Protective effect of turmeric on the oxidative damage caused by chronic sodium nitrite intoxication in Rats.

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Abstract

The present study was carried out to examine adverse effects of nitrite toxicity in female rats and the use of turmeric (***Curcuma longa***) in alleviating these effects. Rats were randomly divided into four equal groups (12 rats each) as follows: Group (1) considered as a control group. Group (2) received sodium nitrite 2g/1 L drinking water. Group (3) received turmeric10g/kg diet. Group (4) received sodium nitrite 2 g/ L drinking water and turmeric 10g/kg diet, animals were daily treated for six months. Five animals of each group were randomly selected for blood sampling and decapitated for pathological examination after 3 and 6 months from the beginning of experiment. Sodium nitrite treated group showed erythrocytosis, thrombocytosis leucocytopenia and increase in ALT, AST, ALK.ph., urea and creatinine with increase in unconjugated bilirubin after six months, administration of turmeric ameliorate adverse effect of sodium nitrite after three months by rise Hb, WBCs and lower AST, ALT, ALK. ph., urea and creatinine but these effects become weak after six months in addition to the decrease of the level of unconjugated billirubin as compared with control group. Turmeric treated group showed decrease in Hb, polythemia, increase in AST, ALT, ALK.ph. and decrease cholesterol and total protein as compared with control group. Histopathological changes of chronic sodium nitrite showed kidney degeneration of tubular epithelial cells and expansion of Bowman's space, diffuse vacuolization of hepatic parenchyma, spleen showed presence of hemosiderin laden macrophage. Therefore, even low dose of sodium nitrite has adverse effect on liver function, blood picture and immunity. Oral administration of turmeric (10g/kg diet) for female rat for long time had adverse effect on liver and decreased hemoglobin concentration, Erythrocytosis is the first sign of exposure to oxidizing agent or generation of free radicals and it could be useful in its diagnosis. Methemoglobinemia alone does not suffice to cause red cell destruction. Administration of turmeric in conjugation with sodium nitrite after three months showed some improvement in kidney and liver picture but had no effect on pathological changes caused by sodium nitrite toxicity in spleen. After six months Administration of turmeric in conjugation with sodium nitrite showed no amelioration of pathological changes. So, no count on turmeric to have an influential antioxidant in processed food products against used sodium nitrite as a preservative.

Key words:

Sodium nitrite, Turmeric, Histopathology, Haematology, Biochemistry.

Introduction

Sodium nitrite is a salt that is used to preserve meats like ham, bacon and hot dogs, Nitrite serves a vital public health function: it blocks the growth of botulism-causing bacteria and prevents spoilage **(Sen and Baddoo, 1997)**. Nitrite also gives cured meats their characteristic color and flavor **(Niehs, 1970).** Because of the use of more than one type of such food, the percentage of nitrite content of the daily food consumption may be higher than the admissible level **(Bilczuk *et al.,* 1991).** In fact, the amount of nitrate in some vegetables can be very high. Spinach, for example, may contain 500 to 1900 parts per million of nitrate; the nitrate to nitrite conversion process from eating vegetables makes up 85 percent of the average human dietary nitrite intake **(NIEHS, 1970; WHO, 1985).** Nitrites and nitrates are environmental pollutants present in food and water and it is suggested that they may contribute to the etiology of liver and kidney diseases and problems related of immunity in domestic fowls **(Ibrahim *et al.,* 1999)**.

Curcumin is widely used for our preparation of food. Curcumin can be found in turmeric, which is the powdered form of Curcuma ***(Longa Rhizomes),*** (Curcumin has been shown to have anti-inflammatory activity when applied topically in EIU **(Gupta *et al.,* 2001)**. Curcumin was found to have a strong antioxidant effect **(Amani *et al.,* 2010)**.

The present investigation aimed to study the protective effect of turmeric on rats treated with sodium nitrite (food additives). The study will include complete hematological examination, kidney and liver functions test and pathological investigation. In addition the study will investigate the toxicological effects of curcumin which is largely used in Egypt.

Materials and Methods

1. Chemicals

Sodium nitrite was obtained from the El-Gomhoria Co. and turmeric powder was obtained from local market at Alexandria city, Egypt.

2. Animals.

Forty eight, clinically healthy, female Albino rats weighing about 80-100g were used throughout this study. Animals were housed in clean metal cages, fed on standard pellet diet and water ad-libitum. They were acclimatizing for 1week, prior to the experiments.

3. Design of experiment.

Rats were randomly divided into four equal groups (12 rats each) as follows:

Group (1) considered as a control group.

Group (2) received sodium nitrite 2g/1 L drinking water according **Kohn *et al.,* (2002).**

Group (3) received turmeric10g/kg diet according to **El-Wakf *et al.,* (2011).**

Group (4) received sodium nitrite 2 g/ L drinking water and turmeric 10g/kg diet.

Animals were daily treated for six months. Five animals of each group were randomly selected for blood sampling and decapitated for pathological examination after 3 months and 6 months from the beginning of experiment.

4. Hematological Examination.

Blood samples were taken from the retro-orbital venous plexus of rats. The two blood samples were collected one with EDTA for hematological analysis and other for separate serum for biochemical analysis. Erythrocytic count (RBCs) was performed using improved Neubauer Haemocytometer and Gower's fluid as a diluting fluid according to **Feldman *et al.,* (2000).** Total leukocytic count (WBCs) were done using improved Neubauer Haemocytometer with turkey's solution according to **Jain, (1993).** PCV% was determined by using microhematocrite centrifuge and microhematocrite capillary tubes method according to **Thrall *et al.,* (2004).**The blood hemoglobin was estimated using the cyanomethemoglobin colorimetric technique according to **VanKampan and Zijlistra, (1961).** The values of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin concentration (MCHC) were calculated by standard formula according to **Dacie and Lewis, (1977).** The prepared fixed thin blood films were stained with

Diff&Quick stain. The film then examined for morphological changes in RBCs and WBCs according to **Thrall *et al.,* (2004).** The percentage values of differential leukocytic count were then calculated according to **Jain, (1993).**

5. Serum Biochemical Studies.

Serum total protein was determined using kit provided by Diamond Diagnostics according to **Doumas *et al.,* (1981).** Serum albumin was determined using kit provided by Diamond Diagnostics according to **Doumas *et al.,* (1971).** Serum globulines was obtained mathematically and Albumin / Globulins ratio (A/G ratio) was calculated.

Serum aspartate aminotransferase (AST), Serum Alanin aminotransferase (ALT) activities were determined according to **Reitman and Frankels, (1957).** Serum alkaline phosphatase, (Alk.ph.) activity was determined using kits of bioMerieux/ France according to modified method of **Kind and King, (1954).** Serum Total Billirubin was determined using kits provided by RANDOX according to **Sherlock, (1951).** Serum urea level was determined using kits provided by bioMerieux according to **Patton and Crouch, (1977)**. Serum creatinine was determined using kits provided by bioMerieux according to **Husdant and Rapaport, (1958).** While serum cholesterol was determined as mentioned by **Fossati and Medici, (1987).** Lipid peroxidation (LPO) was measured by the method of **Buege and Aust, (1978).**

6. Pathological examinations.

Five rats from each group were sacrificed after 3and 6 months from the beginning of experiment and subjected to careful post mortem examination. Suitable tissue samples were taken from liver, kidney and spleen. The samples were immediately fixed in 10 % neutral buffered formalin then processed for microscopical examination and stain with Heamatoxylin and Eosin, according to **Bancroft *et al.,* (1996)**.

7-Statistical analysis.

All data are represented as means + SE. One way analysis of variance (One-way ANOVA) followed by Least Significant Difference (LSD) test was used to determine differences among means of investigated groups. The differences were considered to be statistically significant at $P<0.05$. Data were analyzed by computer using SPSS version 16.0 for windows. (Statistical Package for the Social Sciences Inc, Chicago, Illinois).

Results

Hematological and biochemical results:

- Results after 3 months was showed in (Table1) and (Table 2).

Group treated with Sodium nitrite characterized by significant ($p<0.05$) increase in RBCs, PCV and platelets and significant decrease in MCV, MCH and WBCs as compared with control group, these results indicates to erythrocytosis with low content of Hb inside red blood cell.

Administration of sodium nitrite with turmeric led to increase in RBCs, Hb and PCV and decrease in WBCs and platelet as compared with control and sodium nitrite group. These finding indicates to erythrocytosis with normal content of Hb inside red blood cell. Administration of Turmeric only caused increase in RBCs, WBC (lymphocytosis) and platelets (polycythemia) and decrease in Hb, MCV and MCH as compared with control group.

Administration of sodium nitrite led to increase in AST, ALT and ALK.ph. enzymes, urea and creatinine as compared with control group.

Administration of sodium nitrite with turmeric led to decrease in AST, ALT, ALK.ph. Cholesterol, urea and creatinine and increase in total protein as a compared with sodium nitrite group. Administration of turmeric only led to increase in AST, ALT and ALK.ph. but decrease in total protein and cholesterol as compared with control group.

- Results after 6months was showed in (Table 3) and (Table 4).

Group treated with sodium nitrite characterized by slight increase in RBCS only but MCH and MCV was decreased (microcytosis, hypochromacia), WBCs and platelets was decreased (leucocytopenia and thrombocytopenia). Group treated with sodium nitrite with turmeric characterized by increase in Hb, platelets and WBCs as compared with sodium nitrite group.

Group treated with turmeric characterized by increase in RBCS, WBCs (lymphocytosis) and platelets, (polycythemia) decrease in Hb, PCV, MCH and MCV. Sodium nitrite group characterized by increase in ALT enzyme, total bilirubin, direct, indirect bilirubin, and decrease in cholesterol, total protein, globulin as compared with control group.

