The protective effect of vitamin A against sodium nitrate induced toxicity in liver and kidney of albino rats: histological and ultrastructural study

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Abstract: The aim of the present study was to investigate the effect of sublethal dose of nitrate in a short-term experiment (orally and daily for 8 weeks) and the possible ameliorative effect of vitamins A. 120 adult male Albino rats were divided into 4 groups of 30 rats each and treated daily for 8 weeks as follows: Group I (control) received distilled water, Group II received 10,000 I.U/ rat vitamin A, Group III received 50 mg/kg $NaNO_3$ and Group IV received 50 mg/kg $NaNO_3$ +10,000 I.U/ rat vitamin A. All the animals were sacrificed after 8 weeks of treatment. Specimens from liver and kidney of rats were collected for the optical and electron microscope study. In the present study, light microscopic examination revealed that "Sod. nitrate" caused kidney damage represented by shrinked glomerular tuft, degeneration of some tubules and epithelial lining cells. Formation of focal fibrosis and infiltrated with a number of inflammatory cells. The damage also extended to the hepatic cells including cytoplasmic vacuolization and dialated congested veins. The liver showed hydropic degenerated hepatocytes, necrotic areas infiltrated with a number of inflammatory cells, in addition to the presence of mononuclear cell infiltration and dilated sinusoids. Ultrastructural results of the kidney nitrate treated animals showed that irregular thickening of glomerular basement membrane. Mitochondria were obviously swollen and having disintegrated organelles. Moreover, the proximal tubules contained very dense mitochondria with numerous closely packed cristae, thick basal lamina, vacuoles, distruction of microvilli and irregular nucleus. Electron microscopic examination of the liver of rats treated with sodium nitrate showed swollen hepatic cells with cell sap of low density, scant endoplasmic reticulum and swollen mitochondria. There was segregated organelles in a membrane bound structure and variable size lipid droplets. Condensed nucleus was seen. In addition, myelin figure (finger-print appearance), apparently formed by the concentric lamellar arrangement of rough endoplasmic reticulum. The Light microscopy, and ultrastructural result that the treatment with vitamin A led to repair of almost all the damaged tissues of the liver and kidneys.

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1. Introduction

In recent years, considerable attention has been paid to the problem of nitrate due to the intensive use of nitrates as agricultural fertilizers which reach to humans and animals by different routes **(Stoate *et al.*, 2001; Awodi *et al.*, 2005; Manassaram *et al.*, 2006; Mande *et al.*, 2012)**. In some parts of the world where the soil is impoverished, crop farming is done solely with the application of large quantity of nitrogenous fertilizers. Nitrogenous fertilizers, especially nitrates and nitrites are of great importance and concern to man and animals because they possess mutagenic, carcinogenic, teratogenic and embryotoxic activities **(Antipina *et al.*, 1990; Kasyanenko *et al.*, 1992).** Nitrates accumulate in plants when excessive fertilizers are applied to the soil and when there are unfavourable conditions such as drought, deficiency of soil micro- and macro-elements and treatment of crops with herbicides **(Kovaleva and Talanova, 1988; Khmelnitsky, 1990).** Nitrates in the soil eventually end up in different quantities in the foodstuffs of plant origin consumed by man, and their excess quantity in foodstuffs adversely affects man, and animals **(Antipina *et al.*, 1990).** In fact certain foodstuffs such as maize, guinea corn, carrots, potatoes, sunflower, pumpkins and cabbage are known to accumulate large quantity of nitrates even at the normal fertilizer application rate of 150 kg/ha. **(Khmelnitsky, 1990; Savina, 1993; Awodi *et al.*, 2005; Manassaram *et al.*, 2006).** Workers in fertilizer factories and farmers in some parts of the world, who use bare hands to apply fertilizers in crop farming, are at increased risk of exposure through inhalation and skin contact **(Antipina *et al.*, 1990; Spalding and Exner, 1993; Awodi *et al.*, 2005).** The major source of nitrate in the human body is through intake of food and water **(IPCS, 1999).** Vegetables may account for more than 70% of the nitrates in a typical human diet **(ATSDR, 2001).** Drinking water may contain variable amounts of nitrates which accounts for up to 21% of total nitrates intake in a typical human diet **(Wogan *et al.*, 1995; ATSDR, 2001, Manassaram *et al.*, 2006).** Nitrates are used as

food additives to fix the colour of meat, to inhibit oxidation and to prevent toxigenesis **(Hotchkiss *et al*., 1992).** The major dietary sources of nitrates are vegetables, fruits and juices, milk and dairy products, bread and water **(White, 1975)**.

According to a published report, the poisoning of nitrates were claimed to its conversion into nitrites within the alimentary tract **(Manassaramm *et al*., 2006**). Nitrites may react with amines in the food to form potentially cytotoxic carcinogenic nitrosamines **(Newell *et al.,* 1985 and Muller *et al.,* 1986)**. Nitrates and nitrites were shown to alter homeostasis of epithelial cell membranes by reacting with thiol (oxidation) and amino groups (nitroso compound formation) or heme iron (redox) of the small intestine **(Grudiniski, 1990b, 1991b; Grudziniski; Szymauski 1991b)**. **Glebova (1998)** in an experiment in which sodium nitrate at a rate 9.6 g/kg b. wt given to rats, suggested that nitric oxide has a significant role in the liver as a factor resulting in accumulation of peroxidation products. Sodium nitrite given to Wistar rats at levels of 800 ppm and 1600 ppm in a pellet diet for 646 experimental days was associated with hepato-cellular carcinoma in the liver **(Aoyagi *et al.,* 1980).**

It is known from previous publications that nitrate and nitrite and dimethyl amine may react in the gastrointestinal tract synthesizing, the harmful dimethynitrosoamine substances which had adverse effects on animal and human organs **(Swan, 1975).** Nitrate and nitrite were also reported to cause pathological changes in rat liver ***(Gracia et al., 1987)*** and kidney (**Gojer & Sawant, 1992; Anthony *et al*., 1994)**, pleasant chicks liver and kidney **(Storand and Persin, 1983).**

Also, nitrite reacts with amines to produce nitrosamines and with amides to produce nitrosamides. Nitosamines and nitrosomides constitute the N-nitroso compounds (NNC) and the reaction with nitrite is called nitrosation, nitrosamines readily induced tumours of the liver, oesophagus, kidney, nasal cavity and pancreas and notrosamine chiefly induce tumours of glandular stomach, small intestine and nervous as well as lymphoid systems. The tissue affections depend on the species (NNC) and the treatment system **(Sidney, 1986).**

Excessive quantities of nitrate and nitrite consumption through food can be harmful to health (**Leszczy-nska *et al*., 2009 ; Chan, 2011**). Several clinical studies documented hepatorenal and/or hepatotoxicity in humans were associated with excess nitrate intake **(Parada *et al*., 2009).** Nitrates, in case of their oral absorption, are reabsorbed rather quickly in intestines and over 80% are released in a mass in the urine **(Hill, 1991)** and the high rate of absorption is may be due to the shortness of the nitrate ion radius.

