Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 56 (1): 56-68. Jan. 2018
DOI: 10.5455/ajvs.278511

Comparative Studies on Turmeric and Vitamin C on Sodium Nitrite treated Rats

Shahenaz M.H. Hassan[1], Nahed F. Zaglool[2], Sanaa A. El-shamy[3]

Clinical pathology department[1], Biochemistry department [2], Pathology department[3] -Alexandria, Animal Health Research Institute.

Key words:
Sodium nitrite, Turmeric, Vitamin C, Lipid peroxidation, Superoxidedismutase, Glutathione reductase,

Correspondence to:
eshahenaz@yahoo.com

ABSTRACT

The current study was performed to investigate the comparative effect of Turmeric and vitamin C on the oxidative damage caused by sodium nitrite in rats. Sixty albino rats were used, divided into four equal groups, group (1) considered as a control group, group (2) received sodium nitrite ($NaNO_2$) 30mg/kg.bw., group (3) received (NaNO2) 30mg/kg.bw. and vitamin C 200 mg /kg bw., group (4) received ($NaNO_2$) 30mg/kg.bw. and turmeric 0.5g/ kg.bw. Animals were daily treated for five weeks. Sodium nitrite treated Rats showed a microcytic hypochromic anemia , significant increase ($p \leq 0.05$) in total leukocytes (WBCs), total billirubin , lipid peroxidase(LPO), superoxidedismutase (SOD), significant decrease in glutathione reductase (GR), globulin (β, γ globulin) and vitamin C value as compared to control group. ($NaNO_2$) with Vitamin C treated Rats showed significant increase in RBCs count, HB value, globulin (β, α) and vitamin C value ,significant decrease in total billirubin, (LPO)and (SOD)as compared to ($NaNO_2$) treated group. ($NaNO_2$) with turmeric treated Rats showed slight ameliorating effect through significant increase in RBCs count but hemoglobin value was declined, significant decrease in total billirubin, (LPO)and (SOD), significant increase in globulin (γ globulin) and (GR) as compared to ($NaNO_2$) treated group. Histopathological changes of sodium nitrite treated rats after five weeks exhibited cytoplasmic vacuolization of hepatocytes, dilated congested veins and mononuclear cells infiltration. The damage also extended to kidney represented by shrinkage glomerular tuft, distention of Bowman's space, degeneration of lining tubular epithelial, The results declared that vitamin C preceded the turmeric in ameliorate the toxic effect of sodium nitrite on rats.

1. INTRODUCTION

The use of sodium nitrite as a preservative is common in cooked meat and sausages. Nitrite serves a vital public health function: it blocks the growth of botulism-causing bacteria and prevents spoilage. Nitrite also gives cured meats their characteristic color and flavor. 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 permissible level (Bilczuk et al., 1991). 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).

(Halliwell & Gutteridge 1999) mentioned that Vitamin C is an important dietary antioxidant, it significantly decreases the adverse effect of reactive oxygen and nitrogen that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, neurodegenerative diseases and cataractogenesis. Turmeric is widely used for our preparation of food, which is the powdered form of Curcuma longa rhizomes contain curcumin which has been shown to have anti-inflammatory activity(Gupta et al.,2008). Turmeric has shown to be a powerful antioxidant through inhibiting generation of reactive oxygenspecies (ROS) both in vitro and in vivo (Joe & Lokesh 1994).Therefore, the present study aimed

to investigate and compare the ameliorating effect turmeric and vitamin C against the adverse effect of sodium nitrite in rats.

2-MATERIALS AND METHODS

2.1- Chemicals:

a. Sodium nitrite was obtained from the El-Gmhoria Co®. , **b**- vitamin C 20% (powder) was obtained from veterinarian pharmacy. **c**- Turmeric powder was obtained from local market at Alexandria city, Egypt.

2.2 –Animals:

Clinically healthy, sixty, female albino rats weighing about 160-175g were used throughout this study. Animals were housed in clean metal cages, fed on pellet diet and water ad libitum. They were acclimatized for 2weeks prior to the experiments.

2.3-Design of experiment:

Rats were randomly divided into four equal groups (15 rats each) as the following:

Group (1) kept as a control negative group. Group (2) received sodium nitrite 30mg/kg.bw via gastric intubation according to (Helal et al., 2008). Group (3) received sodium nitrite 30mg/kg.bw and vitamin C 200 mg /kg bw. via gastric intubation according to (Eteng et al.,2006). Group (4) received sodium nitrite 30mg/kg.bw. and turmeric 0.5g/ kg.bw via gastric intubation according to (Anand et al., 2007). All rats were treated with the respective regime daily for five weeks. Blood and tissue samples (liver and kidney) were collected from all rats at the end of the experiment.

2.4- Hematological analysis:

Blood samples were taken from the retro-orbital venous plexus from each rat of all groups. The two blood samples were collected one with EDTA for hematological analysis and other for separation of serum for biochemical analysis. Erythrocytic count (RBCs) was performed using improved Neubauer Haemocytometer and Gower,s fluid as a diluting fluid , leukocytic count (WBCs) were done using improved Neubauer Haemocytometer with turkey's solution , 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 & Zijlistra, (1961). The values of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin concentration (MCHC) were calculated by standard formula , The prepared fixed thin blood films were stained with Geimsa stain. The film examined for morphological changes in RBCs and WBCs and The percentage values of differential leukocytic count were calculated according to Thrall et al. (2004).

2.5- Serum biochemical analysis :

Serum total protein was determined according to Doumas et al. (1981). Serum albumin was determined according to Doumas et al. (1971) using kit provided by Diamond Diagnostics. Serum globulins were obtained mathematically and Albumin / Globulins ratio (A/G ratio) was calculated. Also α, β and γ Ig were determined according to (Narayanan ,1982 and Price et al., 1983). Serum aspartate aminotransferase (AST), Serum Alanin aminotransferase (ALT) activities were determined according to Reitman & Frankels (1957) . Serum urea level was determined according toPatton & Crouch (1977). Serum creatinine was determined according to Husdant & Rapaport (1958) using kits provided by bioMerieux . Lipid peroxidation (LPO) was measured by the method of Buege (1978) using kits provided by (*Biodiaguostic*)®. glutathion reductase activity was measured following the standard procedure (Goldberg & Spooner, 1983).Superoxide dismutase (SOD) activity was determined by the degree of inhibition on the reduction of cytochrome c by superoxide anion generated by the xanthine oxidase/ hypoxanthine system (Flohe, & Otting, 1984). determination of Vitamin C (Brody 1994) .

2.6-Pathological examinations:

All rats were sacrificed at the end of experiment and subjected to careful post mortem examination. Tissue specimens were collected from liver and kidney. The specimens were immediately fixed in 10% neutral buffered formalin and then processed, stained with Heamatoxylin and Eosin and microscopically examined according to Bancroft et al. (1996) .

2.7-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 was analyzed by computer using SPSS version 16.0 for windows. (Statistical Package for the Social Sciences Inc, Chicago, Illinois).

