart, liver, and kidney (16).

So that, the present research was conducted to determined nitrite content (ppm) in some meat products, determined the effect of nitrite on liver and kidney functions and histopathological effect on internal organs of rats.

2- MATERIAL AND METHODS:

2.1. Samples

A total of 120 random samples of meat products represented by luncheon, Pastrami, Sausage, Beef burger, Kofta and Corned beef (20 of each) were collected from different supermarkets in Menofyia governorate for determination of their nitrite content (ppm). The collected samples were directly transferred to the laboratory without undue delay and examined as rapidly as possible. All collected samples were subjected to the chemical examination to estimate the nitrite content (ppm). to evaluate the acceptability of such meat products according to the specification stipulated by Egyptian Standard Specification or Egyptian Organization for Standardization and Quality Control (17) and compare the content on the label with the obtained results. Also the effect of these nitrite on lab animal was determined to measure the margin of safety on the consumer. Accordingly, the following examinations were carried out;

2.2. Determination of Nitrite (18);

Ten grams of the homogenized sample were put into a 100 ml capacity beaker and macerated with 5 ml borax solution and 70 ml of hot distilled water (not lower than 70 ˚C). The mixture was Heated on a boiling water bath for 30 min with continuous shaking was applied. After cooling, 2 ml of Potassium ferrocyanide trihydrate solution (10%) and 2 ml Zinc acetate dehydrate (2%) as well as 75 ml hot distilled water was added. The pH of the supernatant solution

was adjusted at 8.3 and the mixture was left to stand for 30 minutes. However, the supernatant fluid was filtered through a filter paper to obtain clear filtrate. The filtrate was transferred into 100 ml flask to which 60 ml distilled water, 10 ml sulphanilamide solution (0.1%) and 6 ml concentrated HCL were added and the mixture was left for 5 minutes in a dark place. Furthermore, 2 ml N-1-nephthylethelene diamine dihydrochloride solution were added and thoroughly mixed for 3 minutes. The absorbance of the solution was measured by spectrophotometer at wave length 538 nm. The calibration curve was prepared by using 10 ml of each of the diluted standard solutions of Sodium nitrite (25, 50 & 100 ug Sodium nitrite). From the calibration curve, the number of Ug of Sodium nitrite (ml) equivalent to the absorbance of the test sample was recorded.

2.3.Calculation;

Sodium Nitrite (ppm) = 200 (m1 ÷ m0) x V
Where:
m0 = Weight of sample (g)
m1 = Number of Ug of Sodium nitrite equivalent to the absorbance of the test sample.
V = Volume of the filtrate used in the test (ml)

2.4. Experimental part

The present study was carried out on 10 white male albino rats of 3 months old and weighed between 100- 115 gm. Animals were kept at a constant environmental and nutritional condition throughout the period of investigation (2 weeks) and housed in a clean separate steel cages and fed on balanced diet throughout the period of the experiment. Water was offered *adlibtium*.

2.5. Experimental design;

The rats were divided into two groups, placed in individual cages and classified as follows
Group 1 (Control group); Composed of 5 rats were fed on cooked meat free from any preservatives (used as control for all the experimental groups). Group 2 (Nitrite supplemented group at the highest level detected during chemical estimation); Included 5 rats were fed on cooked meat with nitrite where nitrite was added in a dose equal to the highest level detected during chemical estimation (172 ppm).

2.6. Sampling

Blood samples were taken from all groups at the end of experimental period and at overnight fasting, rats were weighted and heparinzed blood samples (20 IU/ ml) were collected from the median canthus of the eye. Each sample was centrifuged at 3000 r.p.m. for 10 minutes for plasma separation. The clean and clear plasma was separated and received in dry sterile sample tube using sterilized pipette and processed directly for determination of Glutamic Oxaloacetic Transaminase (GOT) Glutamic Pyruvic Transaminase (GPT), Alkaline phosphatase, Urea, Serum creatinine and Uric acid. Tissue specimens for histopathological examination (19); Immediately after blood sampling, the animals were sacrificed and the liver, kidneys, spleen and heart animals were carefully removed immediately, washed with saline and blotted between filter papers. Small specimens of liver 1x1.5 and 5mm in thickness, half of the kidney, whole spleen and half of the heart were immediately fixed in 10% neutral buffered formalin. A paraffin section of 5-7 micron-thick were prepared and stained with routine H&E stain for histopathological finding.

3- RESULTS

Table (1). Statistical analytical results of Nitrite levels (ppm) in the examined meat products samples (n= 20 of each).