Group treated with sodium nitrite with turmeric group led to increase in ALT, AST enzymes, total bilirubin, uncongugated billirubin but total protein, globulin and cholesterol was decreased as compared with control group but AST was increased and ALT, ALK.ph, total bilirubin, uncongugated bilirubin total protein, albumin and cholesterol was decreased as compared with sodium nitrite group. Group treated with turmeric, ALT, AST and ALK.ph. Enzymes were increased and cholesterol, total protein was decreased as compared with control group. Lipid peroxidase in liver tissue was significant decreased in sodium nitrite with turmeric treated group as compared with other groups along the experimental period.

Pathological examination.

Clinical symptom.

All over the time of experiment, group 2 treated animals showed poisonous signs demonstrated as dullness, animals usually huddle together and appear depressed. Group 4 appeared inactive and lost their vitality.

Post-mortum examination.

On postmortem examination, There were no significant macroscopic findings in the group 1 (control) used in the experiment. group 2 and group 4 livers and heart showed darker in colure, smaller in size and fragile in texture, the spleen was mildly enlarged and the kidney appeared small, dark brown in color.

Table (1). Hematological parameters (Values are mean ±SD) of 5 rats in each group after 3 months from start of experiment. The mean differences are significant at 0.05 level.

	Control group	SOD. NIT. group	Turmeric group	SOD. NIT. Turmeric group
RBC's (10^6/µl)	6.05±0.28	$8±0.7^{A}$	$6.9±0.13^{A}$	$7.3±0.3^{Aa}$
HB (mg/dl)	12.86±0.3	$13±1.2^{a}$	$11.8±0.21^{A}$	$14.3±0.5^{Aa}$
PCV%	38.8±1.6	$45.2±6.3^{A}$	36.4±0.45	$47.7±1.6^{A}$
MCV (fl)	64.2±0.5	$54.24±3.3^{Aa}$	$56.9±5.6^{A}$	$65.8±0.7^{a}$
MCH (pg)	21.2±0.8	$15.6±0.3^{Aa}$	$17±0.2^{A}$	$19.5±0.3^{Aa}$
MCHC%	33.1±1.3	34.3±2.2	31.8±0.8	32.8±1.4
WBC'(10^3/µl)	9.6±0.7	$8.4±0.3^{A}$	$14.6±1.3^{A}$	$8.12±0.3^{A}$
Lymph.%	85.6±0.5	$73±2.8^{A}$	$92.4±3.04^{A}$	$72±1.5^{A}$
Neut.%	6.4±0.5	$20.4±3.6^{A}$	$2.4±1.1^{A}$	$17.6±2.07^{A}$
Mono.%	5.8±0.4	5±1.8	3.8±1.09	$9.2±0.8^{Aa}$
Esino.%	1.6±0.5	1.4±0.8	0.8±0.8	1.2±1.2
Baso.%	0.6±0.5	0.2±0.4	0.6±0.5	0.4±0.5
Platelets (10^3/µl)	1275±101	$5157±187^{A}$	$5166±563^{A}$	900±47

A $p< 0.05$ group compared with control.

a $p<0.05$ (sod. nit. turmeric) group compared with (sod.nit.) group.

Table (2). Biochemical parameters (Values are mean ±SD) of 5 rats in each group after three months from start of experiment. The mean differences are significant at 0.05 level.

	Control group	(SOD. NIT.) group	(Turmeric) group	SOD. NIT. Turmeric group
S.AST u/l	119±6.8	236.4±3.6^{Aa}	148.6±4.4^{A}	151±0.4^{Aa}
S.ALT u/l	35.2±0.8	64±3.8^{Aa}	42±2.5^{A}	49.4±2.9^{Aa}
S.ALK.ph. u/l	315.6±7.9	363.8±13.5^{Aa}	369.2±31.6^{A}	187.6±8.7^{Aa}
Total bilirubin mg/dl	0.2±0.05	0.2±0.03	0.2±0.03	0.2±0.02
Conjugated b.	0.1±0.08	0.1±0.02	0.1±0.01	0.1±0.03
Uncongugated b.	0.1±0.05	0.1±0.01	0.1±0.01	0.2±0.04
Cholesterol mg/dl	121±2.3	128.6±12.9^{a}	86±2.1^{A}	93±0.7^{Aa}
Total protein g/dl	8.6±0.7	8.6±0.2^{a}	7.8±0.3^{A}	9.3±0.3^{Aa}
Albumin g/dl	4.5±0.03	3.7±0.02	4.7±0.01	4.7±0.1
Globulin g/dl	4.1±0.05	4.8±0.08	3.6±0.01	4.5±0.1
A/G ratio	1.2±0.03	0.7±0.03	1.2±0.06	1.03±0.02
Urea mg/dl	64±4.3	88.2±2.2^{Aa}	63.8±4.5	63±1.1^{a}
Creatinine mg/dl	1.2±0.2	1.5±0.4^{Aa}	0.74±0.1^{A}	0.73±0.08^{Aa}
Lipid peroxidase in liver tissue mmol/mg protein	0.255±0.005	0.196±0.001^{Aa}	0.203±0.002^{A}	0.165±0.002^{Aa}

A p< 0.05 group compared with control.
a p< 0.05 (sod. nit. turmeric) group compared with (sod.nit.) group.

Table (3): Hematological parameters (Values are mean ±SD) of 5 rats in each group after six months from start of experiment. The mean differences are significant at 0.05 level.

	Control group	SOD.NIT. group	Turmeric group	SOD. NIT. Turmeric group
RBCs(10^6/µl)	6.1±0.2	0.2±7^{A}	6.8±0.2^{A}	0.3±6.4^{A}
HB (mg/dl)	0.3±12.9	0.2±12.8^{a}	0.2±11.8^{A}	0.3±13.7^{Aa}
PCV%	±1.339.1	0.3±38.6	0.4±36.2^{A}	0.4±38.6
MCV(fl)	0.6 ±64	1.2±55^{Aa}	4.6±55.4^{A}	1.9±60.6^{Aa}
MCH(pg)	0.8±21.2	0.3±18.3^{Aa}	0.5±17.3^{A}	0.5±21.6^{a}
MCHC%	1.2±33	0.5±33.3^{a}	0.9±31.8^{A}	0.5±35.6^{Aa}
WBCs(10^3/µl)	0.7±9.6	0.4±6.9^{Aa}	1.3±14.7^{A}	0.6±11.3^{Aa}
Lymph.%	85±0.6	70±2.5^{A}	94±1.5^{A}	77.4±1.8^{A}
Neut.%	8±0.6	22.4±2.5^{A}	3±1.4^{A}	11.6±2.07^{Aa}
Mono.%	4.8±0.4	6±1.5	2±1.3	9.2±0.8^{Aa}
Esino.%	1.8±0.5	1.3±0.6	0.6±0.5	1.2±1.2
Baso.%	0.4±0.5	0.2±0.4	0.4±0.2	0.4±0.5
Platelets(10^3/µl)	1275.4±101	847^{Aa}±37.9	5128.2^{A}±593	1251.6^{a}±32.6

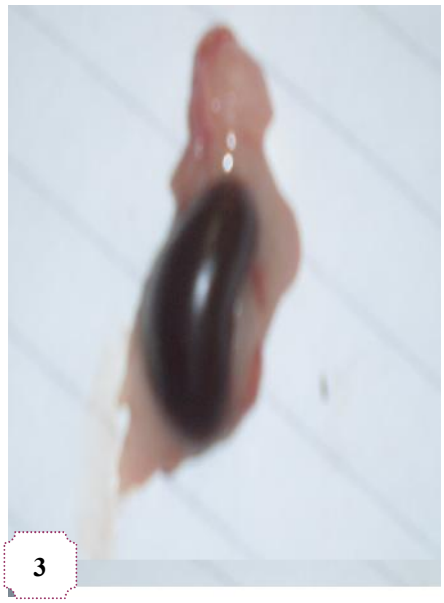
A p< 0.05 group compared with control.
a p< 0.05 (sod.nit. turmeric) group compared with (sod.nit.) group.

Table (4). Biochemical parameters (Values are mean ±SD) of 5 rats in each group after six months from start of experiment. The mean differences are significant at 0.05 level.

	Control group	SOD.NIT. group	Turmeric group	SOD. NIT. Turmeric group
S.AST u/l	118±7.9	111.6±4.4^{a}	150±3^{A}	168.8±5.6^{Aa}
S.ALT u/l	35.8±1.5	55.6±3.6^{Aa}	42.6±1.9^{A}	48.2±2.4^{Aa}
S.AlK.ph. u/l	311.2±9.4	210.6±7.5^{Aa}	369.4±31.9^{A}	149.2±5.6^{Aa}
Total bilirubin mg/dl	0.2±0.04	0.7±0.05^{Aa}	0.22±0.01	0.4±0.03^{Aa}
Conjugated b.	0.1±0.03	0.2±0.03^{aA}	0.1±0.07	0.1±0.01^{Aa}
Uncongugated b.	0.1±0.01	0.5±0.04^{Aa}	0.1±0.06	0.3±0.03^{Aa}
Cholesterol mg/dl	117.4±9.9	91.4±4.4^{A}	87.2±2.4^{A}	86.6±4.7^{A}
Total protein g/dl	8.6±0.7	7.3±0.4^{Aa}	7.9±0.3^{A}	6.5±0.3^{Aa}
albumin g/dl	4.5±0.3	5.1±0.3^{Aa}	4.3±0.2	4.4±0.3^{a}
globulin g/dl	4.1±0.5	2.2±0.1^{A}	3.6±0.2^{A}	2.1±0.2^{A}
A/G ratio	1±0.08	2.4±0.08^{Aa}	1.2±0.07	2.2±0.3^{Aa}
Urea mg/dl	63.2±4.6	52.8±3.1^{A}	62.2±4.5	56.4±4.4^{A}
Creatinine mg/dl	1.1±0.3	0.7±0.04^{A}	0.7±0.08^{A}	0.8±0.05^{A}
Lipid peroxidase in liver tissue mmol/ mg protein	0.249±0.01	0.233±0.002^{Aa}	0.200±0.001^{A}	0.189±0.005^{Aa}

A p< 0.05 group compared with control.
a p< 0.05 (sod. nit. turmeric) group compared with (sod.nit.) group.