3. RESULTS

3.1. Heamatobiochemical results:

As shown in table (1) sodium nitrite treated rats showed a significant decrease in RBCs count, Hb, PCV, MCV value, neutrophil %(neutropenia), Significant increase ($p \leq 0.05$) in MCH, MCHC, total leukocytes compared to control group . ($NaNO_2$) and vitamin C treated group showed improved hematological marker through significant increase in RBCs count, HB, PCV value and neutrophil % as compared to sodium nitrite treated group. ($NaNO_2$) with turmeric treated rats showed slight ameliorating effect through significant increase in RBCs count , PCV value and neutrophil% but hemoglobin value was declined compared to sodium nitrite treated group (fig.1). As shown in table (2,3) ($NaNO_2$) treated Rats exhibited a significant increase in AST, ALT, total billirubin ,conjugated billirubin, unconjucated billirubin and creatinine , lipid peroxidase and superoxide dismutase, Significant decrease ($p \leq 0.05$) in vitamin C value, glutathione reductase , total protein, albumin, and globulin (β, γ globulin) compared to control group. ($NaNO_2$) and vitamin C treated rats showed improved biochemical serum marker through significant decrease in AST, ALT, total billirubin ,conjugated billirubin, unconjucated billirubin and creatinine, significant increase in total protein ,albumin, globulin (β, α) and vitamin C value ;in addition, antioxidant status showed a significant improvement where a significant decrease in lipid peroxidase and superoxide dismutase compared to ($NaNO_2$) treated rats. ($NaNO_2$) and turmeric treated rats showed significant decrease in AST, ALT, total billirubin ,conjugated billirubin, unconjucated bilirubin, lipid peroxidase and superoxide dismutase , significant increase in total protein and globulin (γ globulin) and glutathione reductase compared to ($NaNO_2$) treated rats . Mentioned above results indicated vitamin C preceded the turmeric in ameliorate the toxic effect of sodium nitrite on rats.

3.2. Pathological examination:

3.2.1 Liver :

Histopathological findings of liver of sodium nitrite treated rats after five weeks exhibited a marked loss in its characteristic architecture, congestion, hepatocellular damages, and disruption of the endothelial lining of central vein (fig.2 a) liver of a rat treated with nitrite showing hydropic degenerated hepatocytes (fig.2b). Vacuolar degeneration in hepatocyte with pyknotic nuclei and focal necrotic area (fig.2c) and infiltered with mononuclear leukocytes were noticed (fig.2d). Activation of kupffer cells scattered through degenerated tissue (fig.2e) diffuse vacuolar degeneration of hepatocyte, pyknotic or karyolitic nuclei (fig.2f). Administration of vitamin C with sodium nitrite resulted in marked improvement in the histological structure of central vein , sinusoid and hepatic cells with mild centrilobular congestion (fig.3a). inflammatory cells infiltration(fig. 3b). (fig.3c) showed normal histological appearance with activation of Kupffer cells. Liver of sodium nitrite treated rats with turmeric showed congestion of central vein and hepatic sinusoid (fig.4a) besides congested portal vein (fig.4b). Livers exhibited dilatation and congestion of hepatic sinusoids (fig.4c).

3.2.2. Kidney:

Microscopic examination of the kidney of rats treated with sodium nitrite for five weeks showed alterations of kidney parenchyma such as the renal glomeruli showed distention of Bowman's space and contraction of the glomerular tuft (fig 5a). Mononuclear cellular infiltration in-between degenerated tubules (fig.5b). Administration of vitamin C with sodium nitrite resulted in marked improvement in renal tubules and glomerulus. The tubules appeared with their normal structure, The lining epithelium of some proximal and distal convoluted tubules in renal cortex appeared mild degenerated and exhibited mild vacuolar degeneration (fig.6). Kidney of sodium nitrite and turmeric treated rats showed some shrinkage of glomerular tufts with disruption of Bowman's capsule and expansion of the space (fig.7a). necrosis of the tubular epithelium ,destruction and desquamation of epithelial lining in their lumen (fig.7b) interstitial lymphocytic cellular infiltration, eosinophilic cytoplasm with shrunken, pyknotic nuclei (fig.7c)

Table (1): Hematological parameters (Values are mean ±SD) in the control and different treated groups after 5 weeks from start of experiment. N=5

	Control group	**Sodium nitrite group**	**Sodium nitrite Vitamin C group**	**Sodium nitrite Turmeric group**
RBCs(10^6/µl)	7.1±0.2 a	5±0.2 c	6.8±0.1 a	5.7±0.4 b
HB (mg/dl)	14±0.1a	11.8±0.1c	13.4±0.5b	11.3±0.48d
PCV%	43.8±0.8 a	29.2±0.8 c	42±2.7 a	34.4±1.9 b
MCV(fl)	61.3±2 a	58.1±1.6 b	62.5±1.4 a	59.6±2.2 b
MCH(pg)	19.6±0.4 b	23.6±0.8a	19.5±0.5 b	19.3±1.5b
MCHC%	32±0.7b	40.4±0.8a	31.6±0.5b	32.2±1.3b
WBCs(10^3/µl)	8.6±0.6b	10.8±0.7a	8.2±0.4b	9.5±0.9a
Lymph.%	83.5±0.3b	92.5±0.4a	58.8±0.5c	81.2±0.3b
Neut.%	13.6±0.6b	4.3±0.7c	36.3±0.4a	15.1±0.4b
Mono.%	2.5±0.5c	2.6±0.6c	4.5±0.3a	3.1±0.6b
Esino.%	0.4±0.4b	0.6±0.2a	0.4±0.1b	0.6±0.2a
Baso.%	0	0	0	0

Values superscripts with different letters (a-d) were significantly different (P≤ 0.05).

Table (2): Biochemical parameters (Values are mean ±SD) in the control and different treated groups after 5 weeks from start of experiment. N=5

	Control group	**Sodium nitrite group**	**Sodium nitrite vitamin C group**	**Sodium nitrite Turmeric group**
S.AST u/l	84±7.9 **c**	158.2±26.7**a**	71.2±4.5 **c**	103.6±6.7 **b**
S.ALT u/l	33±2.3d	56±2.7a	40±1.5c	44±2b
Total billirubin mg/dl	1.3±0.01[d]	2.4±0.01[a]	1.5±0.01[c]	1.7±0.01[b]
Conjugated b.	0.57±0.02d	1.27±0.02a	0.65±0.02 c	0.79±0.02b
Uncongugated b.	0.74±0.02c	1.12±0.02a	0.79±0.02c	0.92±0.02b
Urea mg/dl	61±1.6a	54.2±1.3c	61±1.6a	56.6±2.3b
Creatinine mg/dl	0.13±0.01c	0.68±0.05a	0.26±0.03b	0.29±0.04b
Vitamin C level (mg / dl)	1.79±0.1[a]	0.65±0.05[c]	1.82±0.28[a]	1.28±0.08[b]

Values superscripts with different letters (a-d) were significantly different (P≤ 0.05).