Meat products	Nitrite			DS above P.L		ND		DS not prescribed on label	
	Min.	Max.	Mean ± SE	No.	%	No.	%	No.	%
Beef burger	58	131	94.04 ± 5.20	6	30	6	30	2*	10
Luncheon	68	127	98.65± 3.41	9	45	-	-	1	5
Canned corned beef	25	62	42.30± 2.47	5	25	-	-	-	-
Sausage	72	172	127.15± 6.24	17	85	-	-	-	-
Pastrami	28	94	48.69± 4.68	5	25	4	20	-	-
Kofta	37	123	79.56± 6.25	3	15	11	55	1	5

Permissible limit of nitrite in canned corned meat product (50 ppm) and (100ppm) for pastrami and other meat products according to E.S.S (2005). PL= permissible limit, DS= Detectable samples ND= Not detected. *One sample above PL

Table (2). Serum analysis of *control group

Group Serum	Group 1(Control)		
	Min.	Max.	Mean ± SE
GOT	134	170	148.8 ± 6.04
GPT	59	80	71.8 ± 3.62
Alkaline phosphatase	581	791	705 ± 48.86
urea	25	40	31.6 ± 2.48
Uric acid	1.97	2.31	2.11 ± 0.06
Serum creatinine	0.79	0.92	0.84 ± 0.02

* Control group: rats were fed on cooked minced meat without preservatives.

Table (3). The effect of Nitrite (172 ppm) on liver and kidney functions

Group / Serum	Group 2(feed on nitrite 172 ppm) for 2 weeks		
	Min.	Max.	Mean ± SE
GOT	150	239	201.6 ± 16.21
GPT	51	76	62.2 ± 4.35
Alkaline phosphatase	415	518	453.8 ± 17.8
urea	38	43	40.4 ± 0.93
Uric acid	4.11	5.4	4.7 ± 0.22
Serum creatinine	0.98	1.21	1.11 ± 0.04

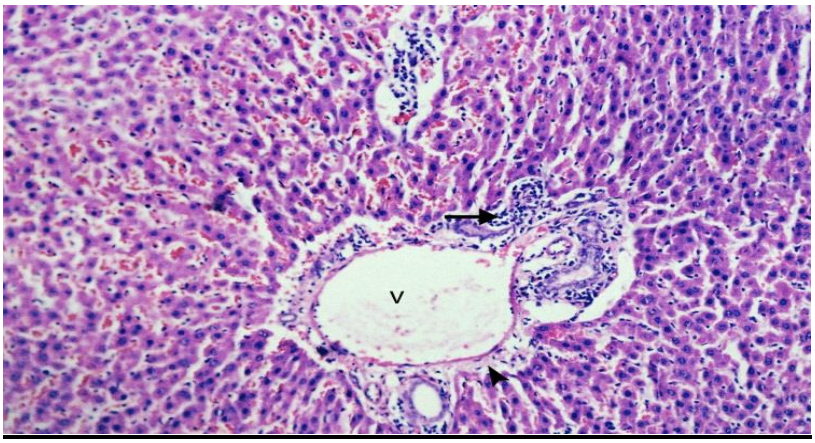

Fig. (1): Liver of nitrite treated rat (Group II) showing dilatation and congestion of the portal vein (V) and hepatic sinusoids with edema (arrow head) mixed with small numbers of lymphocytes (arrow) in the portal areas. H&E stain x 200.

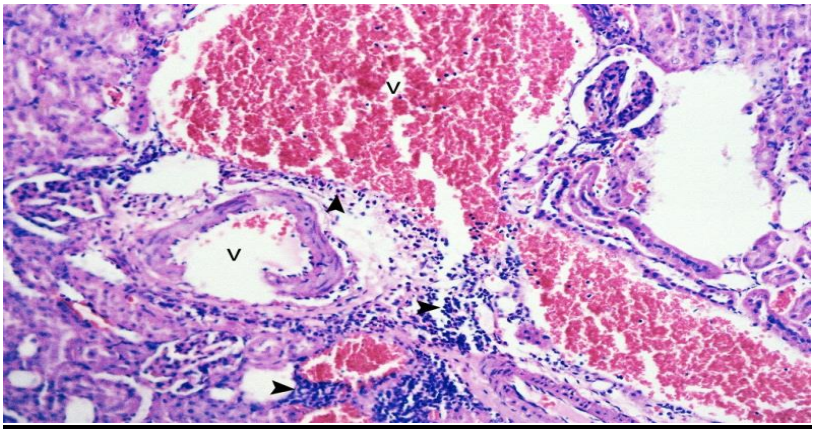

Fig. (2): Kidney of nitrite treated rat (Group 2) showing severe congestion of cortical blood vessels (V) and perivascular interstitium expanded by oedema and aggregates of inflammatory cells (arrow head). H&E stain x 200.