Photo (1). Showing blood samples taken from the retro-orbital venous plexus of rats.
Photo (2). Rat treated with sodium nitrite after three months showing various degrees of congestion within viscera.



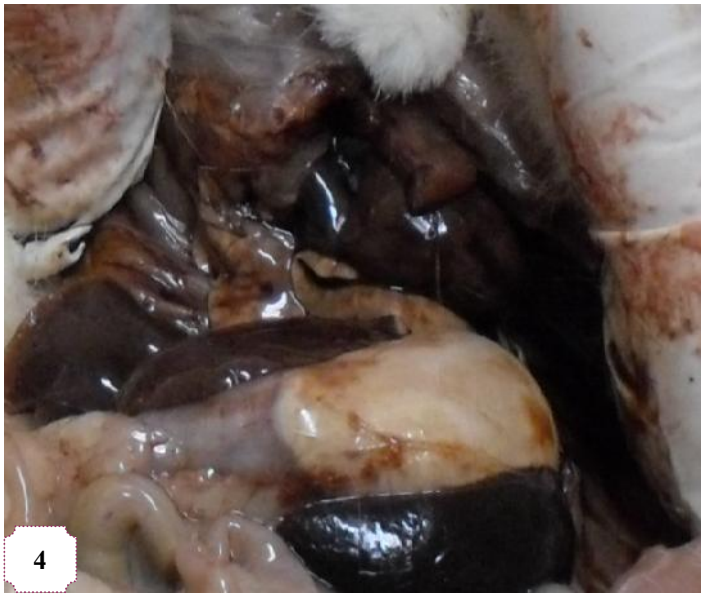


Photo (3). Rat treated with sodium nitrite after three months showing blackish discoloration of kidney.
Photo (4). Viscera of rat treated with sodium nitrite and turmeric after six months showing blackish discoloration due to the extensive haemorrhages within the viscera.

Histopathological finding.

There were no significant microscopic findings in the control untreated group along experiment period. The histopathologic examination of parenchymatous organs in different treated groups revealed that:-

1. Kidney.

The most histopathological changes of sodium nitrite accumulation on kidney after three months showed cytoplasmic degeneration of tubular epithelial cells (Fig.1a). Both proximal and distal convoluted tubules were dilated (fig.1a). Focal cellular necrosis was observed (Fig.1 a). Individual cell necrosis scattered in renal tubules (Fig.1 a &b). The renal blood vessels showed degenerative change, thickening of the wall, preivascular mononuclear cellular infiltration (Fig.1b). The renal glomerulai showed distention of Bowman's space and contraction of the glomerular tuft (Fig.1 a &b).

Six months after of sodium nitrite accumulation on the kidney showed degeneration and vacuolation in the cellular lining of the renal tubules (Fig.2a). The tubules represent necrobiotic changes of epithelial lining and mononuclear leucocytes inflammatory cells infiltration in between the degenerated renal tubules (Fig.2b&c). Focal haemorrhage between the tubules and also in the corticomedullary portion (Fig.2d). Glomerulai showed red blood cells inbetween the glomerular tuft with periglomerular inflammatory cells infiltration (Fig.2e).

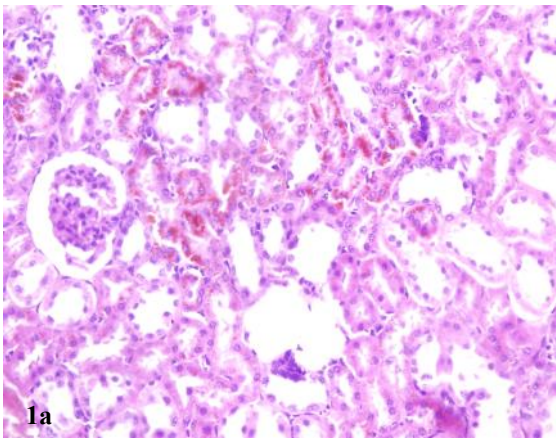
Kidney of turmeric treated rats showed degeneration and necrosis of renal tubules, also distention of glomerular space accompanied with atrophy of glomerular tuft (fig.3a & b). Focal haemorrhage between the tubules (fig.3c).

Administration of turmeric conjugated treated with nitrite accumulation after three months showed mild cytoplasmic vacuolation in the renal tissues (Fig.4a). Six months for nitrite and turmeric treated rats kidney showed Cloudy swelling degeneration and vacuolation of renal tubules (Fig.4b&c)). Red brown granules inside some of renal tubular epithelium cells or yellow red in others, these granules may be haemsidrin laden macrophage (Fig. 4d&e). Focal haemorrhage between the tubules was observed (Fig.4f). Glomerulai showed red blood cells in between the glomerular tuft with periglomerular inflammatory cells infiltration (Fig.4g&h).

Table 5. Scoring of architectural damage on histopathological examination of the rat kidneys in the different treatment groups.

Treatments	Control	Sod. Nit.	Turmeric	Sod. Nit. + Turmeric
Degeneration of renal tubules	-	++	++	++
Vacuolation of renal tubules	-	++	++	+++
Glomerular tuft atrophy	-	++	+++	+++
Focal haemorrhage	-	+++	++	+++
Individual cell necrosis	-	+++	++	++++

(–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++) indicates extremely severe.



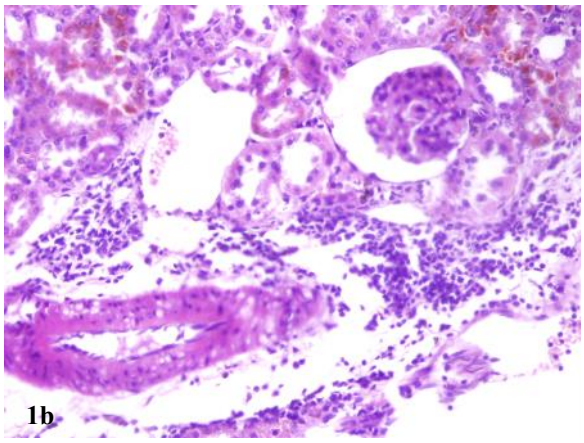
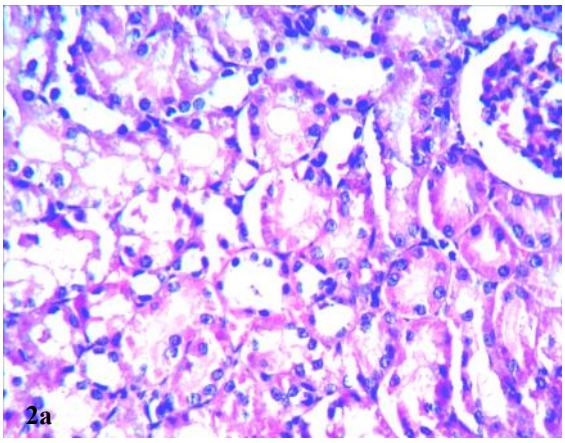
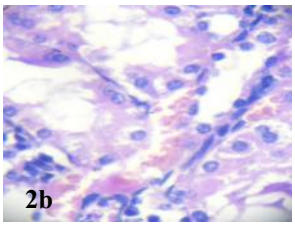
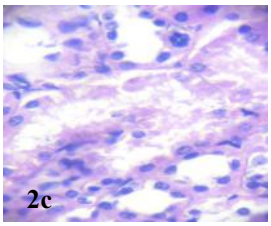
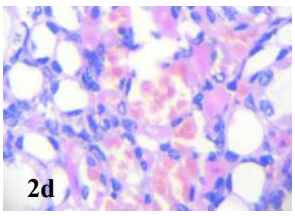


Fig. 1a: Kidney of $NaNO_2$ treated after three months showing sporadic necrosis of some of tubular epithelial of renal convoluted tubules. H&E X200.
Fig. 1b: Kidney of $NaNO_2$ treated after three months showing mononuclear cellular infiltration, The renal glomerulai showed distention of Bowman's space and contraction of the glomerular tuft with periglomerular inflammatory cells infiltration. H&E X200.









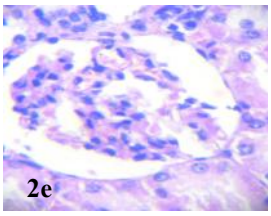
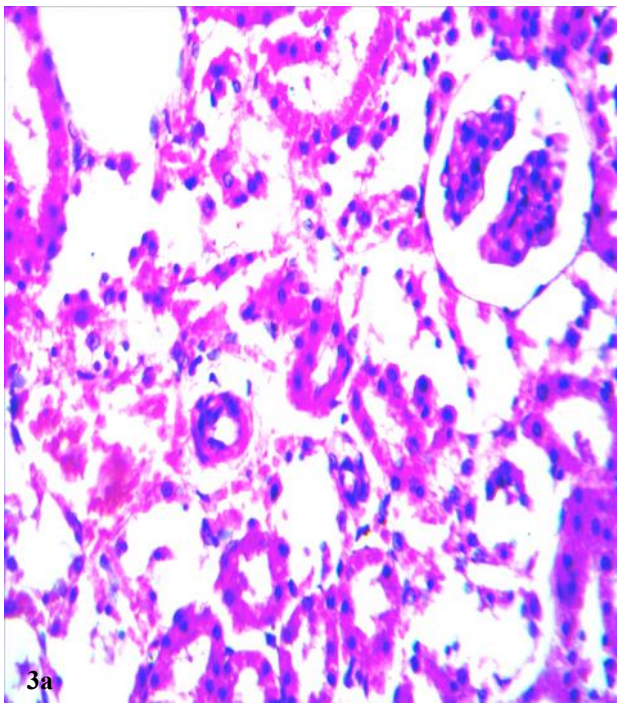
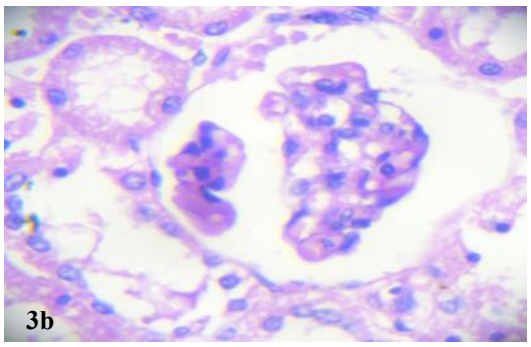


Fig. 2a: Kidney of $NaNO_2$ treated rat after six months showing vacuolation of renal tubules .H&E X250.
Fig. 2b& c: Kidney o $NaNO_2$ treated rat after six months showing necrobiotic changes in renal tubule .H&E X400.
Fig. 2d& e: Kidney of $NaNO_2$ treated rat after six months showing focal haemorrhage, periglomerular inflammatory cells infiltration. H&E X400.