Nitrate may be endogenously transformed into nitrite which in turn can react with amines and amides to produce nitrosamines and free radicals **(Babsky and Shostakovskaya, 1992; McAlliater *et al*., 1995; Oladele *et al*., 1997; Singhal *et al*., 2001; Manassaram *et al*., 2006).** Such products may increase lipid peroxidation, which can be harmful to different organs including liver and kidney **(Yordanov *et al*., 2001; choi *et al*.,2002 ; Santamaria, 2006;).** These have been related to an increased risk of gastric, liver, esophageal, nasopharyngeal and bladder cancers **(Kim *et al.,* 2002; Choi *et al*., 2007 and WHO, 2012).** Methemoglobinemia is another health hazard attributed to nitrite, which is a condition where reduced iron (Fe_2) in hemoglobin is oxidized by nitrite to become Fe_3, thus reducing the total oxygen-carrying capacity of the blood **(Santamaria, 2006; Manassaram *et al*., 2007).**

The ability of animals to resist the toxic effects of environmental agents is dependent on the detoxication and antioxidant systems. Recently, several nutrients and other chemicals are effective antioxidants, such as vitamins, trace elements, amino acids and their derivatives, fatty acids and plant phenolics **(Bhattacharya *et al.,* 1987; *Son et al.,* 2004; Ayo *et al*., 2006 ; Suteu *et al*., 2007).**

Vitamins are known to be potent antioxidants **(Geecha and Fagan, 1992; Williams, 1997; Whitehead and Keller, 2003; Son *et al.,* 2004; Ayo *et al*., 2006; Suteu *et al*., 2007).** Thus, their administration may augment the function of endogenous free radical scavengers and, consequently, decrease the deleterious effects of nitrates and nitrites on body cells.

The aim of the present study was to investigate the effect of sub-lethal dose of nitrate in a short-term experiment (orally and daily for 8 weeks) on albino rats and manifested microscopically in liver and kidney. In addition, the role of vitamin A as an antioxidant to counteract the toxic effect of nitrates was taken into consideration.

2. Materials and Methods

Materials:

Experimental animals:

120 male white albino rats of weights ranging from 150 – 180 g were used in the present study; animals were supplied by the breeding unit of Egyptian Organization for the Biological and Vaccine Production A.R.E. Science. Diet and water was given *ad-libitum.*

Chemicals and drugs:

Sodium Nitrate 98% pure ($NaNO_3$) M.W. 84.99 was provided by the British Drug Houses LTD.

B.D.H. Laboratory Chemicals Division Pool England.

Vitamin A: Vitamin "A" capsules (A-Viton) were supplied by Kahira Pharm. and Chem. Ind. Co. Cairo-Egypt (each contains 50,000 I.U.).

Dosage and route of administration:

-Sodium Nitrate; experiments using rats have shown that the LD_{50} to be 5 mg/g body weight **(Wanntorp and Swahn, 1953).** The dose used was $\frac{1}{10}$ of the LD_{50} which is equal to 500 mg/kg b. wt. Sodium nitrate solution (10%) was prepared by dissolving in distilled water, and was given to animals orally and daily for 8 weeks.

-Vitamin A was given per os at the level of 10000 I.U. / rat.

Experimental schedules:

After two weeks acclimatization period, animals were divided into 4 experimental groups which were treated as follows:

1-Negative control group.

2-Control vitamin A (10,000 I.U. / rat) group.

3-Nitrate group at $\frac{1}{10}$ of the LD_{50} (500 mg/kg b. wt).

4-Nitrate at $\frac{1}{10}$ of the LD_{50} and vitamin A group.

Animals of each group were scarified after 8 weeks of treatment. Animals behaviour, or any clinical signs were carefully observed, and any deaths were recorded during the experimental period.

Histological preparations:

Preparation of paraffin sections:

For the histological preparations, animals were anaesthetized under light diethyl ether and dissected to remove the liver and kidney at the end of the experiment. Liver and kidney tissues were cut into small pieces and then fixed in 10% neutral buffered formalin for 24 hours. The tissue was routinely processed and sectioned at 4 to 5 µm thickness with a microtome and stained with haematoxylin and eosin for histopathological studies.

Electron Microscopic Preparations:

Tissue specimens (liver and kidney) were cut into pieces measuring about 1 mm3 and immediately fixed in fresh 3% glutaraldehyde-formaldehyde at 4°C for 18-24 hours. the specimens were then washed in phosphate buffer (pH 7.4) and then post-fixed in isotonic 1% osmium oxide for one hour at 4°C **(Mercer & Birbeck, 1966).** Serial dehydration in alcohol was carried out in the following order: 30 minutes in 50% alcohol, 2 times in 70% alcohol each for 15 minutes, then for another 15 minutes in 80% alcohol, 15 minutes in 90% alcohol, then 2 times in absolute alcohol each for 30 minutes. The specimens were then passed through propylene oxide solutions 2 times each for 10 minutes. Embedding the specimens in spurr resin started by passing the specimens in propylene oxide-resin mixture at the ratio of 3: 1 for 1 hour, then in propylene - oxide-resin mixture at the ratio 1: 1 for 1 hour, then in propylene oxide-resin mixture at the ratio of 1: 3 overnight. The samples were left in fresh pure resin at room temperature overnight. Next day the specimens were transferred to capsules containing fresh resin and placed in the oven at 60°C for one day for polymerization to obtain hard blocks.

Semi-thin sections were cut from the prepared blocks 1.0 mm thickness by Ultracut Reichert Jung Ultramicrotome with the aid of glass knives. The sections were stained with toluidine blue stain and examined by light microscope to detect the area of interst. Ultrathin sections were then prepared using the ultramicrotome glass knives, stained with uranyl acetate and lead citrate **(Reynolds, 1963)** and examined with a Joel CX100 transmission electron microscope operated at an accelerating vollage of 60 KV.

3. Results

1 Histopathological Study:

A) Kidney of Control Rats

In both control untreated and vitamin A treated rats, the microscopic examination of the kidney showed normal histological structure of the tissue. The renal parenchyma can be differentiated into cortex and medulla. The cortex contains glomeruli, proximal tubules and distal tubules(Fig.1a)

B) Kidney of Sod.nitrate Administered Rats.

In Sod. nitrate-administered group, microscopic alterations were detected at the end of eight weeks, these alteration were in the form of congestion of glomerular tuft (Fig.1b), Dissolution in the cortex (Fig.1c). Highly vacuolated glomerular tuft invaded with a number of red blood cells and inflammatory cells infiltration (Fig.1d)., periglomerular oedema, degeneration of epithelial cells lining in the renal tubules (Fig.1e) and shrinked glomerular tuft, degenerative changes in the renal tubules (Fig. 1f). Formation of focal fibrosis and infiltrated with a number of inflammatory cells (Fig.1g). Dilated blood vessel engorged with RBCs and enlargement of renal corpuscle with atrophied glomerular tuft (Fig.1h). The simultaneous administration of vitamin A plus nitrate resulted in nearly nomal renal tubules and glomerulus. The tubules regained their normal appearance and there was no areas of dissolution in the cytoplasm (Figs. 1i&1j).