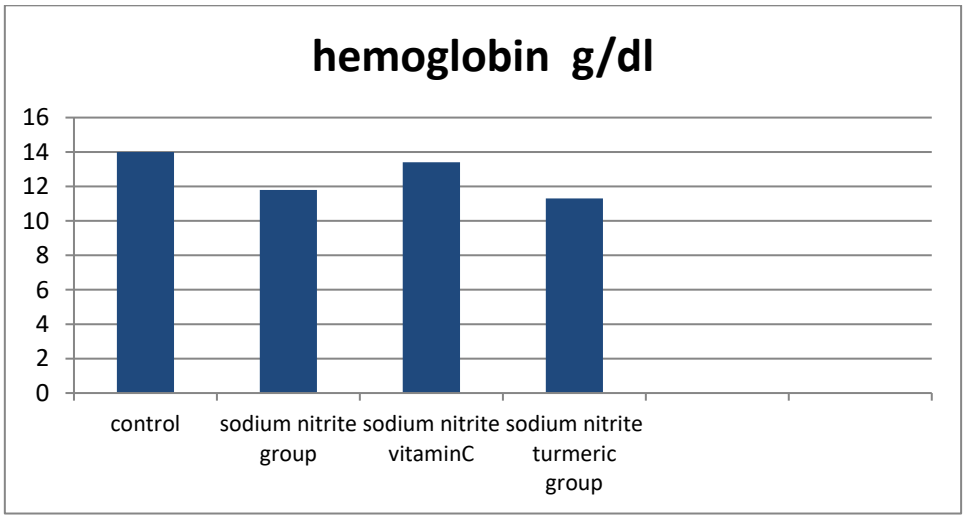

Fig. 1: Hemoglobin value in the control and different treated groups after 5 weeks from start of experiment.

Table (3): biochemical parameters (Values are mean ±SD) in the control and different treated groups after 5 weeks from start of experiment. The mean differences are significant at 0.05 level. n=5

	Control group	Sodium nitrite. group	Sodium nitrite vitamin C group	Sodium nitrite. Turmeric group
Glutathion reductase (U /mg protein)	4.04± 0.29[a]	2.5± 0.18[d]	3.3± 0.08[c]	3.6± 0.23[b]
Lipid peroxidation concentration (U /mg protein)	0.58±0.02[d]	1.24±0.1[a]	0.79±0.04[c]	0.91±0.01[b]
Superoxide dismutase (SOD) activity (U/mg protein)	25.2±1.7[d]	64.3±3.9[a]	35.9±2.9[c]	46.2±1.6[b]
Total protein(g/dl)	5.5±0.08[c]	4.2±0.2[d]	6.3±0.1[a]	6±0.2[b]
Albumin (g/dl)	2.9 ±0.12[a]	2.4±0.36[b]	2.7±0.20[a]	3±0.13[a]
Globulin (g/dl)	2.5±0.04[c]	1.7±0.39[d]	3.4±0.33[a]	2.9±0.25[b]
A/G	1.16±0.01b	1.4±0.04a	0.79±0.01d	1.03±0.01c
Gamma Globulin (g/dl	1.3±0.07[b]	0.4±0.02[d]	1.6±0.15[a]	1.1±0.02[c]
Beta Globulin (g/dl)	0.75±0.03[b]	0.63±0.08[c]	0.91±0.1[a]	0.91±0.07[a]
Alpha Globulin (g/dl)	0.54±0.01[b]	0.66±0.32[b]	1±0.12[a]	0.94±0.17[a]

Values superscripts with different letters (a-d) were significantly different (P≤ 0.05).

4. DISSCUSSION

As shown in table (1) administration of sodium nitrite 30mg/kg.bw daily to rats for 5 weeks revealed to significant decrease in RBCs count, Hb, PCV, MCV compared to control group which indicate to a microcytic hypochromic anemia and Significant increase ($p \leq 0.05$) in MCH and MCHC compared to control group, this was accompanied by an increase of total billirubin , unconjucated billirubin and this might be as a result of heamolysis. These results are in agreement with those obtained by Mahboob et al. (2001); Bassuny et al. (2004). Hemolysis attributed to increase in the activity of the endothelial heme oxygenase by nitric oxide, which degrades heme to carbon monoxide and biliverdin (Foresti et al.,1997). Administration of ascorbic acid concurrent with sodium nitrite improved all the blood parameters measured. This was clear in vitamin C treated rats which revealed significant increase in RBCs count, Hb, PCV, MCV as compared to ($NaNO_2$) treated group. Similar results were reported by Shehata (2005) found that ascorbic acid reduced methaemoglobin and increased haemoglobin concentrations in humans who drank water naturally polluted with nitrate.

Additionally, Hirneth & Classen (1984) reported that vitamin C inhibited the production of plasma nitric oxide and methaemoglobin in female rats fed with a diet containing 5% NaNO3.

Administration of turmeric concurrent with sodium nitrite ameliorated effect of ($NaNO_2$) by slight increase in RBCs, PCV value. On the other hand, it lead to lower Hb level(fig.1) , these results were consistent with Sanaa et al.(2014) they said that administration of turmeric concurrent with ($NaNO_2$) for six months ameliorated the hemolytic effect of nitrite. This ameliorative effect could be explained by increased stability of the lysosomal membrane resulted in enhancement of blood cell survival. Similar explanation has been reported by Banji et al.(2011). Additionally, Yan et al. (2009) mentioned that curcumin decreased iron levels in the bone marrow and spleen. In the current study.

There was significant increase ($p \leq 0.05$) in total leukocytes, lymphocytes (lymphocytosis) with neutropenia in rats treated with sodium nitrite compared to control group. This finding was consistent with Sharma & Hemlata (2012) . On the other hand, Tan et al. (1992) reported that decrease in WBCs count after treatment with sodium nitrite and they explained this finding by failure of the hematopoietic tissues to produce new WBCs. As shown in table (2) sodium nitrite treated rats revealed a significant increase in AST, ALT these findings are in agreement with Bassuny et al. (2004) ; Sanaa et al.(2014) which indicate hepatocytic necrosis (Allis et al, 1990).