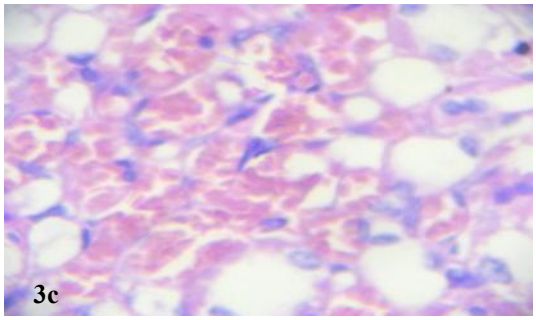
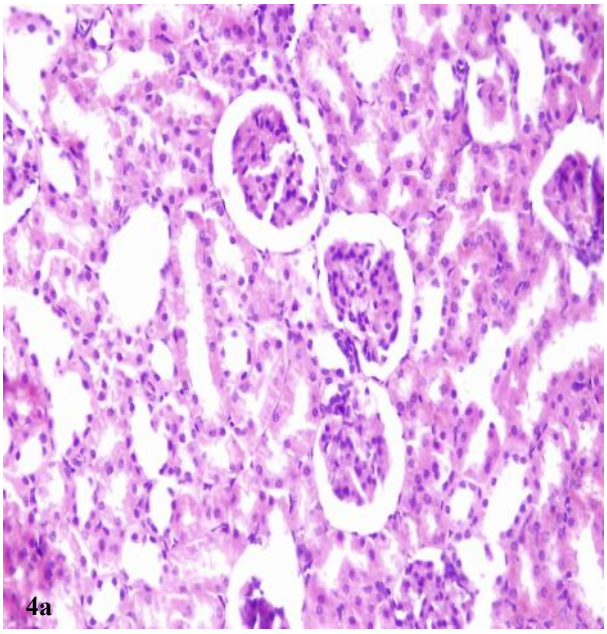


Fig. 3a: Kidney of turmeric treated rats showing degeneration and necrosis. H& E X 250
Fig. 3b: Kidney of turmeric treated rats showing glomerular tuft atrophy, H& E X 400.
Fig. 3c: Kidney of turmeric treated rats showing focal haemorrhage between tubules. H& E X 400.



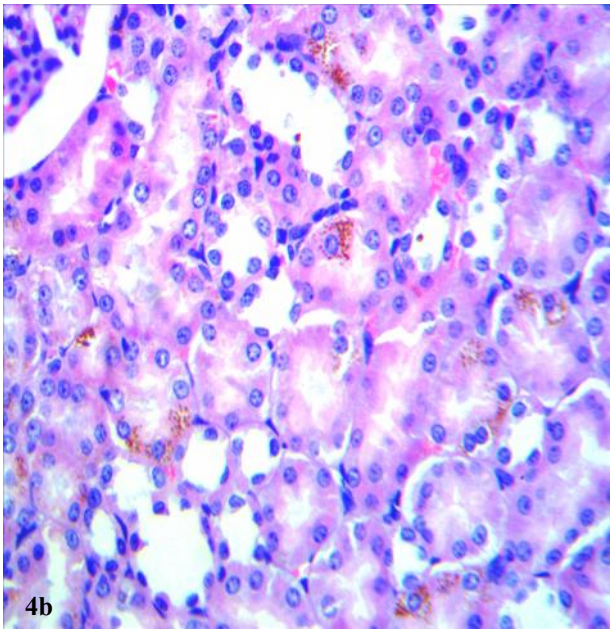
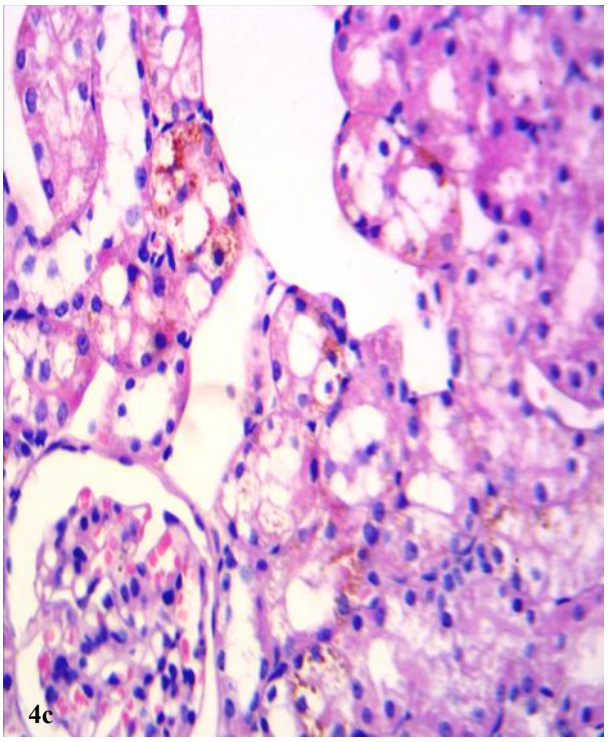
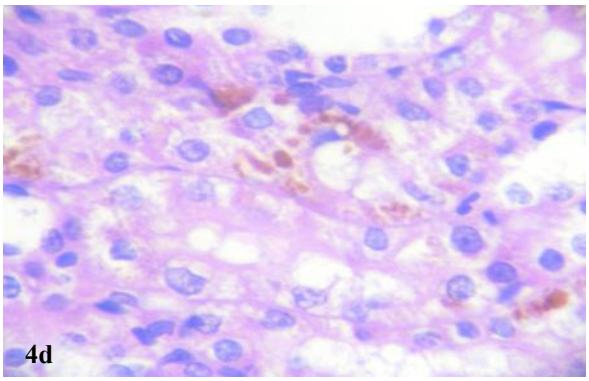
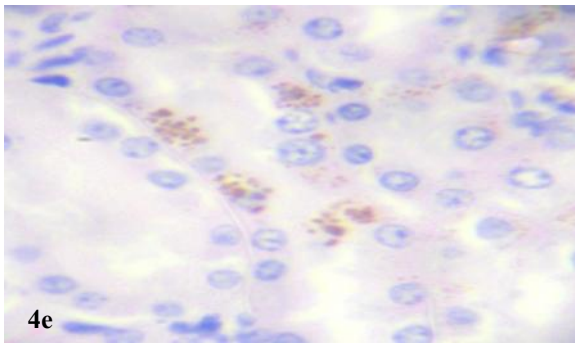
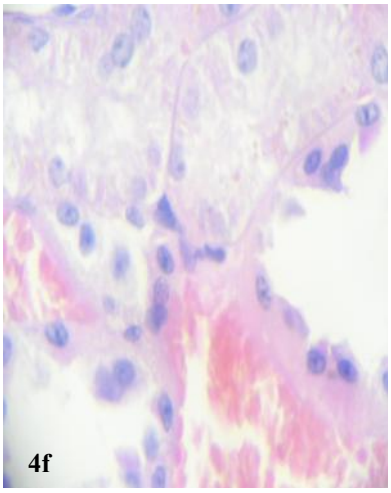


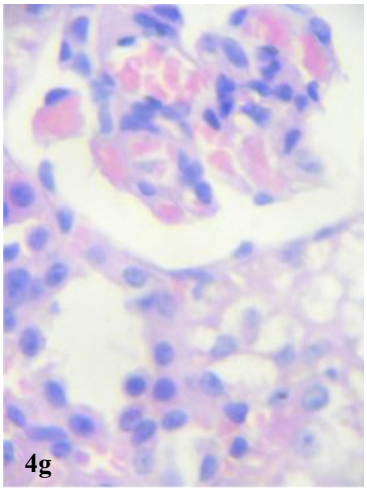
Fig. 4a: Kidney of $NaNO_2$ treated rats concomitantly administered turmeric after three months showing mild renal tubular degeneration. H&E X 200.
Fig. 4b: Kidney of $NaNO_2$ treated rats concomitantly administered turmeric after six months showing cloudy swelling. H&E X250











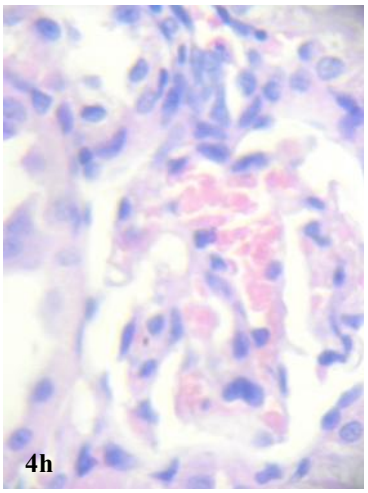


Fig. 4 c: Kidney of $NaNO_2$ treated rats concomitantly administered turmeric after six months showing renal tubular vacuolation . H&E X250.

Fig. 4d&e: Kidney of $NaNO_2$ treated rats concomitantly administered turmeric after six months showing Individual cell necrosis contain red granules. H&E X 400.