C) Liver of Control Rats

The liver in normal untreated and vitamin A treated rats is covered by a capsule of connective tissue. The parenchyma is formed of a more or less

regular cords arranged radially from the hepatic vein (Fig.2a) and consisted of large polyhedral hepatic cells having an eosinophilic finely granular cytoplasm and one or two prominent nuclei (Fig.2b). A fine network of reticular tissue penetrates the parenchyma bounding sinusoidal blood vessels. The portal triad representing branches of the portal vein, hepatic artery and bile duct are distributed at the corners of the lobules (Fig.2c).

D) Liver of Sod.Nitrate Administered Rats.

Light microscopic examination revealed that sod.nitrate induced severe alteration of rat liver when compared to liver of control rats.In the liver of a rat treated with nitrate showing hyperemic hepatic tissue with hypertrophied hydropic degenerated hepatocytes (Fig.2d). In addition, focal necrotic areas infiltrated with a number of inflammatory cells in the hepatic tissue (Fig.2e), the other noticed changes were degenerated hepatocytes and the anucleated others (Fig.2f). The examined liver showed dilated central vein congestion (Fig.2g), hydrophic degenerative hepatocyte infiltrated with a number of inflammatory cells (Fig.2h) besides the wide hypermic portal vein and inflammatory cells infiltration (Fig. 2i), administration of vitamin A plus nitrate resulted in nearly normal structure of the central vein, sinusoids, portal vein, and hepatic cells(Figs. j&k)

2)Electron Microscopic Findings of the Kidney:

A)Normal renal corpuscles of the kidney.

The cortex of normal as well as vitamin A treated rat kidney consists mainly of renal corpuscle and proximal tubule with normal appearance. The renal corpuscle appears as dense round tuft of capillaries. The outer layer of Bowman's capsule is the parietal layer and the inner visceral layer applies closely to the glomerualr capillaries. Podocytes surround the capillaries. Each capillary loop is lined by endothelial cells. Podocytes give rise to primary processes which in turn give numerous secondary foot processes or pedicels that rest on thin basal lamina. Each podocyte has a large nucleus and abundant cytoplasm. The pedicles are separated by split pores with slit membranes. The filtration barrier consists of capillary endothelial inner layer, thin glomerular basement membrane and podocyte layer (Fig. 3a).

B) Renal corpuscle of rat treated with sodium nitrate.

Electron micrograph of a glomerulus of rat treated with nitrate for 8 weeks showing thickening of basement membrane, irregular nucleus with dark condensation of heterochromatin, in addition the presence of swelling mitochondria (Fig. 3b).

Treatment with vitamin A showed mild improvement in the structure basement membrane amorphous particuolated matrial in the glomerular space, where the nucleus appeared idented with dark clumps of heterochromatin adjacent to the nuclear envelope (Fig. 3c)

c) Normal proximal tubules

The proximal tubules are lined by cuboidal cells having intense cytoplasm due to a high content of organelles. The nucleus is spherical and centrally located. Patches of heterochromatin are observed at the nuclear envelope. Numerous elongated mitochondria fill the perinuclear and subnuclear cytoplasm with highly dense matrix and closely parallel cristae (Fig. 4a).

D) Proximal tubules of rats treated with sodium nitrate.

By using sodium nitrate some cells of the proximal tubules revealed contained very dense swelling mitochondria, thickening of basement membrane and irregular nucleus (Fig. 4b), in addition to the presence of some fragmented microvili and vacuoles (Fig.4c). Electron microscopical examination of the kidney of rats treated with both sodium nitrate and vitamin A showed most of the proximal tubular cells revealed a better preservation compared to those treated with nitrate alone. The nucleus showed normal appearance, and the mitochondria were numerous and with closely parallel cristae. Moreover, the microvilli appeared normal (Fig.4d).

3) Electron microscopic findings of the liver:

A) Nornal Liver

In control as well as in vitamin A treated rats, the ultrastructural characteristic features of the hepatocytes was mainly represented by the presence of a large number of mitochondria in close association with rough endoplasmic reticulum occupying most of the cytoplasm. The mitochondria appeared normal in size and have regularly arranged cristae. Large round nucleus was centrally located in the hepatocytes, the nucleus contained chromatin which involves euchromatin (dispersed chromatin) and heterochromatin (condensed chromatin) (Figs. 5a&5b)

B) Electron microscopic findings of the liver treated with sodium nitrate.

Electron microscopic findings of the liver treated with sodium nitrate showed a disintegration of most cytoplasmic organelles. Note the lipid droplet, condensed nucleus, disintegnation of rough endoplasmic reticulum (Fig.5c). Variable size of lipid droplet and Lysosome (Fig.5d). In addition to the presence of myelin figure (finger-print appearance), lysosomal structures and the swollen mitochondria (Fig.5e). Amarked change of hepatocytes represented by irregular nucleus and vacuoles (Fig.5f). After using of vitamin A there is marked improvement of hepatocytes structures (Fig.5g).