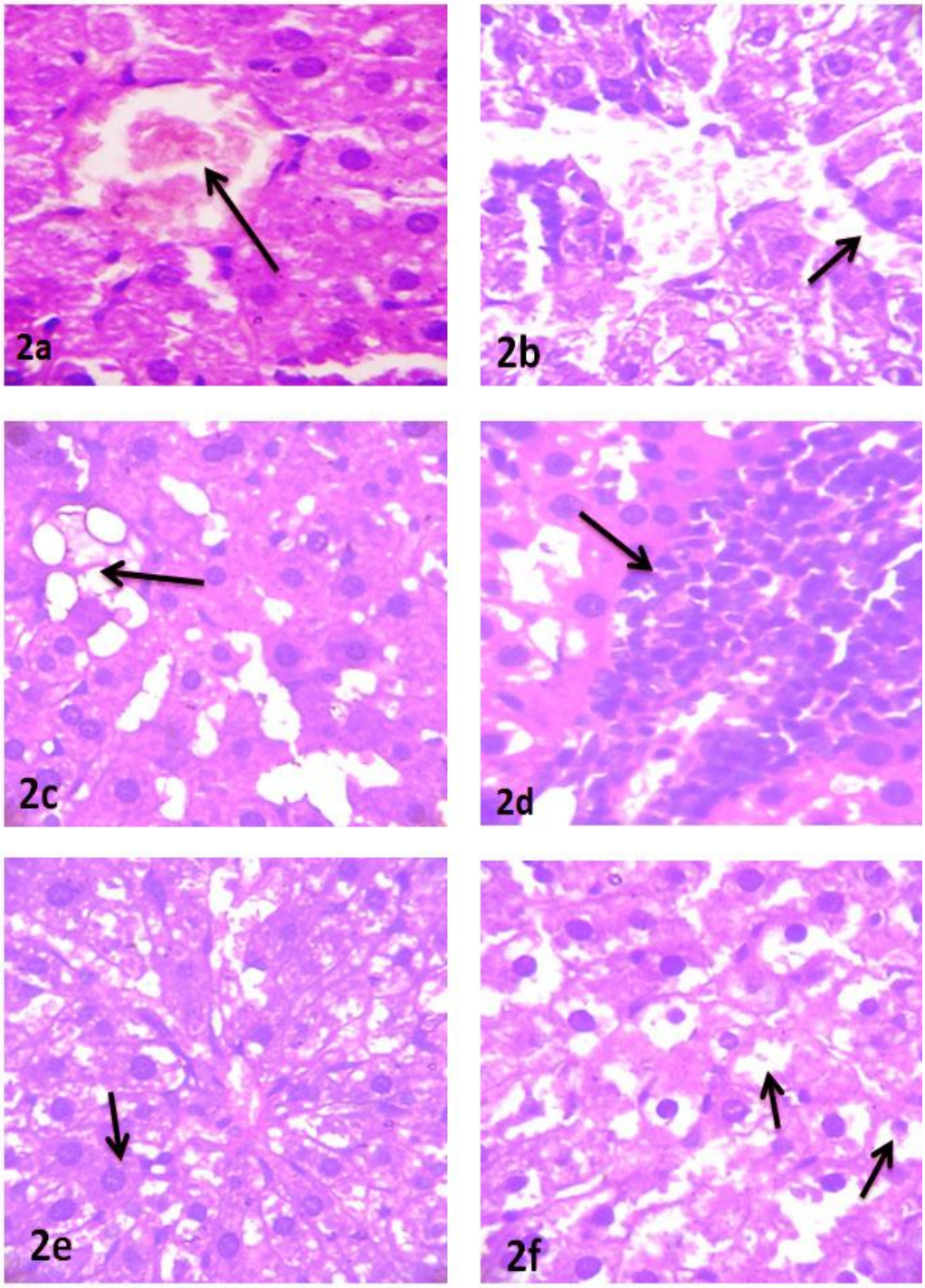

Fig. 2: Liver of NaNO2 treated after five weeks showing (a&b) congestion of central vein and hepatic sinusoid. (c) vacuolar degeneration in the cytoplasm of hepatocytes (d) infiltration of mononuclear leukocytes (e) kupffer cells scattred through degenerated tissue (f) diffuse vacuolar degeneration of hepatocyte, pyknotic nuclei.H&E X 400.

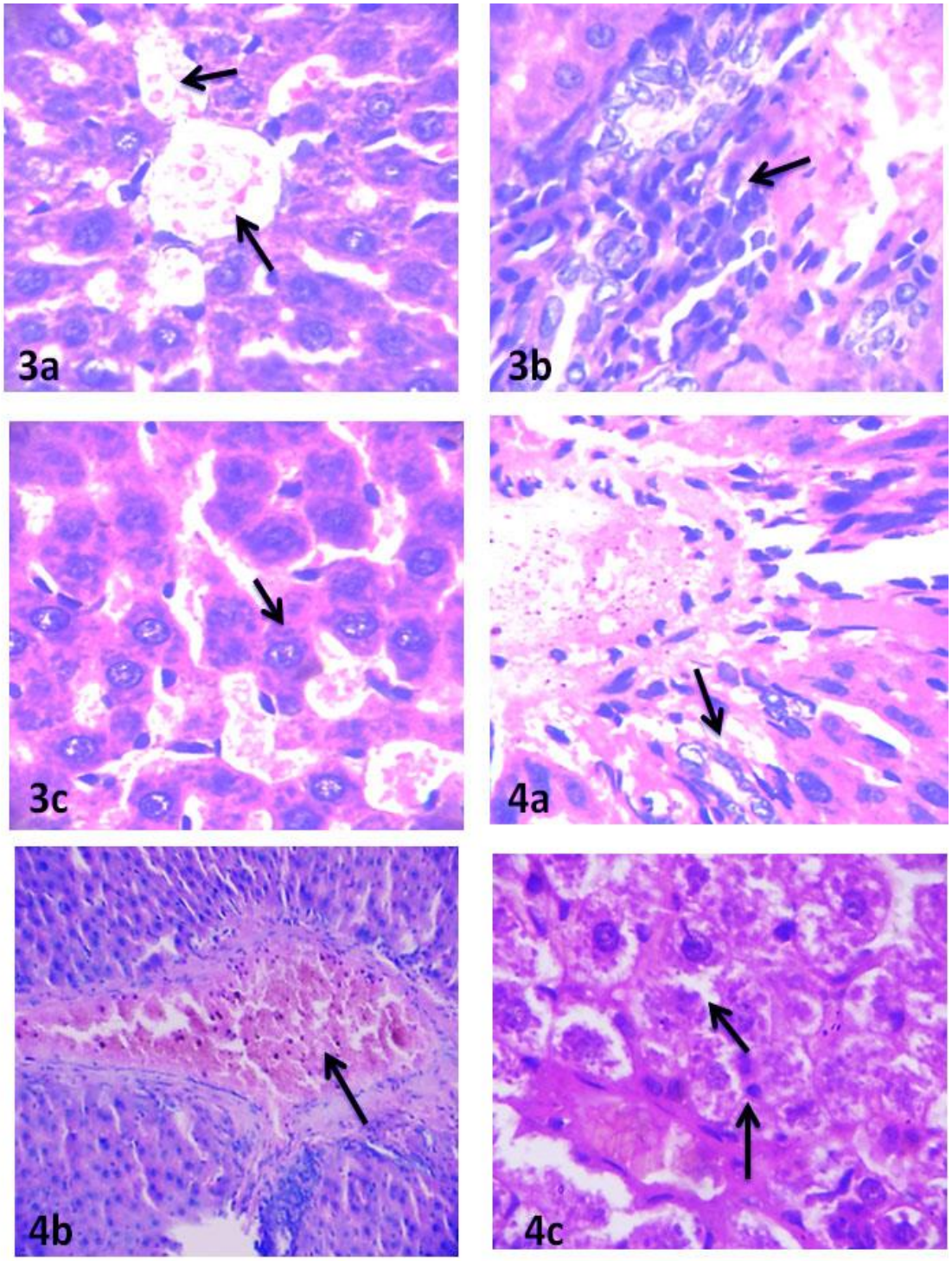

Fig.3: Liver of NaNO2 and vitamin C treated after five weeks showing (a) mild Congestion of the central veins , hepatic sinusoid (b) inflammatory cells infiltration (c) Kupffer cells proliferation .H&E X400. **Fig. 4**: liver of NaNO2 turmeric treated rats after five weeks showing (a X 100) degenerative changes of central vein and hepatic sinusoid. (bX 400) congested portal vein (c) dilatation and congestion of hepatic sinusoids in-between degenerated hepatic cells and pyknosis and kreorhexis of nuclei H&E X400.

