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Ameliorative Effects of Ascorbic Acid (Vit. C) Against Sodium Nitrite Toxicity in Albino Rats: Hematological, Biochemical and Histopathological Studies

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Abstract: Nitrite toxicity can reach to human, animal, bird and experimentally to laboratory animals by direct and indirect methods. Our experiment evaluated the ameliorative effects of ascorbic acid treatment against sodium nitrite toxicity in female Sprague drawly rats. The rats orally given normal distilled water served as control (gp 1). The rats orally given sodium nitrite at 1.5 mg/rat equivalent 1/20 from LD50 (200 mg/kg b.wt.) dissolved in 1 ml distilled water daily for 6 months (gp 2) and simultaneously with vitamin C administration at 10 mg/kg b.wt. dissolved in 1ml distilled water (gp 3) daily for 6 months. Sodium nitrite-treated animals exhibited significant increase in white blood cells. In addition to, significant decrease in red blood cells count, PCV% and hemoglobin concentration. Furthermore, significant increases in serum biochemical parameters related to liver injury alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Also there were elevation in the levels of markers related to renal injury urea and creatinine. Histopathologically, sodium nitrite toxicity induced degenerative changes in renal tubules with congestion of glomerular capillary and vacuolar degeneration of hepatocyte with congestion and dilation of portal vein and blood sinusoid associated with inflammatory cell infiltration. It could be concluded that ascorbic acid treatment can ameliorate the toxic effects of sodium nitrite in albino rats.

Key words: Ameliorative · Biochemical · Sodium Nitrite · Ascorbic Acid · Hematology · Histopathology

INTRODUCTION

Nitrate is an inorganic chemical that is highly soluble in water. Major sources of nitrate in drinking water include fertilizers, sewage and animal manure. Most nitrogen containing materials in natural waters tend to be converted to nitrate. Nitrates also occur naturally in the environment, in mineral deposits, soil, seawater, freshwater systems and the atmosphere. Nitrate and nitrite are commonly used as a preservative and for color enhancement of processed meats, although the amounts added to these products have been substantially reduced from the levels once used [1].

Infection and illness can cause the body to produce even greater level of nitrate [2]. Shallow wells are more susceptible to nitrate contamination than bedrock wells. Wells close to sources of heavy fertilizer use or concentrated animals manure, such as farms and golf courses, are at greater risk. Other contaminant sources include malfunctioning septic systems and construction sites using explosive [3].

Sodium nitrite is naturally present in many fruits and vegetables, its use as a food preservative can be damaging to your health. This additive is present in foods like bacon, lunch meat and hot dogs and knowing more about it will help you make healthier food choices [4]. Sodium nitrite inhibits the formation of a toxin by the anaerobic spore forming bacteria, clostridium botulinum it imparts a pink color to nitrite-cured meats and stabilizes the flavors of stored meats, therefore, it is used as a color fixative and preservative in meats and fish [5]. It is also used in manufacturing diazo dyes, nitroso compounds and other organic compounds, in dyeing and printing textile fabrics, in bleaching fibers in photography as a laboratory reagent and a corrosion inhibitor in metal coatings for phosphatizing and detinning and in the

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manufacture of rubber chemicals sodium nitrite also has been used in human and veterinary medicine as a vasodilator, bronchial dilator, an intestinal relaxant and an antidote for cyanide poisoning [6]. Humans are constantly exposed to sodium nitrite through the oral route because it is used as a food additive, some vegetables, such as spinach and beets and some well water contain high concentrations of nitrates which may be reduced to nitrites by the action of microorganisms before and after ingestion [7].

The aim of the work was to investigate the ameliorative effect of ascorbic acid (Vitamin c) against sodium nitrite toxicity.

MATERIALS AND METHODS

Materials

Experimental Animals: Forty five adult female albino rats weighing 150±10g at 10-12 weeksold were obtained from Laboratory Animal House belonging to Egyptian Company for Production of Antisera, Vaccines and Drugs, Helwan, Egypt. Prior to the experiment, the animals were exposed to adaptation period for 2 weeks in metal cages with wire mesh covers under normal environmental conditions of temperature and humidity. Animals were supplied commercial basal diet and water and provided ad libitum throughout the experiment period. The rats were manipulated according to Experimental Animal Ethics approved by South Valley University, Qena, Egypt.