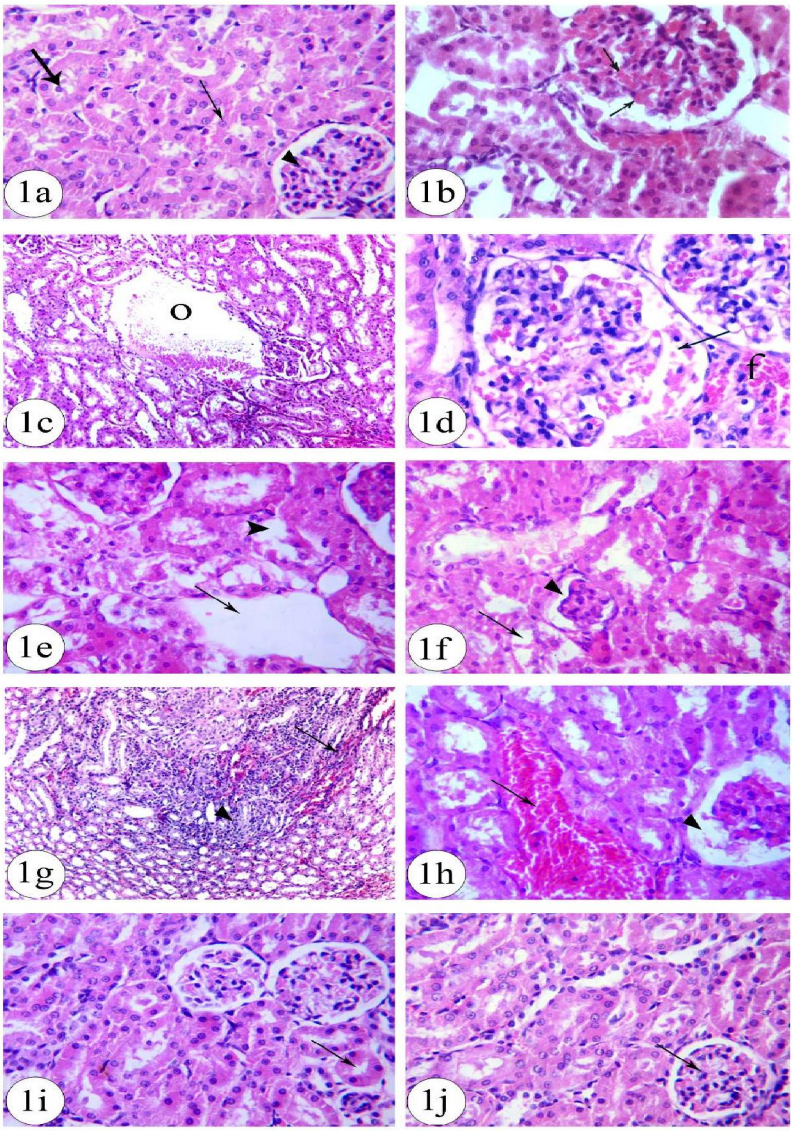
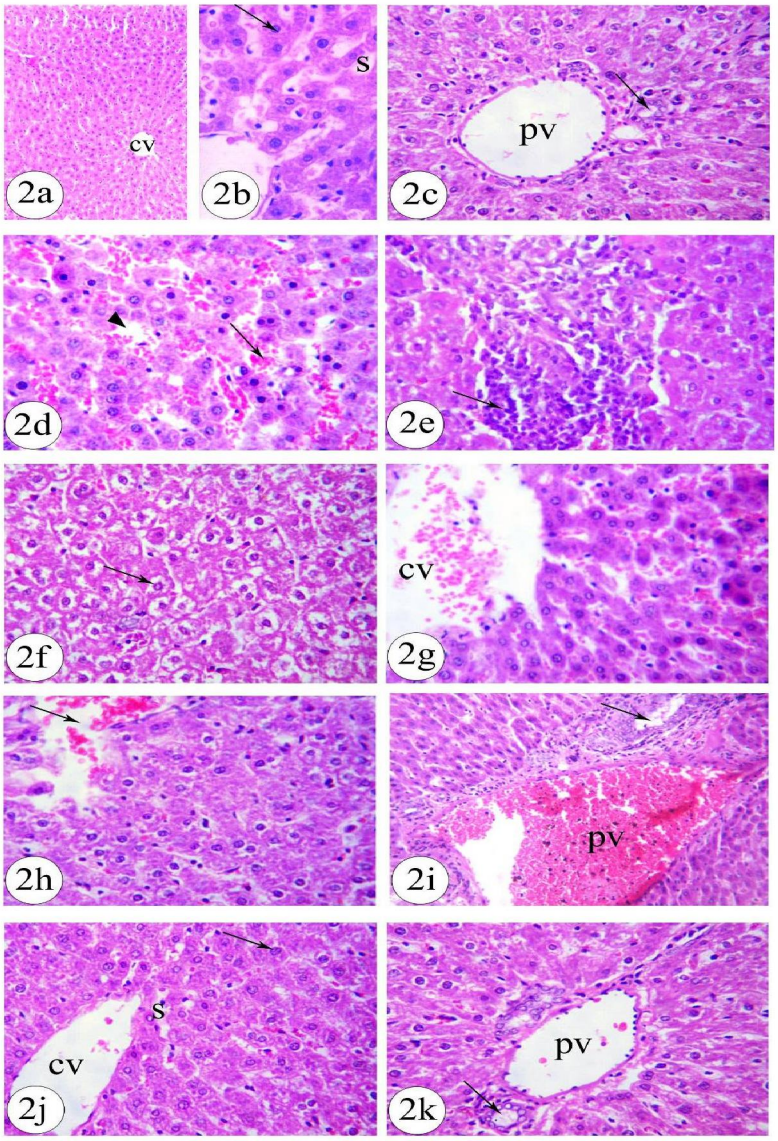


Fig (1a): Section in the kidney of control rat showing malpighian corpuscles with its glomerulus(►) in addition to proximal tubules (↑)). H & E X100.
Fig (1b): Section in the kidney of a rat treated with nitrate showing congestion of glomerular tuft (↑). H & E X400.
Fig (1c): Section in the kidney of a rat treated with nitrate showing dissolution in the cortex (→). H & E X100.
Fig (1d): Section in the kidney of a rat treated with nitrate showing highly vacuolated glomerular tuft (►) invaded with a number of red blood cells and inflammatory cells infiltration (↑). H & E X400.
Fig (1e): Section in the kidney of a rat treated with nitrate showing periglomerular oedema (↑) and degeneration of epithelial cells lining in the renal tubules. H & E X400.
Fig (1f): Section in the kidney of a rat treated with nitrate showing shrinked glomerular tuft(►) and degenerative changes in the renal tubules(↑). H & E X100.
Fig (1g): Section in the kidney of a rat treated with nitrate showing focal fibrosis(►) and infiltrated with a number of inflasmmatory cells (→) at the medullary portion. H & E X100.
Fig (1h): Section in the kidney of a rat treated with nitrate showing a dilated blood vessel engorged with RBCs (→) and enlargement of renal corpuscle with atrophied glomerular tufts (►). H & E X400.
Figs (1i)&(1j): Section in the kidney of a rat treated with nitrate showing nearly normal tubules (Ii) and glomerulus (Ij). H & E X400.



Figs (2a) & (2b): Showing the normal histological structure of the hepatic cords and central vein (CV) (Fig 2a X100). A higher magnification of control liver section illustrating the sinusoids (S) and hepatocytes (↑). H & E X400.
Fig (2c): Showing normal histological structure of the portal vein (PV) and bile duct (↑). H & E X100.
Fig (2d): Section in the liver of a rat treated with nitrate showing a hyperemic hepatic tissue (↑) with hypertrophied hydropic degenerated hepatocytes (►). H & E X400.
Fig (2e): Section in the liver of a rat treated with nitrate showing focal necrotic areas (→) infiltrated with a number of inflammatory cells in the hepatic tissue. H & E X400.
Fig (2f): Section in the liver of a rat treated with nitrate showing degenerated hepatocytes (→) and the anucleated others. H & E X400.
Fig (2g): Section in the liver of a rat treated with nitrate showing dilated central vein (CV) congestion. H & E X400.
Fig (2h): Section in the liver of a rat treated with nitrate showing hydrophic degenerative hepatocytes (→) infiltrated with a number of inflammatory cells. H&E X 400.
Fig (2i): Section in the liver of a rat treated with nitrate showing wide hypermic portal vein (PV) and inflammatory cells infiltration (↑). H & E X400
Fig (2j): Section in the liver of a rat treated with nitrate plus vitamin A showing nearly normal structure of the central vein (CV), sinusoids (S) and hepatic cells (→). H & E X400.
Fig (2k): Section in the liver of a rat treated with nitrate plus vitamin A showing nearly normal structure of the portal vein (PV) and bile duct (↑). H & E X400.