Fig. 4f: Kidney of $NaNO_2$ treated rats concomitantly administered turmeric after six months showing area of hemorrhage between degenerated renal tubular . H&E X 400.

Fig. 4 g & h: Kidney of $NaNO_2$ treated rats concomitantly administered turmeric after six months showing capillary tuft haemorrhage with periglomerular inflammatory cells infiltration. H&E X 400.

2. Liver.

The histopathological changes in liver after three months of sodium nitrite accumulation characterize by diffuse vacuolization in hepatocytes, nuclear picknosis in the majority of hepatic parenchyma (Fig.5a&b). The central veins and sinusoids were dilated and congested, (Fig. 5b, c&d). Few cells around central vein appeared necrosed (Fig.5c&d). Periportal individual cell necrosis of hepatic parenchyma mostly observed in portal areas in which the cytoplasm became more acidophilic with pyknosis or completely disappearance of nuclei and fibrosis of hepatic parenchyma (fig.5e&f).

Liver nitrite treated rats for six months showed disorganization and disorientation of hepatic plate (Fig. 6a). The hepatocyte appeared vacuolated with necrobiotic changes, pyknosis and kreorhexis of nuclei (Fig. 6b). The central veins and sinusoids were dilated and congested (Fig.6 c). The apoptotic cells were scattered in hepatic parenchyma with proliferation of kupffer cells (Fig. 6d&e).

Liver of rats feed turmeric only showed swelling and degeneration of hepatocyte and presence of eosinophilic precipitate between hepatocyte (fig.7a), dilated central vein and sinusoids which contain haemolyzed blood (fig.7b) .

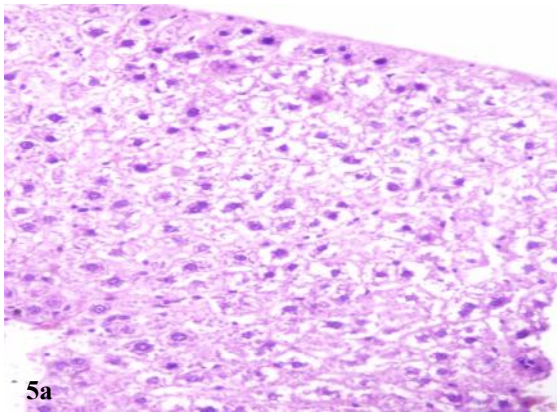
The results of histopathological examination of turmeric on liver sodium nitrite accumulation after three months showed an improvement in hepatic changes (fig.8a&b) except there were some sinusoidal dilatation and multiple vacuoles in hepatic cells (fig. 8a&b) and small areas of intercellular hemorrhage and focal mononuclear cell infiltration (fig.8a).

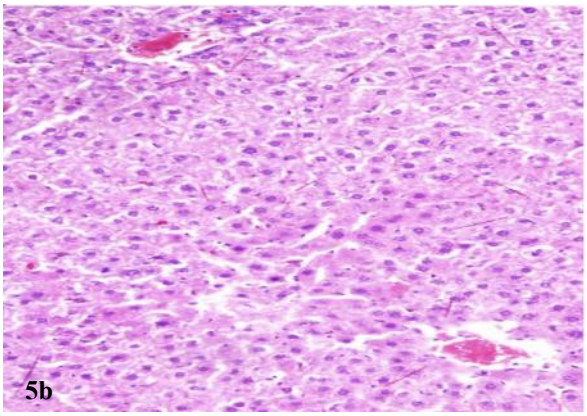
Liver of nitrite treated rats concomitantly administered turmeric after six months showed necrosis of hepatocyte, pyknosis and kreorhexis of nucleus (Fig.9a).Swelling and degeneration of hepatocyte The apoptotic cells were scattered in hepatic parenchyma (Fig.9a) , dilated central vein and Kupffer cells proliferation (Fig. 9b).

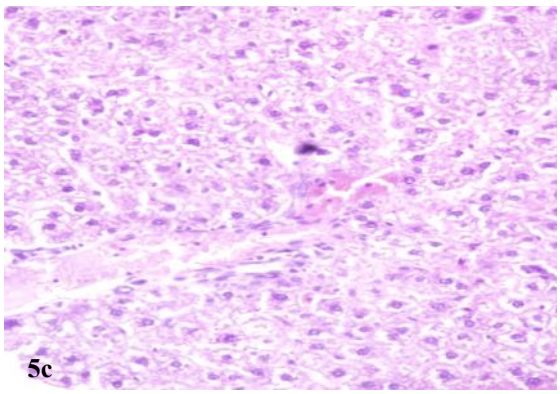
Table 6. Scoring of architectural damage on histopathological examination of the rat livers in the different treatment groups.

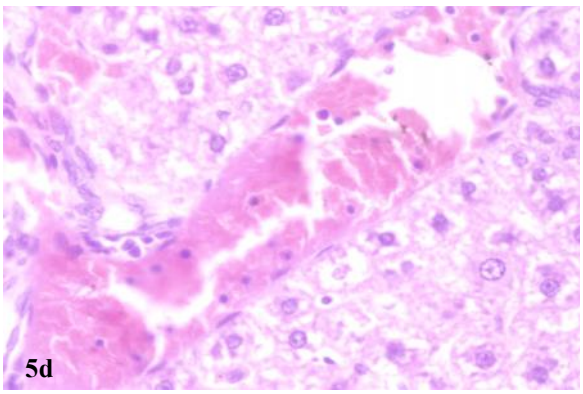
Treatments	Control	Sod. Nit	Turmeric	Sod. Nit.+ Turmeric
Degeneration of hepatocytes	-	++++	++	+++
Vacuolation in hepatocytes	-	+++	++	+++
Necrobiotic changes	-	++++	++	+++
Kupffer cells proliferation	-	+++	++	++
Dilated central vein	-	+++	+++	+++

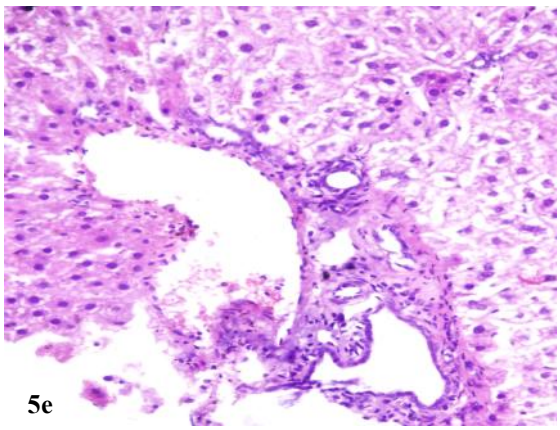
(–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++) indicates extremely severe.











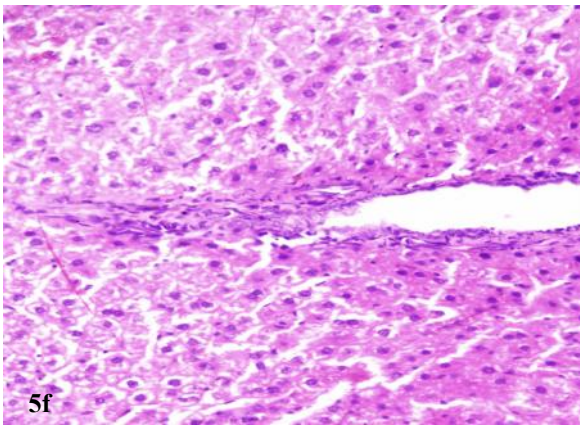


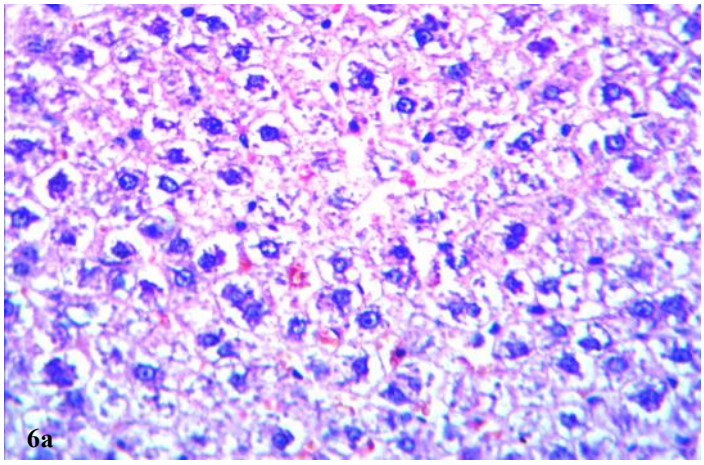
Fig. 5a: Liver of $NaNO_2$ treated after three months showing diffuse vacuolar degeneration of hepatocyte, pyknotic or karyolitic nuclei. H&E X 200.

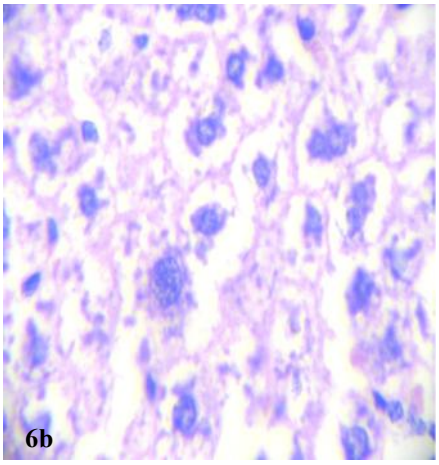
Fig. 5b: Liver of $NaNO_2$ treated after three months showing congestion of hepatic sinusoid. H&E X100.

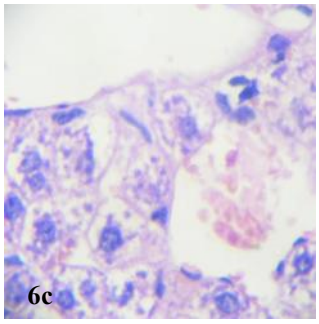
Fig. 5c: Liver of $NaNO_2$ treated after three months showing sinusoidal dilatation and congestion. H&E X 200.