Chemicals

Sodium Nitrite: It is water soluble white or slightly yellowish odorless and stable in dry form at room temperature. It was purchased from EL Gomhuria for Drugs and ChemicalsCompany, Egypt.

Vitamin C: It is white crystalline powder, soluble in water and chemically stable at room temperature. It was purchased from EL Gomhuria for Drugs and Chemicals Company, Egypt.

- Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) kits Bio-Labo kits (Bio-Labo Co., France, (ALT catalog Nos 80227, AST catalog Nos 80225) were estimated using technique delineated by Reitman and Frankel [8]. Alkaline phosphatase (ALP) was assessed using Spectrum kits (Spectrum Co., Egypt) catalog Nos 214002 via method of Belfield and Goldberg [9].
- Serum ure using Bio-Merieux kits (Bio-Merieux Co., France , Catalog nos ,61912) ,creatinine using Bio-Labo kits (Bio-Labo Co., France, Catalog nos 80107) and uric acid using Bio-Labo kits (Bio-Labo Co., France , Catalog nos 80001) were estimated by methods of Fawcett and Scott [10], Bartels *et al*. [11] and Barham and Trinder [12] respectively.

Methods

Experiment Design: Animals were equallydivided into three groups, with each group containing fifteen rats. Each five animals were housed in metal cages with stainless steel cover.

Group (1): The rats were orally given normal distilled water, it served as a control.

Group (2): The rats were orally given sodium nitrite at 1.5 mg/rat equivalent 1/20 from LD50 (200 mg/kg b.wt.) dissolved in 1 ml distilled water daily for 6 months according to Boink [13].

Group (3): The rats were given orally sodium nitrite at 1.5 mg/rat equivalent1/20 from LD50 (200 mg/kg b.wt.) plus Vit. C at 10 mg/kg b.wt. dissolved in 1ml distilled water daily for 6 months.

The experiment was established for a period 6 months. Blood and serum samples were collected at 3rd and 6th month from each five rats of each group for hematological and biochemical evaluation. Also tissues samples from liver and kidneys were collected for histopathological examinations.

Histopathologically: Tissue specimens from the internal organ including liver and kidneys were collected and fixed in 10% buffer formalin solution then dehydrated, cleared and embedded in paraffin wax according to Bancroft and Stevens [14]. Sections at 4-5 microns thickness were cut and mounted on glass slides, then stained with Hematoxylin & Eosin stain and microscopically examined.

Statistics: The data were analyzed using one-way analysis of variance followed by post hoc analysis (Dunnett's test) using SPSS (Statistical package for Social Sciences) version 17. A difference of $P<0.05$ was considered statistically significant.

RESULTS

Clinical Signs: Group 2 which received sodium nitrite showed obvious clinical signs including decrease food

Table 1: The mean and standard error of RBCs, hemoglobin concentration (Hb), packed cell volume (PCV %), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC %) and WBCs of group (1), group (2) and group (3).

	Parameter						
Groups	RBCs (x10^6)	WBCs (x10^3)	Hb. (gm/dl)	PCV %	MCV (fl)	MCH (pg)	MCHC%
				3 months			
Group (1)	7.43±0.31	13.16±0.5	13.13±0.26	42.16±0.56	54.76±0.18	17.55±0.20	32.05±0.28
Group (2)	7.44±0.29	12.30±1.1	12.90±0.15	41.50±0.94	53.51±1.56	17.37±0.98	31.53±0.33
Group (3)	7.06±0.23	13.43±0.1	12.70±0.30	41.50±0.30	56.23±1.15	18.14±1.45	31.50±0.10
				6 months			
Group (1)	7.40±0.32	13.1±0.2	13.26±0.24	42.23±0.52	63.69±1.66	17.92±0.54	32.01±0.83
Group (2)	5.12±0.20*	16.1±0.4*	9.66±0.23*	36.43±0.67*	73.03±0.26*	17.75±1.45	26.47±1.05
Group (3)	6.38±0.03	13.80±0.2	12.16±0.38	40.66±1.02	63.70±1.59	19.98±0.83	30.47±0.38

* Significantly different from normal control group, $p < 0.05$.