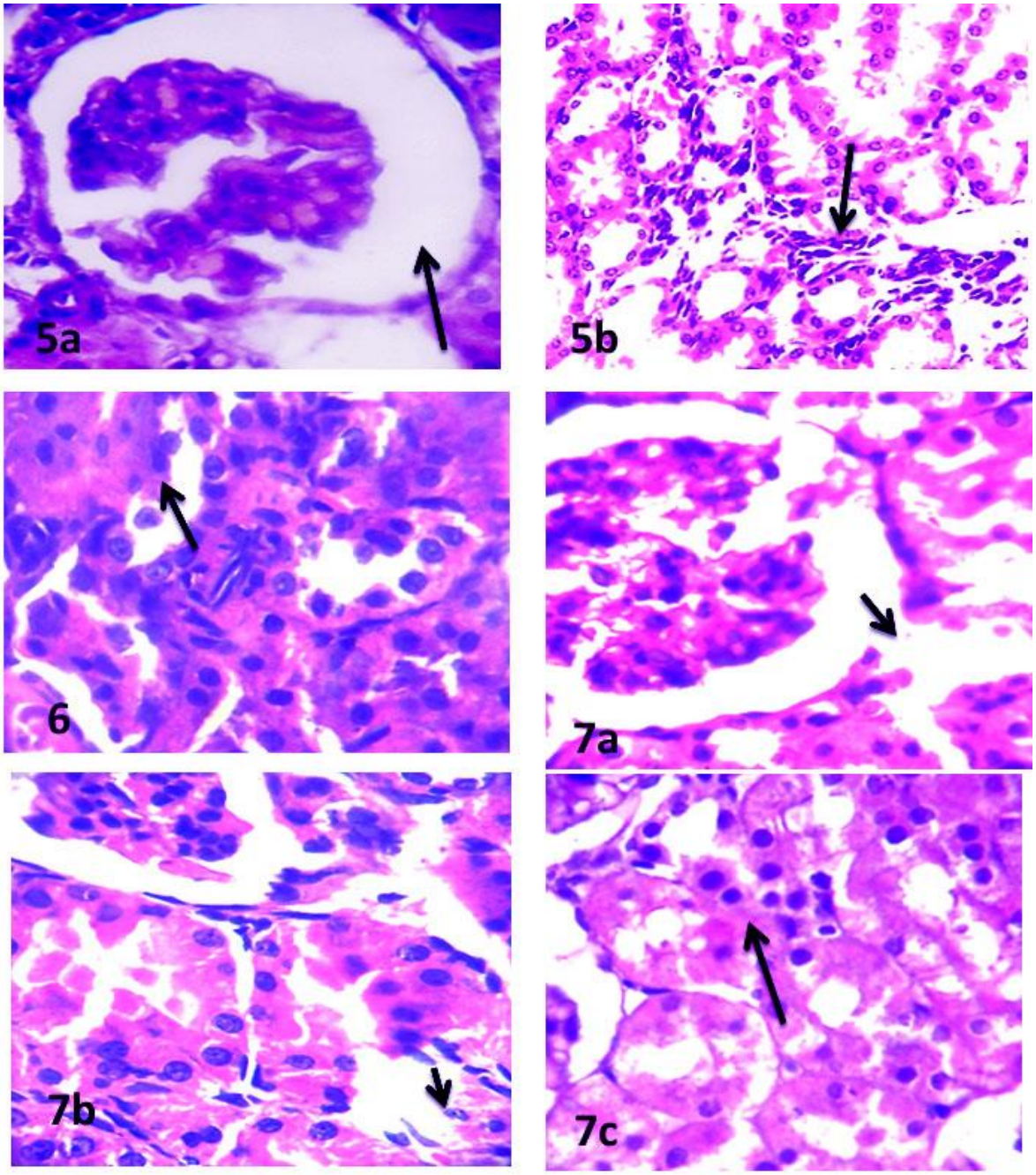

Fig . 5 : Kidney of NaNO2 treated after five weeks showing (a) distention of Bowman's space and contraction of the glomerular tuft (bX250) mononuclear cellular infiltration in-between degenerated tubules **Fig.6**: kidney of NaNO2and vitamin C treated after five weeks showing Mild degeneration in lining epithelium of tubules .H&E X400. **Fig.7**: Kidney of NaNO2 treated rats administered turmeric after five weeks showing (a) shrinkage of glomerular tufts with disruption of Bowman's capsule (b) necrosis of the tubular epithelium ,destruction and desquamation of epithelial lining in their lumen(c) interstitial lymphocytic cellular infiltration, pyknotic nuclei.H&E X100.

The ameliorative effect of vitamin C administration which appeared in the decrease in the activities of liver marker enzymes such as AST, ALT in vitamin C treated rats may be due to the antioxidant effect of vitamin C, which is reported to protect the liver from damage. Vitamin C may protect lipids and lipoproteins in cellular membranes against oxidative damage caused by toxic free radicals, thus may prevent certain types of hepatic cellular Damage (Krishnamoorthy and Sangeetha, 2008). administration of turmeric resulted in slight decrease level of liver enzymes as compared to sodium nitrite treated group indicate to ameliorative effect of turmeric ,These results are in harmony with Hemeida & Mohafez (2008) ; Sanaa et al. (2014) , 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. Administration of ($NaNO_2$) impairs kidney functions which indicated by increase in creatinine but turmeric or vitamin C administration ameliorated this effect. These results are consistent with Abdelaziz and Ashour (2007) ; Sanaa et al. (2014) . As shown in table (3) sodium nitrite treated rats revealed significant decrease in total protein, albumin, globulin (β, γ globulin) . these finding are agreement with Bassuny et al.(2004) ; Shehata (2005). The decrease in total protein, albumin and globulin concentrations may be due to formation of nitric oxide or peroxynitrite, which oxidises proteins impairs liver metabolism (Zraly et al.,1997).

Shehata (2005) mentioned that the decreased globulin concentration caused by nitrate treatment might indicate an immunodepressive response. In contrary, There was significant increase in total protein and globulin in vitamin C treated rats this result was consistent with Tarasub et al. (2012) .As shown in table (2) sodium nitrite treated rats revealed significant increase lipid peroxidase, superoxide dismutase as compared to control group. On other hand, there were significant decrease ($p \leq 0.05$), in glutathione reductase , these findings are consistent with (Krishnamoorthy and Sangeetha, 2008) found that LPO level increased due to sodium nitrite administration but vitamin C treatment significantly reduced the lipid peroxidation level and nitrite content (Santos et al., 2009) . Gonchar et al. (2006) said that over dose of nitrite caused decrease in glutathione reductase (GR). Vitamin C treated rats showed significant decrease in LPO as compared to sodium nitrite treated group. This result was harmony with Krishnamoorthy & Sangeetha (2008) . In current study, vitamin C or turmeric treated rats showed significant increase in glutathione reductase . The increase in glutathion level reveals the better recovery of hepatic cells from the oxidative stress induced by nitrite toxicity. As glutathione is the major cellular nucleophile, it provides an efficient detoxification pathway for a variety of electrophilic reactive metabolites (Biswas et al., 2005; Krishnamoorthy and Sangeetha, 2008) . In contrary, acute high doses of Vit C act as prooxidant and induced oxidative stress that resulted in decreased glutathione reductase activity Marković et al. (2010). In addition, El-wakf & El-kholy(2011) found that LPO was decreased in-group treated with sodium nitrite concurrent with turmeric, this result indicated to use of turmeric counteracted nitrite-induced toxicity. SOD is the scavenging enzymes that remove the toxic free radicals (Wohaieb & Godin, 1987). In the enzymatic antioxidant defense system, SOD is one of the most important enzymes and scavenges O_2 anion to form H_2O_2, thus diminishes the toxic effects due to this radical or other free radicals derived from secondary reactions (Bhattacharya et al., 1999). In the present study, sodium nitrite treated rats showed significant increase in SOD level , this result was consistent with Hazneci et al. (2005) suggested that prominent oxidative stress, particularly in the presence of high levels of O_2^-, leads to high levels of SOD. Sodium nitrite treated rats showed significant decrease in vitamin C level , this result was consistent with Librojo (1978) who reported that rats showed a decrease in blood ascorbic acid levels with increased dietary nitrite. Histopathological findings of liver of sodium nitrite treated rats after five weeks exhibited a marked loss in its characteristic architecture. Severe toxic changes were observed such as congestion, hepatocellular damages, hepatic cord degeneration, and disruption of the endothelial lining of central vein and dilatation of the hepatic