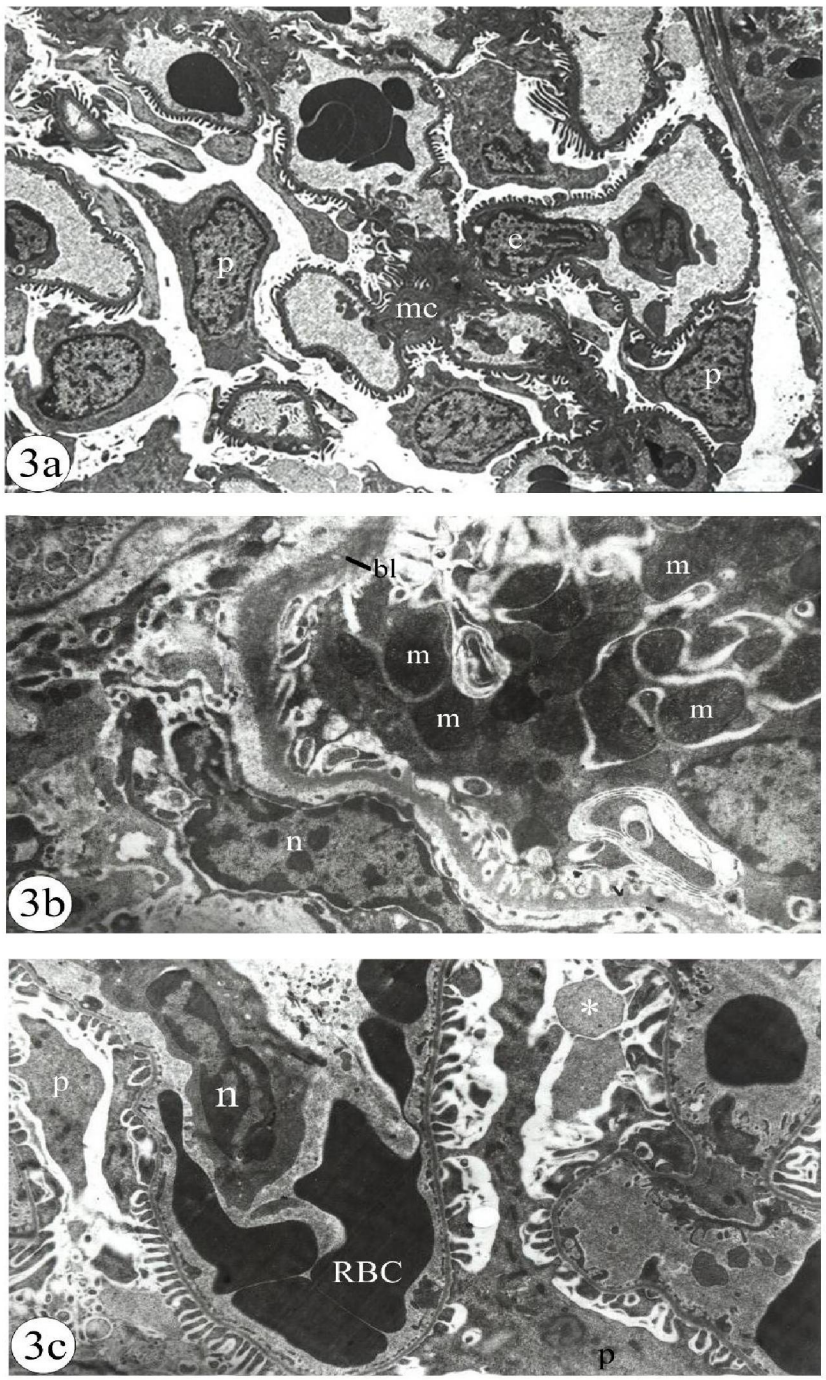


Fig.(3a): Electron micrograph of a renal corpuscle of control rat showing podocytes (p), capillary loops which lined by endothelial cells (e). Mesangial cells (mc) support the capillary loops. (x 3120).

Fig.(3b): Electron micrograph of a glomerulus of rat treated with nitrate for 8 weeks showing thickening basement membrane (bl), irregular nucleus (n) with dark condensation of heterochromatin and swelling mitochondria (m). (x 7100).

Fig.(3c): Electron micrograph of a glomerulus of rat treated with nitrate plus vitamin A showing podocytes (p), amorphous particulated material (*) in the glomerular space, nucleus (n) and red blood (RBC). (x 5680).

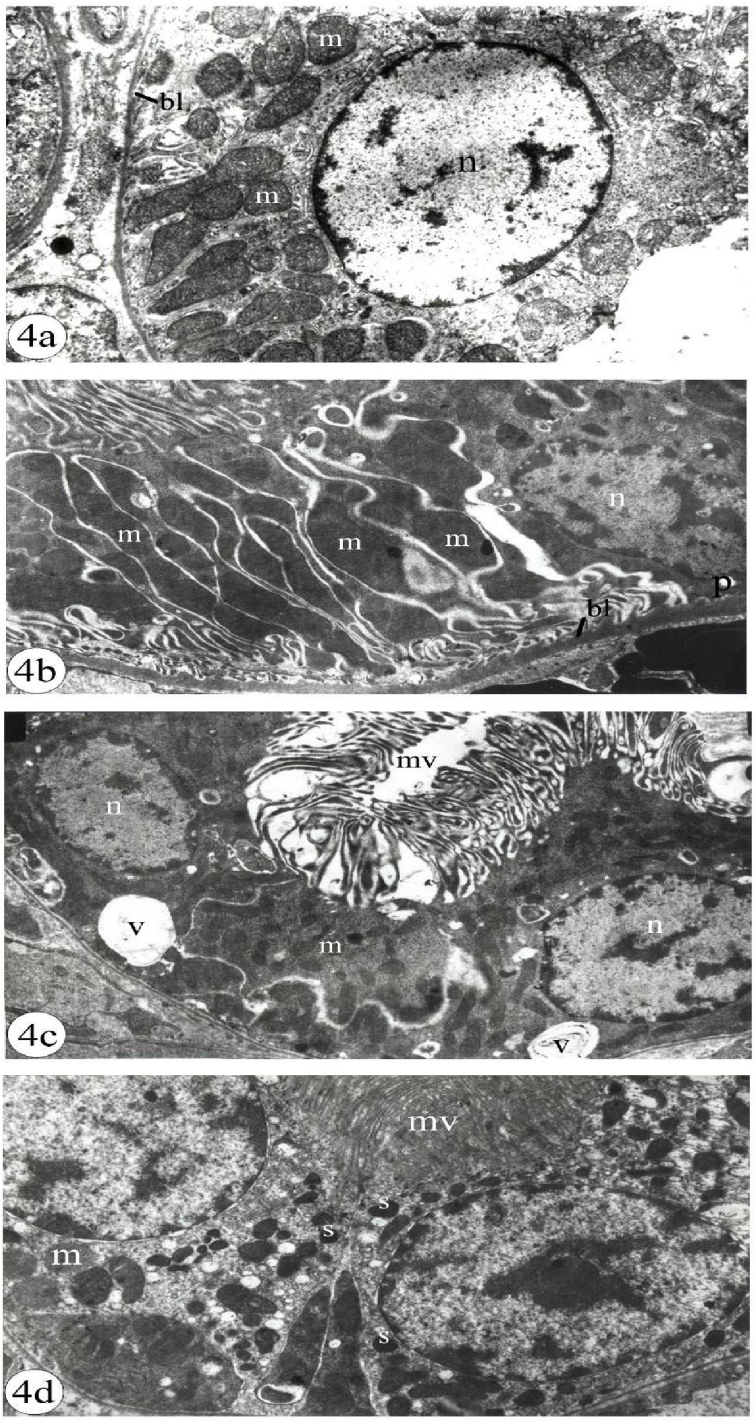


Fig.(4a): Electron micrograph of a proximal convoluted tubule of control rat showing basal membrane (bl), regular nucleus (n) and mitochondria (m). (x 5680).