Table 2: The mean and standard error of serum aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and alkaline phosphatase (ALP) of group (1), group (2) and group (3)

Time / Months	Gps	ALT U/l	AST U/l	ALP U/l
3 months	1	35.33±0.88	96.66±3.38	131.00±1.52
	2	36.33±2.60	94.66±0.33	131.00±2.00
	3	39.00±2.64	93.33±4.66	132.67±4.84
6 months	1	39.66±1.20	97.00±1.15	133.33±1.20
	2	61.33±2.33*	127.67±1.85*	146.67±3.48*
	3	44.66±0.88*	109.33±2.40*	142.33±2.02*

* Significantly different from normal control group, $p < 0.05$.

Table 3: The mean and standard error of serum urea, creatinine and uric acid of group (1), group (2) and group (3)

Time / months	Gps	Creatinine Mg/dl	Urea Mg/dl	Uric acid Mg/dl
3 months	1	0.63±0.03	37.86±1.44	3.56±0.26
	2	0.62±0.05	38.93±0.40	4.00±0.35
	3	0.60±0.01	38.45±1.38	3.10±0.36
6 months	1	0.74±0.04	38.45±3.66	4.33±0.31
	2	1.36±0.12*	57.61±2.29*	6.06±0.38*
	3	0.75±0.03	37.75±3.37	4.83±0.20

*Significantly different from normal control group, $p < 0.05$.

and water intake, decrease in body weight and sluggish movement with cyanosis of mouth and tongue appeared commonly at 6th month post treatment. Treated group with vitamin C showed normal behavior with normal feed and water consumption.

Hematological Findings: Table (1) showed non-significant changes detected after 3 months in the hematological parameters of gps 2 and 3 including red blood cells (RBCs) count, hemoglobin concentration (Hb), packed cell volume (PCV) %, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBCs) count and blood platelets. While, at 6 months there was significant decrease in RBCs count, Hb concentration and PCV% of gp 2 when compared with control. On the contrast, significant increase was detected in MCV values of gp 2when compared with control. MCH, MCHC, WBCs count and blood platelets exhibited non-significant changes when compared with control gp. Gp 3 which received vitamin C revealed non significant changes when compared with control.

Table (2) showed that non-significant changes were recorded after 3 months in liver function tests including AST, ALT and ALP enzymes of gps 2 and 3 when compared with control. While at 6 months, AST, ALT and ALP enzymes were significantly increased in gps 2 and 3 when compared with control, also gp 3 showed significant decrease in ALT, AST and ALP when compared with gp 2.

Table (3) showed that non-significant changes were recorded after 3 months in kidneys function tests including urea, creatinine and uric acid of gps 2 and 3 when compared with control. While at 6 months; urea,

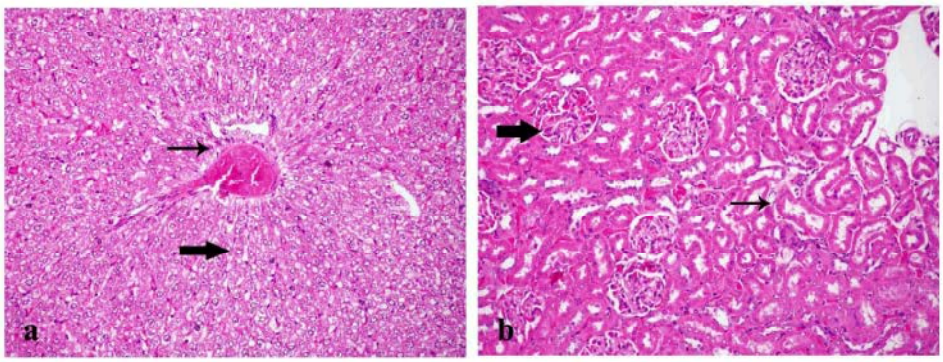

Fig. 1. 1. a.: Liver of group 2 which receiving sodium nitrite showing vacuolar degeneration of hepatocytes, besides congestion in the portal vein and blood sinusoids (H&E., 80), 1. B: Kidneys showing congestion of glomerular capillaries with swelling in the glomerular cells, besides degenerative changes in renal tubules in group 2 (H&E., 150).