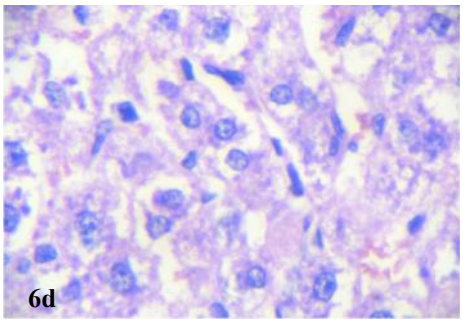
Fig. 5d: Liver of nitrite treated after three months showing hepatic blood vessels contain intravascular haemolysis . H&E X 400.

Fig. 5e&f: Liver treated with NaNO2 after three months showing periportal individual cell necrosis of hepatocyte, fibrosis of hepatic parenchyma .H & E X200.









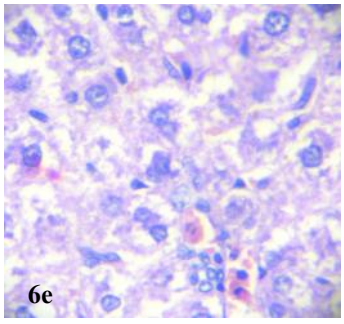
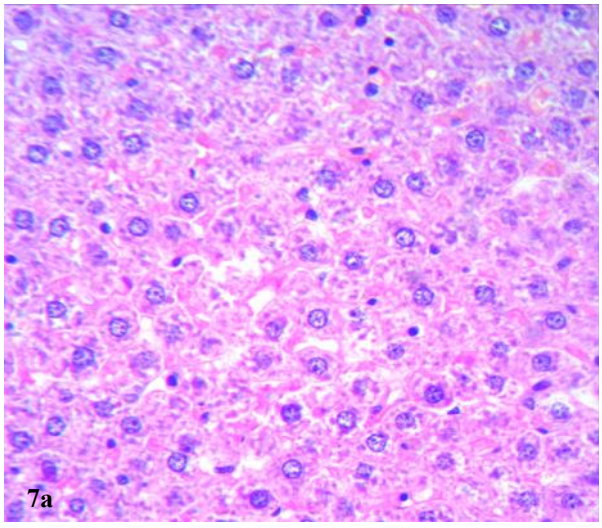


Fig.6a: Liver of $NaNO_2$ after six months showing disorganization and disorientation of hepatic plate, vacuolatin of hepatocytes . H&E X 250.

Fig.6 b, c, d& e: Liver of $NaNO_2$ treated after six months showing pyknotic or karyolitic nuclei, central vein dilatation, proliferation of kupffer cells. H&E X 400**.**



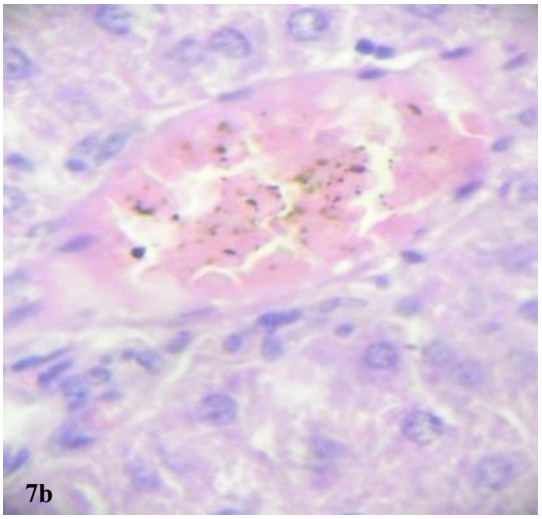


Fig. 7 a: Liver of turmeric treated rats showing swelling and degeneration of hepatocyte. H & E X250.

Fig. 7 b: Liver of turmeric treated rats showing dilated sinusoids. H & E X400.

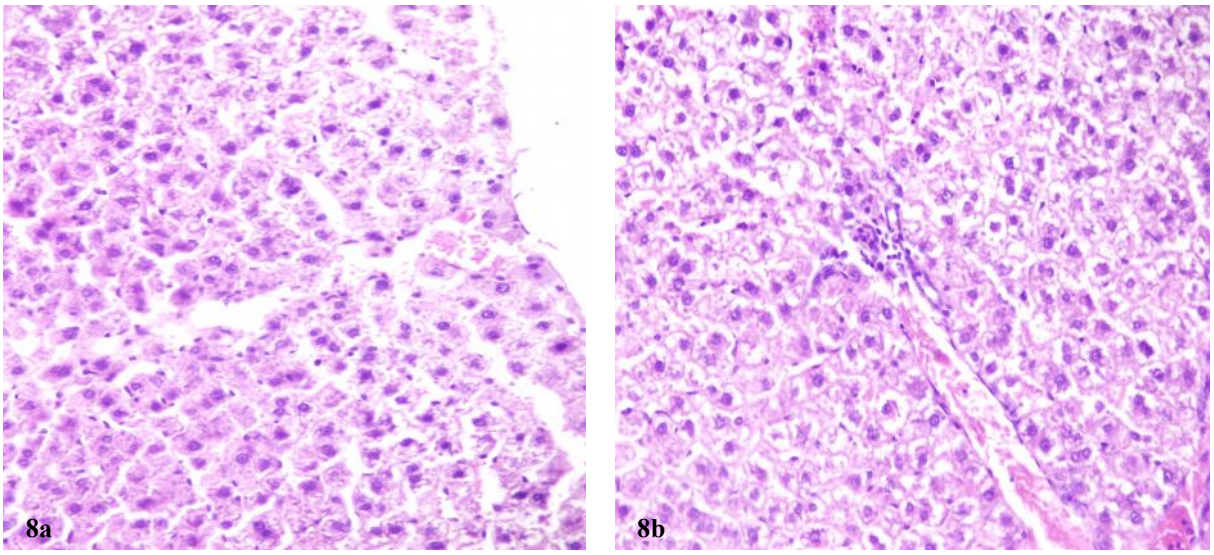


Fig. 8 a&b: liver of $NaNO_2$ treated rats concomitantly administered turmeric after three months showing cytoplasmic vacuolation, pyknosis of some nuclei, intercellular haemorrhage, dilated sinusoids

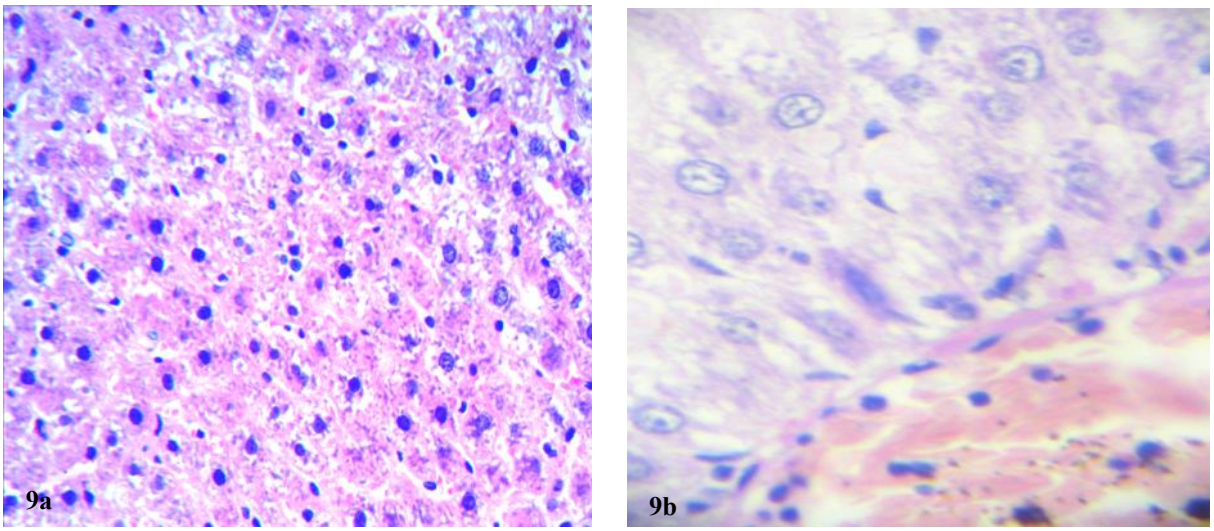


Fig. 9a: liver of $NaNO_2$ treated rats concomitantly administered turmeric after six months showing necrosis of hepatocyte with pyknosis and kreorhexis of nuclei. H & E X250.

Fig. 9b: liver of $NaNO_2$ treated rats concomitantly administered turmeric after six months showing dilated central vein. H & E X400.

3. Spleen.

The spleen of sodium nitrite accumulation showed degenerative changes in the white and red pulp represented as depletion of lymphocytes of lymphoid follicles and depletion of hematopoietic element (Fig.10a). Congestion with infiltration of macrophage (fig. 10a).

The histopathological findings of turmeric on spleen sodium nitrite accumulation group of rats showed depletion of lymphocytes of lymphoid follicles (Fig. 10b). Areas of haemorrhages associated with haemosiderin laden macrophage (Fig. 10 b&c).