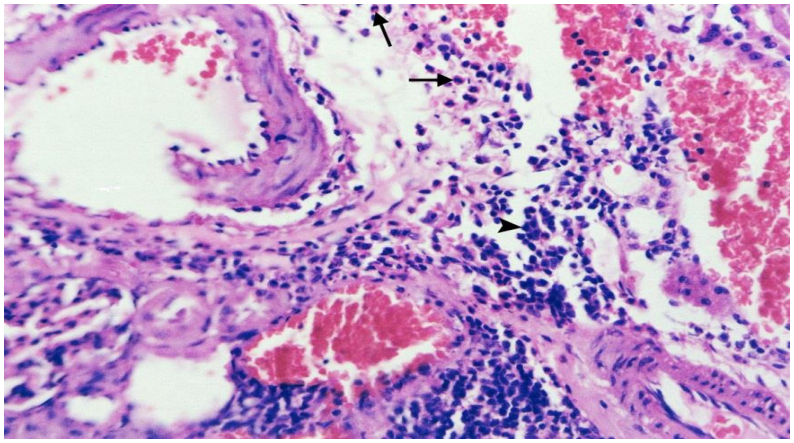

Fig. (3): Kidney of nitrite treated rat (Group 2) showing perivascular interstitial aggregates of inflammatory cells mainly lymphocytes (arrow head) and moderate numbers of neutrophils (arrow). H&E stain x 400.

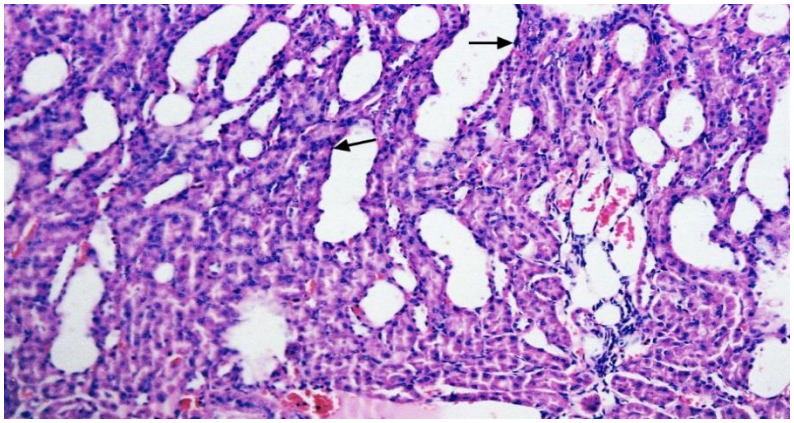

Fig. (4): Kidney of nitrite treated rat (Group 2) showing cystic dilatation of some renal tubules lined by attenuated epithelium (arrow). H&E stain x 200.

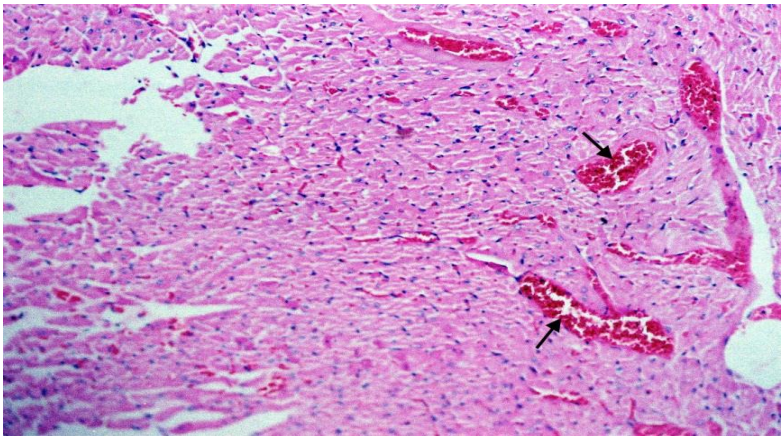

Fig. (5): Heart of nitrite treated rat (Group 2) showing congested blood vessels (arrow) and intermuscular capillaries. H&E stain x 200.