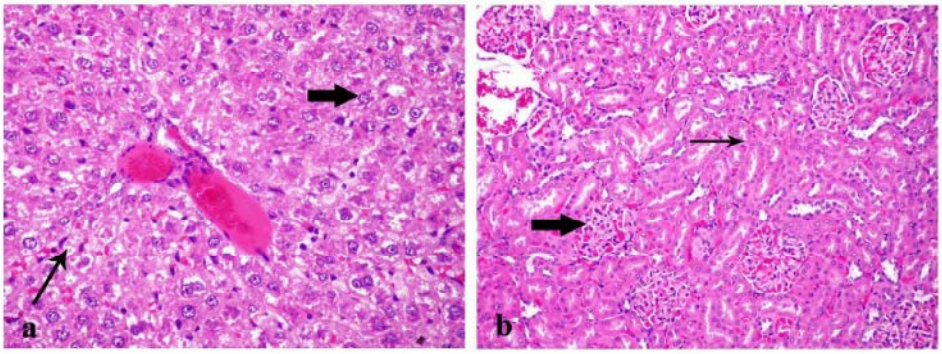

Fig. 2: 2. A:Liver of group 3 which receiving sodium nitrite plus vitamin c showing regeneration of some hepatocytes, with necrosis in other hepatocytes, also fibroblast scattered among hepatic cells (H&E., 300), 2. B: Kidneys showing congestion with dilatation of glomerular capillaries filled bowman's space, besides degenerative changes in renal tubules in group 3 (H&E., 150).

creatinine and uric acid level of gps 2 and 3was higher than control. Gp 3 revealed significant improvement in the renal tests when compared with gp 2.

Pathological Results: Liver of gp 2 showed vacuolar degeneration of hepatocytes characterized with vacuolation of the cytoplasm, besides congestion in the portal vein and blood sinusoids (Fig. 1a). Moreover, kidneys of this gp showed congestion of glomerular capillaries with swelling in the glomerular cells, besides degenerative changes in renal tubules (Fig. 1b).

Liver of gp 3 showed regeneration of some hepatocytes, with necrosis in other hepatocytes, also hepatic fibrosis indicated by fibroblast scattered among hepatic cells (Fig. 2a). Kidneys showed congestion with dilatation of glomerular capillaries filled bowman's space, besides degenerative changes in renal tubules in gp3 (Fig. 2b).

DISCUSSION

Nitrite is known to cause free radical generation [15] as it can stimulate oxidation of ferrous ions in oxyhemoglobin to form methemoglobin as well as various ROS [16]. Nitrite-promoted Ca2+ influx in blood cells activates phospholipases, which increase the proportion of phospholipids with a rigid structure in the membrane [17].

The present study demonstrated the toxic effect of NaNO2 on the hematological parameters in rats. It induced significantly reduction in RBC count, Hb concentration and PCV% with increased MCV. Oxidative damage might be a relevant cause of the initial decrease in RBC count which may be attributed to lysis or shrinkage of erythrocytes in the blood [18]. However, other investigators reported that NaNO2 administration increases methemoglobin without any effect on RBC hemolysis [19].

The increase in the activity of AST, ALT and ALP enzymes in the serum of sodium nitrite treated group, could be attributed to the toxic effect of nitroso-compounds formed in the acidic environment of the stomach causing severe hepatic necrosis [20] or it might be due to anaemia and methaemoglobinemia which induced hypoxic injury to centrilobular hepatocytes that consequently cause enzyme leakage [21]. High levels of AST, ALT and ALP may be due to the escape of these enzymes from the liver cytosol into the blood stream and liver dysfunction and defect in the biosynthesis of these enzymes with change in the permeability of liver membrane takes places [22].

Increased serum urea, uric acid and creatinine concentrations suggested an impairment of kidney function. This is in agreement with many authors who reported the toxicity of sodium nitrite onthe serum urea, uric acid and creatinine concentrations [23]. These effects could be attributed to changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate [24].

Sodium nitrite in group 2 induced degeneration of hepatocytes, besides congestion in the blood vessels. Moreover, there were degenerative changes in renal tubules with congestion of glomerular capillaries. Thus correlated to nitrosamines and free radicals generated by sodium nitrite. Such products may increase lipid peroxidation, which cause harms and damage to the different organs including the liver and kidneys [25].

Co-Administration of vitamin C with sodium nitrite prevented the changes recorded in blood, liver function enzymes activities and serum kidney function parameters as compared with control group. Vitamin C exerted ameliorating effect on sodium nitrite-induced lipid peroxidation (LPO), thus it led to decrease in the LPO level with increased antioxidant enzymes activities [26].

CONCLUSION

It could be concluded that prolonged administration to sodium nitrite impairment liver and kidneys function at 6^{th} dpi. The administration of vitamins C in the inoculated animal displayed possible protection on liver and kidneys against bad effect of sodium nitrite.

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