sinusoids. These changes could be the results of membrane distribution induced by nitrite. Klatskin and Conn, (1993) attributed the dilatation of the blood sinusoids to the direct toxic effect of the toxin leading to their dilatation. Also, the dilatation of the sinusoids may be attributed to hepatic congestion that results from a direct action of the treatment on the vessel wall or the back pressure in the portal space (Azeez et al., 2011). The most striking histological feature recorded in rats of nitrite treatment was vacuolar degeneration in the cytoplasm of hepatocytes, pyknotic nuclei and focal necrotic area infiltered with mononuclear leukocytes. Hepatocellular necrosis is probably due to the direct attached of the cell membranes by the hepatotoxin or by interacting with some specific components of the metabolic pathways leading to the alteration of their structure and function . Klatskin and Conn, (1993). These results are in accordance with Hussein et al.,(2012); Sanaa, and Mahmoud (2012) reported hydropic degenerated hepatocytes, necrotic areas infiltrated with a number of inflammatory cells .Similar changes in the hepatic tissue of adult rats have been reported by the previous findings of Ogur et al., (2005). The morphological features induced by high sodium nitrite completely reflected the classic apoptotic features(Khan et al.,2012). In this result found out that the co-administration of sodium nitrate and vitamin C ameliorated pathological changes caused by sodium nitrate it helped for the healing of hepatic parenchyma and regeneration of hepatocytes and great improvement in liver upon treatment of sodium nitrite intoxicated rats .The report of Krishnamoorthy and Sangeetha (2008) gave credence to the hepatoprotective effect of vitamin C against metallic compounds induced liver toxicity. Fouad et al., (2017) reported treated with vitamin C can ameliorate the toxic effects of sodium nitrite in albino rats. Liver of sodium nitrite and turmeric treated rats showed mild degenerative changes Similar changes in the hepatic tissue of adult rats have been reported by the previous findings of Sanaa et al.(2014). Mathews et al. (2012) mentioned curcumin treatment protection by preserving the structural integrity of the cell and the protective mechanism of curcumin, it helped for the healing of hepatic parenchyma and regeneration of hepatocytes. Several reports have linked its protective action to anti-inflammatory and anti-infectious activities of this plant (Srinivas, et al.,1992). The protective effect of curcumin may be explained by the fact that it prevents cellular damage occurring as a result of oxidative stress (Aly et al., 2009). In the present study, microscopic examination of the kidney of rats treated with sodium nitrite after five weeks showed alterations of kidney parenchyma. Most of these changes were congestion of glomerular tufts, distention of Bowman's space and contraction of the glomerular tuft , dilated convoluted tubules , cytoplasmic degeneration of tubular epithelial cells , mononuclear cellular infiltration in-between degenerated tubules, necrosis of the lining epithelium of tubules , fibroblasts in between renal tubule. Congestion, tubular degeneration and necrosis were described by Sultan et al. (2000) and Abd El-Tawab et al. (2003). Sodium nitrite toxicity induced degenerative changes in renal tubules with congestion of glomerular capillary (Fouad, et al., 2017). 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 increase their blood flow. The permanent vasodilatation and congestion causes cellular hypoxia , which may be followed by fibrosis (Tooke, 1996). Also the tubules come in contact with toxic chemicals during their excretion and elimination by the kidneys (Tisher 1989). The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of the cells (Shimizu et al.,1996). In the present study, it was found that the co-administration of vitamin C. and sodium nitrite led to marked improvement in histopathological changes. Consequently, using ascorbic acid alleviated the histopathological effects of sodium nitrite, by (Reham et al., 2016). Vitamin C exerted ameliorating effect on sodium nitrite- led to increased antioxidant enzymes activities (Krishnamoorthy and Sangeetha, 2008). Kidney of sodium nitrite and turmeric treated rats showed mild degeneration of renal tubules. the same result was mentioned by Sanaa et al.(2014). (Iqbalet al. 2003) found that curcumin protects against chemical toxicity. El-Wakf et al., (2011) noticed that tumeric are not effective in reducing nitrate toxicity. but (Yan et al., 2009) and (Masuda,et al.,2001) demonstrated that curcumin prominently reduced tissue injury. This contradiction could be attributed

to turmeric is the raw material but curcumin is active principle of turmeric.

5. CONCLUSION

It is recommended to limit the use of sodium nitrite as a food preservative for damage caused by it. Moreover, use of vitamin C or turmeric to reduce the harmful effect of sodium nitrite, while taking into account that turmeric negatively affects the level of hemoglobin.

6. REFERANCES

Abdelaziz, I. A, Ashour, A. A. 2007. Biochemical Studies on Albino Rats after Administration of Nitrosamine and the Therapeutic Actions of Vitamin C , Honey Bee or Crushed Citrus Seeds. Islam. Univ. J. 15(2):177–188.

Allis, J.W., Ward, T.R., Seely, J.C., Simmons, J. E. 1990. Assessment of Hepatic Indicators of Sub-Chronic Carbon Tetrachloride Injury and Recovery in Rats. Fund.Applid. Toxicol. 15:558–570.

Aly, H., Mansour, A., Abo-Salem,O., Abd-Ellah, H., Abdel-Naim, A. 2009. Potential Testicular Toxicity of Sodium Nitrate in Adult Rats. Food Chem. Toxicol. 48:572–578.

Anand, P., Ajaikomar B. K., Robert A. N., Bharat B. A., 2007. Bioavailability of Curcumin: Problems and Promises. Mol. Pharmaceut. 4(6):807–818.

Azeez, O., Mahmood, M., Hassan, J. 2011. Effect of Nitrate Poisoning on Some Biochemical Parameters in Rats. Iraq. J. Vet. Sci. 25(2):47–50.

Bancroft, J. D., Stevens, A., Turner, D. R. 1996. Theory and Practice of Histological Techniques. 4th ed. New York Edinburgh Madrid, San Francisco, Tokyo.: Churchill Living Stone.