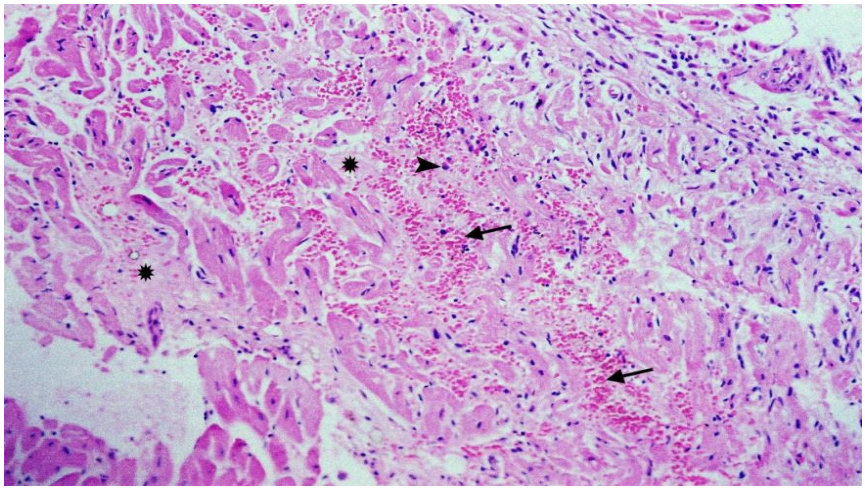

Fig. (6): Heart of nitrite treated rat (Group 2) showing focal areas of oedema (asterisk) and hemorrhages (arrow) admixed with few numbers of macrophages (arrow head) and displaced myocardial muscle fibers. H&E stain x 200.

4- DISCUSSION

Sodium or potassium nitrite is widely used as a curing agent in cured meat products because it inhibits outgrowth and neurotoxin formation by Clostridium botulinum, delays the development of oxidative rancidity, develops the characteristic flavor of cured meats, and reacts with myoglobin and stabilizes the red meat color. As soon as nitrite is added in the meat formulation, it starts to disappear and the nitrite that has not reacted with myoglobin and it is available corresponds to residual nitrite level (20). Regarding the results recorded in table (1) the mean values of nitrite levels (ppm) in the examined meat products as follows 94.04± 5.20 for beef burger, 98.65± 3.41 for luncheon, 42.30± 2.47 for canned corned beef, 127.15± 6.24 for sausage, 48.69± 4.68 for pastrami and 79.56± 6.25 for kofta. The minimum and maximum values of nitrite in meat products (ppm) were reported as 58&131, 68&127, 25&62, 72&172, 28&94 and 37&123 for beef burger, luncheon, canned corned beef, sausage, pastrami and kofta, respectively. Nearly similar results were recorded by (20) who mentioned that the mean values of nitrite contents were 49.76± 2.3 ppm in imported corned beef, while in canned luncheon samples, were 45.5 ±4.3 ppm and were 27.9±2.5 ppm in local canned meat samples, respectively and the obtained results were nearly similar to (21) for the examined luncheon samples, 97.255± 12.66 ppm, while the same authors recorded higher results for the examined pastrami, 67.815± 12.69 ppm,. Also higher results for luncheon samples were reported by (22), (23) and (24) as they recorded 137.6 ±2.4 ppm, 118.9 ppm and134.7± 8.05 ppm, respectively, (25) recorded higher results for sausage, pastrami, corned beef and canned luncheon beef as they found that the mean values of nitrite level in the examined meat products were 120.40 ±7.03, 142.15 ±9.13, 186.27 ±4.42 and 159.96 ±6.73 ppm for sausage, pastrami, corned beef and canned luncheon beef, respectively . Lower result was obtained (26) as they found that the mean value of residual nitrite was 24.31± 1.18 with minimum 11.59 and maximum 36.71 for meat products. Slightly lower results obtained by (27) for the examined luncheon and frozen sausage samples while higher results for canned beef, as mean value of nitrite content (ppm) was 93.15, 116.2 and 98.05, respectively. The detectable samples

above the permissible limit recommended by Egyptian Standard Specification (28) for nitrite contents in meat products were showed in Table (1) that 30% of beef burger samples exceed the permissible limit, 45% of luncheon samples exceed this limit as well as 25% of canned corned beef, 85% of sausage, 25% of pastrami and 15% of kofta. The functions of nitrite in meat curing were recorded to stabilize the color of lean tissues, to contribute the characteristics flavor of cured meat, to inhibit growth of a number of anaerobic food poisoning and spoilage microorganisms and to retard development of rancidity (18). Also (29) realized that up till now no substance has been found to take the place of nitrite with its varied actions in meat products.