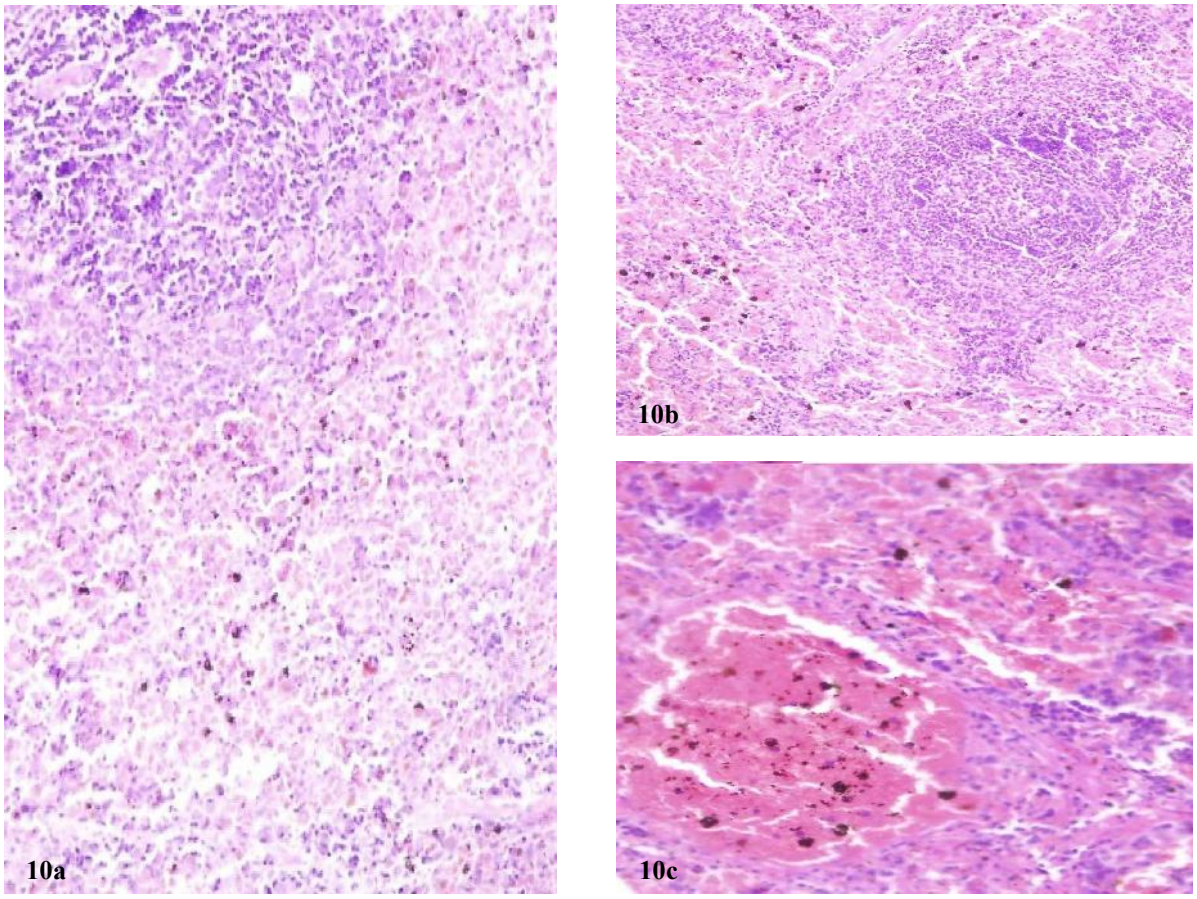


Fig. 10a: Spleen treated $NaNO_2$ after three months showing depletion of lymphocytes and infiltration of macrophage with haemosiderin. H & E X200.

Fig. 10b: Spleen of $NaNO_2$ and turmeric treated group after three months showing depletion of lymphocytes. H & E X100.

Fig. 5c: Spleen of $NaNO_2$ treated rats concomitantly administered turmeric after three months showing areas of haemorrhages associated with haemosiderin laden macrophage . H & E stain X200.

Discussion

The results of the present investigation revealed that, administration of $NaNo_2$ for three months to rats induced increase of R.B.C's count and Hct % (erthrocytosis). These results are in agreement with those reported by **Jirri *et al.*, (1983)** the number of red blood cells decreased during the first two weeks and then slowly increased. The erythrocytosis developed after administration of Na NO2 could be related to oxidant effect of nitrite on Hb resulting in increased formation of methemoglobin with decreased oxygen carrying capcity of the blood, which ultimately induce tissue hypoxia **(Imaizumi *et al.*, 1980; Hirneth and Classen, 1984; Gupta *et al.*, 2001).** The resulted methemoglobin related anemic hypoxia could induce erythrocytosis through increase of erythropiotin **(Stockham & Scott, 2002).**

Administration $NaNO_2$ for 6 months to rats resulted in slight increase in RBCS count, microcytosis and hypochromacia which indicated by decrease in MCH and MCV. Simultaneously with significant increase in uncojugated bilirubin, which could be attributed to haemolysis as explained by **Mahboob *et al.*, (2001) and Foresti *et al.*, (1997).** They mentioned that administration of $NaNo_2$ leads to increase in the activity of the endothelial hemoxygenase by nitric oxide, which degrades heme to carbon monoxide and biliverdin but this finding inconsistent with the finding of

Nyakas *et al.*, (1994) that sodium nitrite increase met Hb, but had no role on RBCs haemolysis. The swinging between erythrocytosis and microcytic hypochromic anemia as a result of sodium nitrite administration was reported by **Gluhchevaa *et al.*, (2012)** and attributed erythrocytosis to β-adrenergic action.

Helal *et al.*, (2008) demonstrated anemia (microcytic hypochromic) induced by $NaNo_2$ administration without mention of erythrocytosis, in our opinion this could be attributed to differences in dose, rout of administration of NaNo2 and gender of rat. This explanation has been augmented by the findings of **Kohn *et al.*, (2002)** they reported that 10% of the hemoglobin is oxidized to the ferric form at a dose of 11.0 mg/kg in female and 15.9 mg/kg in male rats. They also stated that methemoglobinemia was dependant on dose and route of administration of $NaNO_2$.

Administration of turmeric with sodium nitrite for three months to rats resulted in amelioration of the effect of sodium nitrite by decrease level of erythrocytosis and increase in hemoglobin concentration; however **Yan Jiao *et al.*, (2009)** mentioned that Curcumin decreased iron levels in the bone marrow and spleen. On the other hand, **(Modasiya *et al.*, 2011)** mentioned that Curcumin had no adverse effects on the level of erythrocytes count or hemoglobin. A concurrent administration of turmeric with $NaNO_2$ for six months ameliorated the hemolytic effect of $NaNO_2$. This effect could be explained by increased stability of the lysosomal membrane resulted in enhancement of blood cell survival. Similar explanation has been reached by **Banji *et al.*, (2011).**

Administration of $NaNo_2$ for three months to rats induced leucocytopenia. These results are in agreement with those reported by **Mahboob *et al.*, (2001) and Tan *et al.*, (1992)** they also attributed the decrease in WBCs count after treatment with sodium nitrite to the failure of the hematopoietic tissues to produce new WBCs. However, administration of turmeric with had no effect in spite of leucocytosis (lymphocytosis) produced by administration of turmeric alone, Similar Findings were reported in mice **Antony *et al.*, (1999).** The adverse effect of $NaNo_2$ on total WBCs count was ameliorated after six months administration of turmeric.

The present study revealed increase in ALT, AST and ALk.ph. after administration of $NaNO_2$ for three months. These findings are in agreement with those reported by **Popov *et al.*, (1996) and Bassuny *et al.*, (2004)** which indicates hepatocytic necrosis **Allis *et al.*, (1990).**

Table 2 and Table 4 were showed that in spite of administration of turmeric only increase level of AST, ALT and ALK.ph enzymes causing liver damage, Concurrent administration of turmeric with $NaNo_2$ for three months resulted in decrease level of these enzymes indicated to ameliorative effect of turmeric, after 6 month this ameliorative effect of turmeric on liver was masked; These results are in harmony with **El-Bahr, (2007)** with iron overloaded in rat and **(Hemeida & Mohafez, 2008)**, they stated that curcumin administration prevented ALT and AST increases and improved liver function. It was believed that the antioxidant activity of curcumin might be directly or indirectly associated with the maintenance or preservation of membrane integrity, which might help to prevent the elevation of serum marker enzymes **Naik *et al.*, (2011)** in addition disappearance or absence of ameliorative effect of turmeric after six months of administration could be explained based on the fact that low concentrations of curcumin induce antioxidant effects, while higher concentrations of this compound increase the cellular levels of reactive oxygen species (ROS). Moreover, the presence of 2 α, β-unsaturated ketones in the chemical structure of curcumin may also mediate some of its negative properties. These chemical groups are known to react covalently with exposed thiol groups of cysteine residues of proteins through a reaction termed Michael addition; this reaction may explain why curcumin generates ROS by irreversibly modifying the antioxidant enzyme thioredoxin reductase **Burgos *et al.*, (2010).** Moreover, in acidic condition curcumin acts as a powerful hydrogen donor while in basic pH

above 8 enolate form of the curcumin predominates and acts as a powerful electron donor a mechanism more typical for antioxidants **Modasiya & Patel, (2011).**

No significant increase in serum total cholesterol levels in rats treated with sodium nitrite. This finding was in disagreement with **Helal *et al.,* (2008).** Hypocholesterolemic effect of turmeric detected in the present study is in agreement with **Soudamini *et al.,* (1992).** This hypocholesterolemic effect of turmeric is related to its effect on the stimulation of bile acid and biliary cholesterol secretion as well as enhanced excretion of bile acids and cholesterol in feces **Srinivasan & Sambaiah, (1991).**

The results of administration of $NaNO_2$ demonstrated impairs kidney functions which indicated by increase in urea and creatinine. These results are consistent with **Pfeifer & Weber, (1979) and Shehata, (2005)**. Turmeric administration erases this increasing in urea and creatinine, Administration of $NaNo_2$ for 6 months induced significant decrease in total protein and globulin concentrations, which could be attributed to formation of nitric oxide or peroxynitrite that oxidizes proteins and lipoproteins. These results are in agreement with **Gow *et al.,* (1998) and Guzik *et al.,* (2000)**. Oxidative stress refered to an imbalance between intracellular production of ROS and the cellular antioxidant defense mechanisms **Ogur *et al.,* (2000)**. In other words, oxidative stress is responsible for many deleterious effects in cell, including DNA damage **Stohs *et al.,* (2001)**. Nowadays, trends on applying nutritional antioxidants in ailments related to oxidative stress have gained immense interest. Herbal plants such as tumeric are known to exert their health effects by scavenging free radicals and modulating antioxidant defense system. In the present study, LPO was decreased in group treated with sodium nitrite and turmeric, this result is in agreement with **El-Wakf *et al.,* (2011)**. This indicated to use of turmeric counteracted nitrite-induced toxicity.