Banji, D., Jyothi, P., Otilia J., Banji F. , Ranjith, K., Narsi, R. 2011. Evaluation of the Concomitant Use of Methotrexate and Curcumin on Freund's Complete Adjuvant-Induced Arthritis and Hematological Indices in Rats. Indian J Pharmacol. 4(5):546–550.

Bassuny, S., Shehata, S., Bahgat, L., Mohamed, S. 2004. Nitrate Toxicity in Rabbits: 1- Effect of Nitrate in Drinking Water on Digestion, Some Blood Constituents and Growth Performance of Growing Rabbits. Egyptian J. Rabbit Sci. 14:147–158.

Bhattacharya, A., Abhijit C., Shibnath G.l, Salil K. B.. 1999. Antioxidant Activity of Active Tannoid Principles of Emblica Officinalis (Amla). Indian J. Exp.Biol. 37(7):676–680.

Bilczuk, L., Gowin, A. , Ebertowska, Z. Mach, H. 1991. "Nitrate and Nitrite Levels in Daily Food Rations of Children from the Rural Pulowy Regions." Rocz. Panstw. Zaki. Hig. 42(2):139 – 147.

Biswas, S.K., McClure, D., Jimenez, L.A., Megson, I.L., Rahman, I. 2005. "Curcumin Induces Glutathione Biosynthesis and Inhibits NF-Bactivation and Interleukin-8 Release in Alveolar Epithelial Cells: Mechanism of Free." Antioxid Redox Signal. 7:32–41.

Brody, T. 1994. "Nutritional Biochemistry; Academic Press: San Diego, CA; Pp. X and 450-9." Academic Press: San Diego, CA; Pp. X 450–459.

Buege, J.A., Aust, S.D. 1978. "Microsomal Lipid Peroxidation, 52: 302-310." Colowick SP and Kaplan NO (Eds.) Methods in Enzymology. New York, Academic, 52: 302–310.

Doumas, B. T., Bayso, D. D., Carter, R. J., Peter, T. Jr., Schffer, R. 1981. "A Candidate Reference Method for Determination of Total Protein in Serum. I. Development and Validation." Clin. Chem. 27(10):1642–1650.

Doumas, B. T., Walson, W. A.,Biggs, H. G. 1971. "Albumin Standards and Measurement of Serum Albumin with Bromocrysol Green. 31(1):87-96." Clin. Chim. Acta. 31(1):87–96.

El-wakf, Azza M., Waffa M. El-kholy. 2011. "Use of Tumeric and Curcumin to Alleviate Adverse Reproductive Outcomes of Water Nitrate Pollution in Male Rats." Enzyme 9(7):229–239.

Ersoy H., Aysun B. K., Çetin Ö., Kadir B., Gürsoy D., Semsettin K., MD, Muammer E.. 2005. "Hazneci Report et Al. A Comparative Study of Superoxide Dismutase, Catalase, and Glutathione Peroxidase Activities and Nitrate Levels in Vitiligo Patients." Int. J. Dermatology 44: 636–640.

Eteng M.U., Ibekwe H.A., Amatey T.E., Bassey B.J., Uboh F.U., Owu D.U. 2006. "Effect of Vitamin C on Serum Lipids and Electrolyte Profile of Albino Wistar Rats." Niger J Physiol Sci. Jun-Dec; 21(1–2):15–19.

Flohe, L. Otting, F. 1984. "Superoxide Dismutase Assays." Methods Enzymol. 105:93–104.

Foresti, R., Clark, J., Green, C., Motterlini, R. 1997. "Thiol Compounds Interact with Nitric Oxide in Regulation Heme Oxygenase-1 Induction in Endothelial Cells. Involvement of Superoxide and Peroxynitrite Anions." J. Biol. Chem. 272: 18411–18417.

Fouad S. S., Mouchira M. M., Haridy M. A.,Khalil A.M.2017" Ameliorative Effects of Ascorbic Acid (Vit. C) Against Sodium Nitrite Toxicity in Albino Rats: Hematological, Biochemical and Histopathological Studies". American-Eurasian J. Toxicol. Sci. 9 (1): 01-06.

Goldberg, D.M., Spooner R.J. 1983. "Method for the Determination of Glutathione Reductase." Methods o Enzym. Analysis. 3(3):258–265.

Gonchar O., Mankovskaya, I., Klyuchko, E. 2006. "Role of Complex Nucleosides in the Reversal of Oxidative Stress and Metabolic Disorders Induced by Acute Nitrite Poisoning." Indian J. Pharmacol. 38(6):414–418.

Gupta S, Agarwal R, Srivastava S, Agarwal P, Agrawal S, Saxena R, Galpalli N. 2008. "The Anti-Inflammatory Effects of Curcuma Longa and Berberis Aristatafreeze in Endotoxin-Induced Uveitis in Rabbits." Invest Ophthalmol Vis. Sci. 49(9):4036–4040.

Hirneth H., Classen H.G. 1984. "Inhibition of Nitrate-Induced Increase of Plasma Nitrite and Methemoglobinemia in Rats by Simultaneous Feeding of Ascorbic Acid or Tocopherol." Arzneimittelforschung 34:988–991.

Halliwell, B., Gutteridge, J. C. 1999." Free Radicals in Biology and Medicine. 3rd ed. London, England: Oxford Univ. Press.

Helal , E., Zahkok, S, Soliman, GH. Al-Kassas, M; and Abdel Wahed, H. 2008. "Biochemical Studies On The Effect Of Sodium Nitrite and/Or Glutathione Treatment On Male Rats." The Egyptian J. Hospital Med. 30:25–38.

Hemeida, A., Mohafez, M. 2008. "Curcumin Attenuates Methotraxate-Induced Hepatic Oxidative Damage in Rats." J. Egyptian Nat. Cancer Inst. 20(2):141–`148.

Husdant, H., Rapaport, A. 1958. "Estimation of Creatinine. , 14: 222." Clin. Chem. 14:222.

Hussein H., Elnaggar M.and J. 2012. "'Protective Role of Vitamin C against Hepatorenal Toxicity of Fenvalerate in Male Rats,.'" Global Advanced Re-Search J. Environ. Sci. Toxicol.1(4):60–65.

Ibrahim, A., Zaky, Z., Sharkawy, A. Mubarak, M. 1999. "Effect of Vitamin C on Nitrate Toxicity in Ducks. ,." The 15 Th Annual Conference for Egyptian Soci. Of Toxicl. 6–7 Octobe.

Iqbal, M., Sharma, S., Okasaki Y., Fujisawa, M. and Okada, S. 2003. "Dietary Supplementation of Curcumin Enhance Antioxidants and Phase II Metabdizing Enzymes in Male Mice: Possible Role in Protection against Chemical Carcinognesis and Toxicity." Pharmacology Toxicology, 92:33–38.

Ítala M. Sales S., Adriana d. Tomé, G. Barros S., Paulo M. Pinheiro F., Gardenia C. Gadelha M., and Rivelilson M. 2009. "Oxidative Stress in the Hippocampus during Experimental Seizures Can Be Ameliorated with the Antioxidant Ascorbic Acid." Oxid Med Cell Longev. 2(4):214–221.