Fig(4b): Electron micrograph of a proximal convoluted tubule of rat treated with nitrate showing thickening basal membrane (bl) nucleus (n) elongated mitochondria (m). (x 5680).

Fig.(4c): Electron micrograph of a proximal convoluted tubule of a rat treated with nitrate showing dense mitochondria (m), microvilli (mv), nucleus (n) and vacuole(v). (x 5680).

Fig(.4d): Electron micrograph of a proximal convoluted tubule of a rat treated with nitrate plus vitamin A showing condensation of the mitochondria(m), microvilli(mv) and lysosome (S). (x 5680).

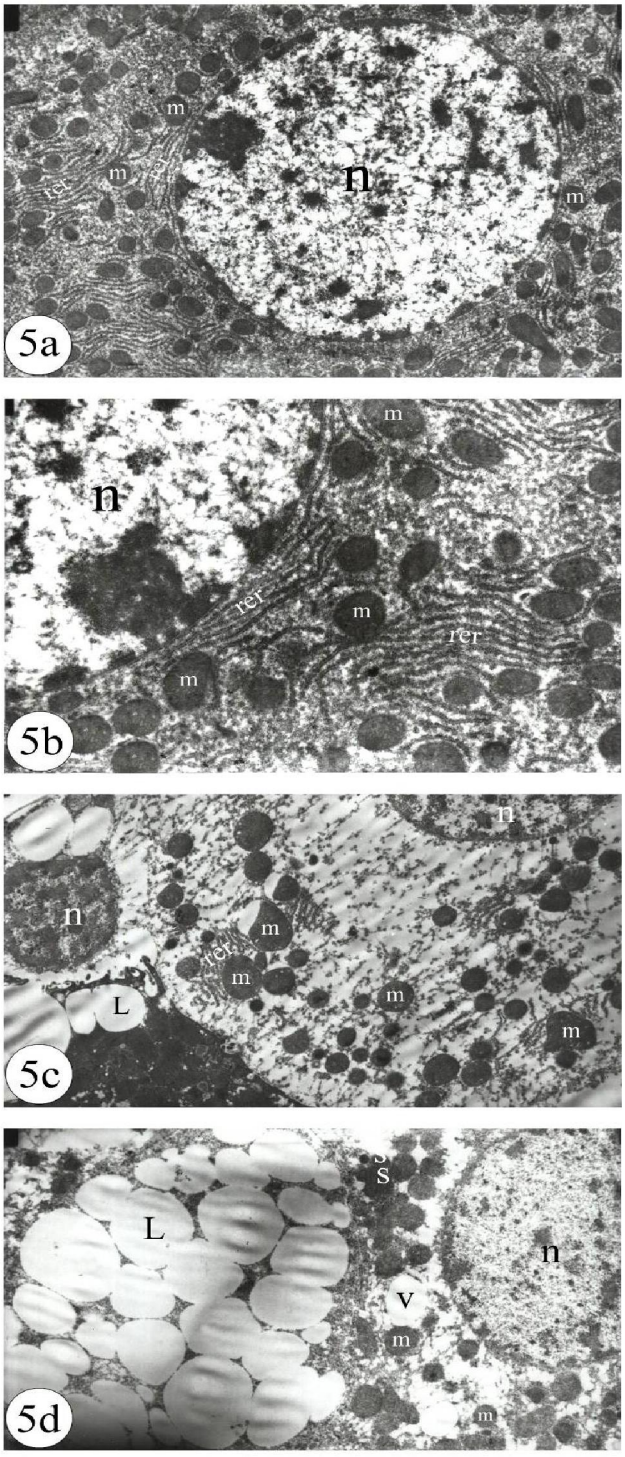


Fig.(5a&5b): Electron micrograph of a hepatocyte of control rat showing mitochondria (m) which appeared normal in size, large round nucleus (n) normal rough endoplasmic reticulum (rer). 5a: (x 9514), 5b: (x 14200).

Fig(5c): Electron micrograph of a hepatocyte of rat treated with nitrate for 8 weeks showing. nucleus (n), mitochondria (m) and rough endoplasmic reticulum (rer) lipid droplets (l). (x 5600).

Fig.(5d): Electron micrograph of a hepatocyte of rat treated with nitrate showing variable size of lipid droplets (l),), mitochondria (m) nucleus (n) and lysosome (S). (x 5680).

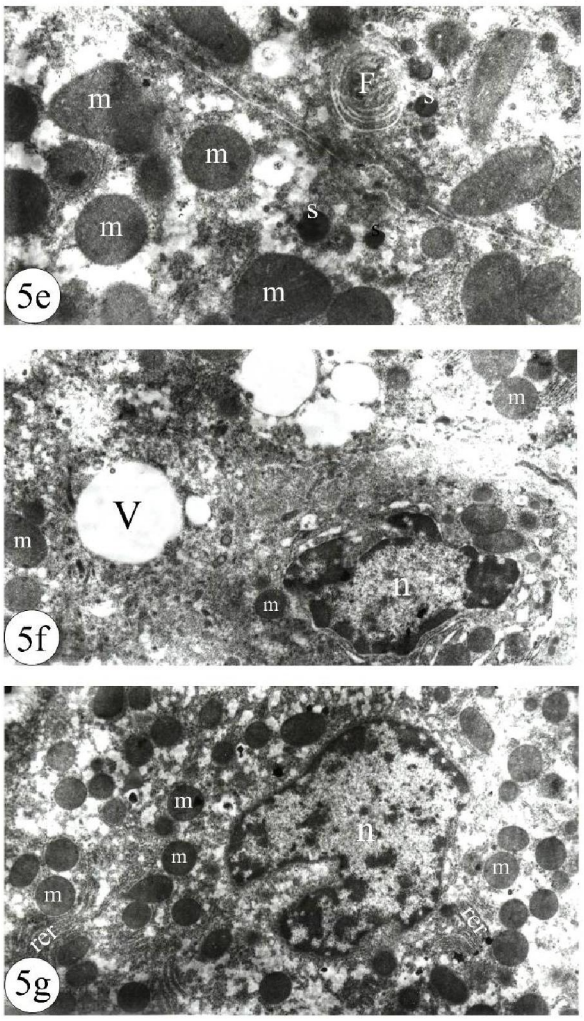


Fig.(5e): Electron micrograph of a hepatocyte of rat treated with nitrate for 8 weeks showing myelin figure (f) (finger-print appearance), lysosome (s) and the swollen mitochondria (m). (x 14200).

Fig.(5f): Electron micrograph of a hepatocyte of rat treated with nitrate showing mitochondria (m), vacuoles (v) and irregular nucleus (n). (x 9514).