The most histopathological changes in the kidney of rats treated with sodium nitrites showed cytoplasmic degeneration of tubular epithelial cells, cellular necrosis and picknotic nucleoli . The renal blood vessels showed degenerative change, thickening of the wall, preivascular mononuclear cellular infiltration .These results were in agreement with those obtained by **Abd El-Tawab *et al.,* (2003)** who reported sodium nitrate induces fibrosis around glomerulus and renal tubules, which may be due to nitric oxide (NO) formations which cause vascular smooth muscle relaxation which leads to dilatations of their lumens and increas their blood flow. The permanent vasodilatation and congestion causes cellular hypoxia and death, which may be followed by fibrosis **Took, (1996)**. **Abd El -Tawab *et al.,* (2003)** found that sodium nitrate causes dilatation and congestion of glomerular and pertubular capillaries. In this study the renal glomerulai showed distention of Bowman's space and contraction of the glomerular tuft. Sodium nitrite induced renal toxicity was in accordance with that obtained by **Hassan *et al.,* (2009)**, this toxicity may be attributed to increased generation of NO that leads to increased lipid peroxidation **Goligorsky *et al.,* (2002)** or may be due to direct effect of sodium nitrite which alters transport of sodium and chloride in the distal nephron and may also adversely affect the renal function **Zaki *et al.,* (2004)** or through changes in the threshold of tubular re-absorption, renal blood flow and glomerular infiltration rate. Histopathological examination of kidney of rats treated with both sodium nitrite and turmeric after three month showed some improvement in kidney picture and absence of some pathological alterations which were detected in sodium nitrite rat group but . Kidney of sodium nitrite and turmeric treated group after six month showed degeneration and necrosis of renal tubules with increase in glomerular space. **Iqbal *et al.,* (2003)** found that curcumin protects against chemical carcinogenesis and other forms of electrophilic toxicity. Our results were in disagreement with **Yun *et al.,* (2009) and Masuda *et al.,* (2001)** who demonstrated that curcumin prominently reduced tissue injury, in present study the histpathological finding demonstrated degenerative changes as result of tur-

meric only (Table 5).

The alternative changes of liver of rats treated with sodium nitrites included diffuse vacuolization in hepatocytes , nuclear pyknosis in the majority of hepatic parenchyma similar result has been observed by **Rees *et al.*, (1990); Zaidi and Phil** (**2010)** who found that sodium nitrite induced hypoxia resulted in extensive vacuolization and degeneration of hepatocytes in periportal region. Also these findings are in agreement also with that mentioned by **Hassan *et al.*, (2009)** recorded liver necrosis induced by sodium nitrite and attributed the liver damage to the toxic effect. **Akpabio *et al.*, (2013)** reported periportal necrosis of hepatocytes with mononuclear cellular infiltration. **Sharma *et al.*, (2013)** Studied mild necrosis of hepatocytes, with infiltration of inflammatory cells in between hepatocytes and bridging necrosis and portal triditis. Hepatic blood vessels was dilated engorged with intravascular haemolysis with perivascular lysis. Moreover, **Sarsour and Hassuneh, (2001)** reported that sodium nitrite causes inflammatory reaction and infiltration of inflammatory cells that subsequently release large quantities of potential oxidants as $H2O_2$ that might induce damage to surrounding tissue and cells. The result of histopathology examination on livers of rats fed sodium nitrite after six months showed periportal necrosis of hepatocytes with mononuclear cellular infiltration. **Mathews, *et al.*, (2012)** mentioned Curcumin treatment protection by preserving the structural integrity of the cell and the protective mechanism of curcumin may due to the strong antioxidant property, it helped for the healing of hepatic parenchyma and regeneration of hepatocytes but treatment by curcumin did not restored normal hepatic histopathology which could be due its antioxidant nature that combines free radical scavenging and metal chelating properties. This results revealed there was some improvement in liver upon treatment of sodium nitrite intoxicated rats after three month with turmeric powde rbut ater six months administration of turmeric in conjugation with sodium nitrite showed no amelioration of pathological changes (Table 6).

The results of histopathological examination of spleen upon treatment of sodium nitrite intoxicated rats after three month showed depletion of lymphocytes of lymphoid follicles and depletion of hematopoietic element. These findings are in agreement with that mentioned by **(Sharm & Hemlata, 2012)** who recorded congestion of white pulp and severe red pulp expansion and infiltration of neutrophils in red pulp. The results of histopathological examination of spleen upon treatment of sodium nitrite intoxicated rats after three month with turmeric powder showed areas of haemorrhages associated with haemosiderin laden macrophage. The result in disagreement with **El-Ashmawy *et al.*, (2006)** who reported Simultaneous treatment that turmeric are useful herbal remedies, especially for controlling oxidative damages and genotoxicity.

Conclusion.

- Even low dose of sodium nitrite has adverse effect on liver function blood picture and immunity.
- Oral administration of turmeric (10g/kg diet) for female rat for long time had adverse effect on liver and decreased hemoglobin concentration.
- In histpathological administration of turmeric in conjugation with sodium nitrite showed after three months some improvement in kidney and liver picture and absence of some pathological alterations but had no effect on pathological changes caused by sodium nitrite toxicity in spleen.
- After six months Administration of turmeric in conjugation with sodium nitrite showed no amelioration of pathological changes.
- Erythrocytosis is the first sign of exposure to oxidizing agent or generation of free radicals and it could be useful in its diagnosis.
- Methemoglobinemia alone dose not suffice to cause red cell destruction.
- So, no count on turmeric to have an influential antioxidant in processed food products against used sodium nitrite as a preservative.

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استخدام الكركم للحد من الاعراض الجانبية للتسمم المزمن بنيتريت الصوديوم في الفئران.

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الملخص العربى

أجريت الدراسة الحالية لمعرفة الآثار السلبية لسمية النيتريت في إناث الفئران واستخدام الكركم في التخفيف من حدة هذه الاثار .تم استخدام اربع مجموعات من إناث الفئران تحتوى 12 فار لكل منهم، المجموعة الاولي (المجموعة الضابطة) ، المجموعة الثانية تلقت 2 جرام من نيتريت الصوديوم في واحد لتر من مياة الشرب. المجموعة الثالثة اعطيت 10 جرام من الكركم لكل واحد كحم من العليقة. اما المجموعة الرابعة اعطيت بالإضافة إلى2 جرام من نيتريت الصوديوم في واحد لتر من مياة الشرب 10 جرام من الكركم لكل واحد كحم من العليقة يوميا يوميا لمدة 6 اشهر. وأظهرت المجموعة التي تلقت العلاج نيتريت الصوديوم بعد 3شهورزيادة عدد الكريات الدم الحمراء ،زيادة الصفائح الدموية وانخفاض في عدد كريات الدم البيضاء و زيادة في انزيمات الكبد ALT، AST، ALP ، اليوريا و الكرياتينين بالمقارنة بالمجموعة الضابطة ، اظهرت المجموعة المعاملة بالكركم تحسين التأثير السلبي لنيتريت الصوديوم بارتفاع الهيموجلوبين والكريات البيضاء ، وانخفاض AST، ALT . ALP واليوريا و الكرياتينين ولكن هذا التأثير أصبح ضعيفا بعد6 شهر ، بالإضافة إلى أن تخفيض مستوى الصفراء بالمقارنة مع المجوعة الثانية, أظهرت المجموعة التي تلقت الكركم انخفاض في الهيموجلوبين ، وزيادة في كل خلايا الدم ، وزيادة في AST، ALT، ALP وانخفاض الكوليسترول و البروتين الكلي مقارنة مع المجموعة الضابطة . أظهرت التغيرات الهستوباثولوجية للفئران المعالجة بنيتريت الصوديوم المزمن بعد3 شهور افات متعددة حيث كانت في شكل تنكرزخلايا الأنابيب الكلوية مع تغلظ نوويي ، انتشار الفجوات في خلايا الكبد ، كما أظهر الطحال وجود خلايا الميكروفاج الحاملة للهيموسيديرين .اثبت فحص الفئران التي عوملت بالكركم مع نيتريت الصوديوم تحسنا بعد الشهر الثالث في كل من الكلي و الكبد و اظهرت عدم استجابة في الطحال. ولكن بعد ستة أشهراظهرت جميع الأعضاء عدم الأستجابة للكركم و كانت التغيرات الباثولوجية في المعاملتين متشابهة الي حد كبير.

ولذلك فان نيتريت الصوديوم حتي ولو في جرعة بسيطة له تأثير سيء علي وظائف الكبد وصورة الدم و المناعة. والكركم بصورتة المتوفرة في الاسواق في جرعة (10 جرام لكل 1 كجم عليقة) له تأثير سيء علي الكبد ويسبب نقص ملحوظ لنسبة الهيموجلوبين في الدم لاناث فئران التجارب لمدة طويلة . وتناول الكركم مع الصوديوم نيتريت يقلل من التأثير السيء للنيتريت ولكن هذا التأثير الايجابي للكركم يقل بعد ستة شهور. الزيادة العددية لخلايا الدم الحمراء تعتبر من المؤشرات الاولية لتعرض الجسم للمواد المؤكسدة ولذلك يمكن الاعتماد علي ذلك في التشخيص. وجود الميتهيموجلوبين والناتج عن تناول نيتريت الصوديوم ليس السبب في تكسير خلايا الدم الحمراء. لذلك لا يعتمد علي الكركم لتفادي سلبيات المواد الحافظة والتى تضاف للمنتجات الغذائية .