Joe, B. Lokesh, B. R. 1994. "Role of Capsaicin, Curcumin and Dietary N-3 Fatty Acids in Lowering the Generation of Reactive Oxygen Species in Rat Peritoneal Macrophages.,." Biochem. Biophys. Acta. 1224:255–263.

Khan R. , Khan M. , Sahreen, S. 2012. "CCl4-Induced Hepatotoxicity: Protective Effect of Rutin on p53, CYP2E1 and the Antioxidative Status in Rat. ." BMC Complement Altern Med 12–178.

Klatskin, G., Conn, H. 1993. Histopathology of the Liver. Oxford and New York.

Krishnamoorthy, P., Sangeetha M., 2008. "Hepatoprotective Effect of Vitamin C on Sodium Nitrite-Induced Lipid Peroxidation in Albino Rats." 45(June):206–208.

Librojo, N. T. 1978. "Nitrite Effects on Ascorbic Acid Metabolism in Guinea Pigs and Rats." Iowa state university, ph.d.,

Mahboob, S., Sheri, A., Shakoor, A., Raza, S. ,Andleeb, S. 2001. "effect of nitrate and nitrite pollution on some haematological parameters of rabbits . J. Agri. Sci. 38:44–46.

Marković D. S., Dragana S. Đ. , Danijela M. C. , Ana D. O. , Jovana B. Ž. , Branka I. O. , Andraš Š. Š., Zorica S.S. and Mihajlo B.S. 2010. "Effects of Acute Treatment of Vitamin c on Redox and Antioxidative Metabolism in Plasma and Red Blood Cells of Rats." Kragujevac J. Sci. 32:109–116.

Masuda, T., Maekawa, T., Hidaka, K., Bando, H., Takeda, Y. and Yamaguchi, H. 2001. "Chemical Studies on Antioxidant Mechanism of Curcumin: Analysis of Oxidative Coupling Products from Curcumin and Linoleate." J. Agric. Food Chem. 49(5):2539–2547.

Mathews, V. V. et al. 2012. "Hepatoprotective Efficacy of Curcumin against Arsenic Trioxide Toxicity." Asian Pacific Journal of Tropical Biomedicine 2(2 SUPPL.).

Narayanan, S. 1982. "Method–comparison Studies on Immunoglobulins." Clin Chem 28:1528–1531.

Ogur, R., Coskun, O., Korkmaz, A. 2005. "High Nitrate Intake Impairs Liver Functions and

Morphology in Rats; Protective Effects of α-Tocopherol." EnviromToxicol Pharmacol, 20:161–166.

Patton, M. P. , Crouch, S. R. 1977. "Determination of Urea (Urease Modified Berthelot Reaction)." Anal. Chem. (49):464–469.

Price, C., Spencer, K., Whicher, J. 1983. "Light-Scattering Immunoassay of Specific Proteins." Are Views. Ann Clin Biochem 20:1–14.

Reham A., Rasha A., Aziza A. 2016. "Protective Effects of Ascorbic Acid and Garlic Oil against Toxic Effects Induced by Sodium Nitrite as Meat Additive in Male Rats ." Global Veterinaria 16(6):508–524.

Reitman, S.,Frankels, S. 1957. "A Colormetric Method for Determination of Serum Glutamic Oxaloacetic Transaminase and Serum Glutamic Pyruvic Transaminase." Am. J. Clin. Pathol. 25–56.

Sanaa, R., Mahmoud, M. 2012. "The Protective Effect of Vitamin A against Sodium Nitrate Induced Toxicity in Liver and Kidney of Albino Rats: Histological and Ultrastructural Study." J. Amer. Sci.: 8(12).

Sanaa A. El-shamy, Nahed F. Zaglool, Shahenaz M. H. Hassan, And Shahera M. ..Rasha. 2014. "Protective Effect of Turmeric on the Oxidative Damage Caused by Chronic Sodium Nitrite Intoxication in Rats." 1st Scientific Conference of Food Safety and Technology pp.Animal Health Research Institute 19–23 June:186–205.

Sharma, M. K. ,Hemlata S.. 2012. "Evaluation of the Haematological Responses to High Nitrate Exposure in Rabbits." J. Clin. Diag. Res. 6(1):145–149.

Shehata, S. A. 2005. "Nitrate Detoxification of Drinking Water by Ascorbic Acid in Growing Rabbits." World Rabbit Science 13(2):93–106.

himizu, S. Eguchi, Y., Kamiike W., Waguri S., Uchiyama Y., Matsuda, H., TsShimizu, S., ujimoto Y. 1996 "Retardation of Chemical Hypoxiainduced Necrotic Cell Death by Bcl-2 and ICE Inhibitors: Possible Involvement of Common Mediators in Apoptotic and Necrotic Signal." Transductions. Oncogene 12(91):2045–2050.

Srinivas, L., Shalini, V., Shylaja, M. 1992. "Turmerin: A Water Soluble Antioxidant Peptide from Turmeric (Curcuma Longa)." Archives Biochem. Biophys., 292:617–662.

Sultan, M., Haleem, H., Ahmed, E. , Darderi, M., Aly, A. 2000. "Some Studies on Nitrate Poisoning in Rats and Possibility of Methylene Blue as an Antidote. J. Comp. Pathol. and Clinic. Pathol. 13(1):157–173.

Tan , YS, Nambiar , R., Yo, SL. 1992. "Prevalence of Protein Calorie Malnutrition in General Surgical Patients." Ann. Acad. Med. Singapore 21(3):334–338.

Tarasub N. , Thongbai J., Chinnawat T., Watcharaporn A. 2012. "Protective Effects of Curcumin, Vitamin C, or Their Combination on Cadmium-Induced Hepatotoxicity." J. Basic Clin. Pharmacy 3(2):273–281.

Thrall, M. A., Campbell, T. W., De Nicola, D., Fettman, M. J., Lassen, E. D., Rebar, A., Wieser, G. 2004. Veterinary Hematology and Clinical Chemistry. edited by Lippincott Williams & Wilkins. Philadelphia, Baltemore, New York, London.: A walters Kluwer Company.

Tisher, C., Brenner. B. 1989. "Renal Pathology with Clinical and Functional Correlation." J. B. Lippincott Company. Philadelphia.

Tooke, J. E. 1996. "Endothelium: The Main Actor or Choreographer in the Remodeling of the Microvasculature in Diabetes." Diabetographia; 104.:745–746.

Wohaieb S. A., Godin D. 1987. "Alterations in Free Radical Tissue Defense Mechanisms in Streptozotocin-Induced Diabetes in the Rat: Effects of Insulin Treatment." Diabetes 36:1014–1018.

VanKampan, E. J., Zijlistra, W. G. 1961. "Estimation of Hemoglobin." Clin. Chem. Acta 6:538.

Yan, J., John, W., Xiumin, D., Wei , W., Heather, H., Nancy, D., Mary, A., Frank M. , Suzy, V. 2009. "Curcumin, a Cancer Chemopreventive and Chemotherapeutic Agent, Is a Biologically Active Iron Chelator." Blood janury 8,113(2):462–469.

Zraly z., Bendova j., Svecova d., Faldikova l., Veznik z., ZajicovA A. 1997. "Effect of Oral Intake of Nitrates on Reproductive Functions of Bulls." Vet. Med. (Praha) 42:345–354.