Fig.(5g): Electron micrograph of a hepatocyte of rat treated with nitrate and vitamin A showing irregular nucleus (n), nearly normal mitochondria (m) and rough endoplasmic reticulum (rer). (x 11360).

4. Discussion

Nitrate and nitrite ions are both highly soluble in water. These ions are considered as a part of the diet and are also produced from nitric oxide. When taken with food, they are readily absorbed from the small intestine **(Walker,1998**). Nitrate salt is partially converted to nitrite by oral bacteria and by stomach acids, helping to reduce gastrointestinal tract infection **(Combs, 2000).** Nitrate is in high concentration in drinking water and meat products which induced methemoglobinemia in infinite. It has been implicated in the formation of methemoglobin and carcinogenic nitrosamine in humans **(Kapor, 2004; chan, 2011).**

Information available shows that nitrites and nitrates are both oxidation products and ready sources of NO, that NO reacts rapidly with superoxide to form highly reactive peroxynitrite ($ONOO^-$) such products may increase lipid peroxidation (LPO) which can be harmful to different organs including liver and kidney **(Chow and Hong, 2002**; **Hassan *et al*., 2009**); **Rocha *et al*.; 2012).** The liver is liable to injury by a variety of causes and its injury may lead to profound metabolic disorders. Liver injury induced by chemicals has been recognized as one of the most toxicological problems **(Cohen, 1982).**

In the present work, treatment of rats with sodium nitrates resulted in obvious changes in the general architecture of the liver which generally, exhibited inflammatory cells infiltration and alterations of sinusoidal lumen. The most striking histological feature recorded in rats of nitrate-treatment, was vacuolar degeneration in the cytoplasm of hepatocytes, pyknotic nuclei and focal necrotic area infiltered with mononuclear leukocytes. These results are in accordance with **(Hussein *et al*., (2007). Klastskin and Oconn (1993)** attributed the dilatation of the blood sinusoids to the direct toxic effect of the toxin leading to their dilatation. Moreover, Liver of the rats fed with tomato –red exhibited sinusoidal congestion as a result of which the radial orientation of the hepatic cords was lost **(Sharma, *et al.,* 2008)** nuclear pyknosis was also observed in some hepatocytes. Hepatocellular necrosis is probably due to the direct attach 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).** A possible mechanisms for the production of these changes is that nitrate relaxes the peripheral arterioles leading to a drop in blood pressure and a diminished blood flow, resulting in stagnant hypoxemia. In addition, excessive dilatation of the walls of the arteries may compress the vasa vasorum, further impairing the nutrition of the media. These lesions may be related to a prolonged hypoxia subsequent to methaemoglobin formation. In addition to the metabolic activation of cytochrome P-450 **(Haschck and Rousseaux, 1998)**. The observed vascular dilatation may represent an adaptive process as an attempt to overcome this oxygen deficiency which, when prolonged, may be the cause of atrophic cell's formation. Atrophied liver cells probably later, results in the presence of necrotic patches. 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)**. Liver damage was also recorded by **Gasteva *et al.* (1996), Cuzzoerea *et al.* (1997)**, **Sultan *et al*.(2000), Mahmoud (2006) and Nehad *et al*. (2010)**, Pathological studies of 3 male Barki sheep given a dose of 60 mg of sodium nitrite / kg b. wt for 2 months and 120 mg/kg b. wt for another 2 months revealed degeneration and necrotic changes in the liver **(El-Ballal *et al.*, 1994)**. The liver of goats which were administered 1% potassium nitrate at the level of 4 mg/kg b. wt orally through stomach tube for 32 days caused dilatation of the central vein and sinusoids, degenerative changes in periaciner zone, beside hyperplasia, hypertrophy and detachment of epithelium lining of the bile duct with mononuclear cell infiltration **(Mondal *et al.* 1999).**

In the present investigation, electron microscopic study of the hepatocytes of nitrate treated rats revealed cytoplasmic vacuolization and alterations of cell organelles. Short lamellae of rough endoplasmic reticulum found in close association with swollen mitochondria or scattered freely within the cytoplasm. In addition, The hepatocyte response to toxic insult was also reflected by irregular shape of nuclei and nuclear condensation. Similar results were reported in rats by **Gordienko & Didenko (1977); Cracium & Rusu (1980)** and in broilers by **Abou El-Magd *et al.,* (1998). Colombini and Wu (1981)** found that erythrosine administration led to increased membrane permeability to calcium, potassium and chloride ions which consequently disturbed the balance of ions in the liver cells and lead to cytoplasmic vacuolation and mitochondrial swelling.

The rough endoplasmic reticulum is particularly liable to the free radical attack, not only because it is considered as a site of radical production but also due to the enrichment of its membrane with polyunsaturated fatty acids which are susceptible to free radical attack **(Slater, 1984).**

In the present study, it was found that sodium nitrate causes dilatation and congestion of glomerular and peritubular capillaries. The lumen of renal tubules (proximal and distal convoluted tubules) becomes dilated with degenerations of its cells. Also, sodium nitrate induces fibrosis around glomerulus

and renal tubules, which may be due to nitric oxide (NO) formations, which causes vascular smooth muscle relaxation. This leads to dilatations of their lumens and increases their blood flow. The permanent vasodilatation and congestion causes cellular hypoxia and death, which may be followed by fibrosis **(Took, 1996).**

In the present study, light microscopic examination of the kidney of rats treated with sodium nitrate showed alterations of kidney parenchyma compared to the control. Most of these changes were congestion of glomerular tufts and the glomeruli suffer from liquefactive necrosis and dissolution. Mass of fibroblasts, histoblasts and mononuclear cells, intertubular renal blood vessels and focal areas of haemorrhage were also observed in the cortex. **Winks *et al.* (1950)** found that degenerative vascular and parenchymatous lesions in organs including the kidneys of rats which given massive doses of sodium nitrite for up to 18 weeks. Congestion, tubular degeneration and necrosis were described by **Sultan *et al.* (2000)** in the kidney of rats given potassium nitrate.The kidney of goats which were administered 1% potassium nitrate showed mild renal tubular degeneration in the cortex and medulla along with congestion of medullary blood vessels **(Mondal *et al.*, 1999)**. Congestion and nephrosis of the kidney were recorded in Barki sheep given a dose of 60 mg/kg b.wt. for another two months; these changes were associated with the occurrence of microcysts **(El-ballal *et al.*, 1994)**. Intragastric introduction of sodium nitrate to rats was followed by the development of considerable disturbances of energy metabolism in kidneys **(Kostenko, 1995)**. Renal dysfunction in rats induced by nitrate have been recorded by **Gasteva *et al.* (1996), Kengatharan *et al.* (1996), Ismail *et al*. (2003) and Hassan *et al*., (2009). Mahmoud (2006)** found congestion and hemorrhage with infiltration in kidney of rats administrated brilliant blue dye. **Jones and Hunt (1983)** suggested that both haemoglobinaemia resulting from excessive blood haemolysis and renal ischaemia may operate synergistically to produce renal damage and insufficiency. The adverse effects of nitrate on kidney could thus be attributed to oxidation of important iron-containing enzymes such as the cytochromes responsible for cellular respiration and other oxidation-reduction processes **(Santamaria, 2006 ; Manassaram *et al*., 2007)** where oxidation of haemoglobin to methaemoglobin **(Kohn *et al*., 2002**; **Fewtrell, 2004**; **Dejam *et al*., 2005** ; **Umbreit, 2007; Azeez *et al*., 2011)** induced hypoxia.