Reduced nitrite levels (40-80 ppm) resulted in decreased nitrosamine formation and appear to be adequate for the other functions of nitrite (e.g. color, flavor, rancidity prevention, etc.). 50 ppm (mg /kg) is initial nitrite level, which is sufficient for the desired meat color, up to 200 ppm, depending on the country and is used for antibotulinal activity, so the result has been the almost total absence of botulism in cured meat. Its effect on non-spore former is distinctly genus dependent (22). As with the organic acids, lower pH noticeably improves the antimicrobial activity of nitrite (30). The recorded high permissible limit in a few of the examined meat products samples may be regarded to an error in the processing of these meat products concerning the extension of the shelf-life is the more important aim for economic income than the adverse effect of the preservatives used on consumer's health (26). Moreover, table (1) indicated that nitrite detected in 2 samples (10%) not prescribed on the label one of which are above the permissible limit for beef burger. Also one sample (5%) of luncheon and one sample (5%) of kofta contain nitrite not prescribed on the label. These data not follow the recommendation of (31) which recommended that if food products contain preservatives the percent of each should be indicated. Biochemical analysis could help to identify target organs of toxicity as well as the general health status of animals. It may also to provide an early warning signal in stressed organism (32). The plasma transaminase GOT&GPT as well as alkaline phosphatase (entering the blood after the cell necrosis of certain organs) can be used to establish the tissue damage of the liver and kidney (33) and (34).

Rises in the serum level of urea and creatinine is indicator of renal failure and rises in GOT is indicator of hepatocellular injury as reported by (35). Also, elevated GOT can be used to diagnose myocardial infarction, arrhythmias and sever angina of heart. So, table (3) discussed the effect of nitrite 172 ppm (Group 2) on liver and kidney functions in comparison to control group in table(2) that was fed on cooked minced meat without Nitrite. After 2 weeks feeding on cooked minced meat contain nitrite (172 ppm) the serum of rats showed increase GOT, urea, uric acid and creatinine in correlation with control group. In which the mean values of GOT, urea, uric acid and serum creatinine were (301.6± 16.21, 40.4± 0.93, 4.7± 0.22 and 1.11± 0.04) for group (2) and (148.8± 6.04, 31.6± 2.48, 2.11± 0.06 and 0.84± 0.02) for group (1) control one. Also reduction in GPT and alkaline phosphatase level was recorded in group (2) (62.2± 4.35 and 453.8± 17.8) in comparison with control group (1) (71.8± 3.62 and 705± 48.86). GOT can be used to diagnose myocardial infarction within 10-48 hours. Other conditions with elevated GOT include arrhythmias and severe angina of the heart and liver damage (36). These changes in the biochemical parameters may be due to damage in the in heart, liver and kidney that proved by the results of the histopathoglogical examination in figures(1,2,3,4,5 and 6) which indicated that

rats treated with 172 ppm nitrite in the food for 15 successive days showed mild histopathological changes in liver, kidneys and heart; no lesions were noticed in spleen as the microscopical examination of the livers revealed normal histological appearance of the hepatocytic parenchyma similar to the control group (No histopathological changes were detected in the examined organs of these rats). However, dilatation and congestion of the central, portal veins and hepatic sinusoids were commonly seen; the portal areas were expanded by oedema mixed with small numbers of lymphocytes (Fig. 1). Rarely, pyknosis of the nuclei of individual hepatocytes were noticed in few cases. The examined kidneys revealed also severe congestion of cortical blood vessels and the perivascular interstitium was expanded by oedema (increased clear space) and aggregates of inflammatory cells (Fig. 2) mainly lymphocytes and moderate numbers of neutrophils (Fig. 3). Rarely, interslitial lymphocytic cellular infiltration was seen in few cases in-between the renal tubules. Moreover, some renal tubules in both cortex and medulla exhibited cystic dilatation and were lined by attenuated epithelium (Fig. 4). The heart showed congested blood vessels and intermuscular capillaries (Fig. 5). Focal areas of edema and hemorrhages admixed with few numbers of macrophages and displaced myocardial muscle fibers were seen in few cases (Fig. 6).

The reported results is similar to the results recorded by (37), (38) and (39), all of them recorded high level of GOT, urea and serum creatinine as a result of high level of nitrite administration. Also (40), (41) and (42) all of them reported that sodium nitrites produce necrotic changes of the liver and deterioration of the liver function and renal tubules.

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