The present study indicated that sodium nitrate induced marked histopathological alterations in the kidney tissues of rats such as tissue impairment, swelling of the lining epithelium of glomeruli, injured brush border of proximal convoluted tubules, necrotic lesions of the urinary tubules and focal hemorrhage between the degenerative renal tubules. Similar results, have been reported by others **(Aughey *et al*., 1984; kjellstrom, 1986; Mitsumari *et al*., 1998; Inkielewicz and Krechniak, 2003).** The mechanism of swelling starts as a decrease in O2 levels which causes a drop in aerobic respiration. To maintain ATP levels, the cells must rely more on glycolysis. Glycolysis leads to lactic acid builds up, which causes the intracellular pH to drop. An acidic environment in the cell causes dysfunction of the Na+/K+ ATPas and consequent cell swelling due to an influx of Na+ and H2o.Persistent ischemia can lead to ca^{++} influx mitochondrial and lysosomal damage, and membrane damage **(Lieberthat *et al*., 1998).** In the present investigation, many renal tubules of the rat kidneys showed marked degenerative lesions under the effect of sodium nitrate. This is justifiable since the renal tubules are particularly sensitive to toxic influences, in part because they have high oxygen consumption and vulnerable enzyme systems, and in part because they have complicated transport mechanisms that may be used for transport of toxins and may be damaged by such toxins. Also the tubules come in contact with toxic chemicals during their excretion and elimination by the kidneys **(Tisher and Brenner, 1989).** Such degenerative changes were markedly pronounced in the proximal convoluted tubules. These findings reinforce those of **Koechel *et al*., (1984)** and **Damjanov (1996),** who found that many chemicals had a direct nephrotoxic action and exerted their effects principally on the proximal convoluted tubules. 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). De Camargo and Merzel (1980)** observed that mice fed with 10 and 500 ppm NaF for 3 months had shrunken kidneys, degeneration of tubular cells, and dilatation in the convoluted tubules. Similar changes were seen in the present investigation. One possible mechanism for the tubular lesions was the direct toxic effect on the cell function (Bold). Damage to the brush border and leakage of alkaline phosphates (ALP) and gammaglutamyle transferase (GGT) enzymes, which are associated with the brush border of the renal tubules, as a result of toxin binding to the brush border and considered as an early marker of toxic tubular insult **(Porter, (1994); Fadel and Larsen, 1994; Edelstein, *et al*., 1995; Davies *et al*., 1995 ;** The damage produced by sodium nitrate in the rough endoplasmic reticulum as reported by **Elewa *et al*., (1999), El-Beih *et al*. (1987) and Berlin (1967).** In addition, **Palla *et al*., (1987),** postulated that in many

kidney diseases, the permeability of the glomerular capillaries is increased leading to increased levels of excreted proteins. They added that any lesions produced in the kidney tubules will eventually cause dysfunction in the transport mechanism to and from the renal epithelium.

The most characteristic changes in the kidneys of nitrate treated rats seen under the electron microscope revealed that the proximal tubules contain a very dense mitochondria with numerous closely packed cristae, thick basal lamina, irregular nucleus, many cytoplasmic vesicles and vacuoles and lipid droplets.

The present ultrastructure of the kidney in sodium nitrate group revealed several changes in the podocytes, proximal tubules. In the present investigation the podocytes displayed fusion of their foot processes which may obliterate the filtration slits. The glomeruli are well adapted structure for filtration process. In this regard, three layers contribute to this process, the capillary endothelium, glomerular basement membrane and podocyte layer **(Burkitt *et al.*,1999).** However, any alteration occurs in such structures causes disturbance in the ultrafiltration process **(Ludere *et al.*,1981**).

In the present study, administration of vitamin A together with sodium nitrate was advantageous to ameliorate the health status of the animal. Vitamin A had a positive effect on the kidney and liver of nitrate-treated rats. High doses of nitrate and nitrite in food products decreased the food intake, body weight, vitamin A in liver and utilization of Bcarotene **(Majcherzak, *et al.* 1991, EL-Tahan *et al.*, 2010).** Forages with a high nitrate content have been reported to affect vitamin A nutrition in animals **(Carner, 1958; Carner *et al.*, 1958; Smith *et al.*, 1961)**. Increased depletion rate of liver vitamin A stores was observed by **O'Dell (1960)** in rats fed a diet containing nitrite. *In vivo* experiments indicate that nitrite is capable of destroying carotene and vitamin A **(Pugh *et al.*, 1962; Olson *et al.*, 1963, Keating *et al.*, 1964)**.

Nitrate was found to decrease liver vitamin A stores in rats **Yadav *et al.(* 1962)** and to be associated with more rapid depletion of liver vitamin A stores **(Carner *et al.*, 1958)**. In rats with adequate vitamin A stores fed a high nitrate silage diet, liver stores of vitamin A were maintained by the addition of vitamin A but declined when cartoene was given **(Smith *et al.*, 1961)**. Liver storage of vitamin A was not affected in rats fed nitrate when vitamin A was administered regardless of whether vitamin A was given orally as an oil solution, as a water solution or injected in an oil solution **(Emerick and Olson, 1962)**. However, nitrate did significantly reduce liver storage of vitamin A with intragastric administration of carotene **(Emerick and Olson, 1962)**. These results may agree with the opinion of the authors, that nitrate acts either to destroy carotene, inhibit the conversion of carotene to vitamin A, or to prevent the absorption of vitamin A in the rat. **Emerick and Olson (1962)** concluded that vitamin A supplementation may overcome this effect of nitrate. A possible mechanism of the action suggested by these authors may be through the goitrogenic effect of nitrate on the thyroid of rats. Regarding the effect of nitrate and nitrite on vitamin A, one theory has been proposed by **Wood (1980)** that nitrite oxidizes iron-containing enzymes such as dioxygenase enzymes thereby decreasing the conversion of beta carotene to vitamin A. **Mitchell *et al.* (1967)** reported that vitamin A is readily destroyed by oxidation and since nitrates are oxidizing agents, nitrates could destroy vitamin A.

The addition of vitamin A to the ration mitigated the toxicity of nitrite in studies involving chicken and turkey **(Sell and Roberts, 1963; Sunde, 1964; Marrett and Sunde, 1968)**. These studies point to the interference with vitamin A utilization as a mode of action of nitrates in chicken and turkeys.

Finally, the present study reveals toxic effects of sodium nitrate on the kidney and liver during the use of this drug. Therefore, more researches must be done on other organs of the body to highlight its effects on these organs.

5. Conclusion:

The present study, concluded that high dose intake of vitamin A 50,000 I.U. / rat result in decrease the toxic effect of sodium nitrate on the histopathology and ultrastructure of liver and kidney. It is recommended that the use of sodium nitrite must be limited and use of vitamin A as antioxidant to prevent the toxic effect.

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