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Comparative Protective Effects of L-Ascorbic Acid and Crude Honeybee Extract Supplements Against Toxic Effects Induced by Sodium Nitrate in Male Rats

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ABSTRACT

Background: Sodium nitrate has been used extensively as a meat additive to keep meat products fresh. Several strategies have been implemented in counteracting such toxicity.

Objective: The goal of the current investigation was to determine if ascorbic acid and crude honey extract can protect male rats from the harmful biochemical and histopathological effects of sodium nitrate.

Materials and methods: 36 male Albino rats were alienated into 6 groups; control was given saline, honey group was given (2.5g/kg/day honey, ascorbic group was given (200mg/kg /day) L-ascorbic acid, $NaNO_3$ group was given 200mg/kg/day, honey/ $NaNO_3$ group rats given honey and nitrate with the former doses, and Ascorbic/ $NaNO_3$ group was given L-ascorbic acid and NaNO3 with the former doses. Organs' relative weight, liver and kidney blood biochemical determinants, serum tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), histology and histochemistry were determined.

Results: The sodium nitrate had significantly increased liver, kidney and spleen relative weights while decreased thymus weight. Higher levels of AST, ALT, urea, creatinine, IL-6 and TNF-α were observed in $NaNO_3$ treated group. Liver, kidney, and spleen of rats treated with $NaNO_3$ revealed histological and histochemical alterations. The treatment with L-ascorbic acid and crude honeybee improved the later dysfunctions with more palliative effect for L-ascorbic acid.

Conclusions: We can conclude that sodium nitrate has to be limited in use. L-ascorbic acid was therefore recommended to be used in reducing the harmful metabolic alterations induced by sodium nitrate better than the honeybee.

Keywords: Ascorbic acid, Honey, Kidney, Liver, Sodium nitrite, Spleen.

INTRODUCTION

Sodium nitrate and sodium nitrite are preservatives found in treated meats like sausage, bacon, and ham, as well as some cheeses [1]. Nitrate can be transformed to nitrite in the food byproducts, as well as in the gastrointestinal tract or mouth, both nitrate and nitrite must be taken into consideration when evaluating any potential hazard [2].

Adding nitrite and nitrate to food byproducts is just one of the various sources of nitrogenous compounds to which human beings are exposed. Smoked foods and salt-preserved fish are other sources of nitrogenous compounds in the human diet[3]. It is believed that low levels of Na nitrate consumption for long period are associated with several health problems with many harmful side effects like carcinogenicity, histopathologic tissue alterations, nitrative tissue injury, and hepatic as well as renal lipid peroxidation, as well as encouraging chromosomal aberrations [4]. The liver, kidney and spleen are one of the potential targets of toxicity after exposure to Na nitrate, through induction of oxidative stress [5].

Animals' ability to withstand the toxic influences of environmental toxicants is reliant on their detoxifying capability and antioxidant schemes.

Vitamins, amino acids and their byproducts, trace elements, plant phenolics and fatty acids have recently been discovered to be effective antioxidants[6]. Ascorbic acid and honeybee are considered potent antioxidants, they play a vital role in diminishing oxidative stress through reactions with different free radicals and inhibition of lipid peroxides formation [7].

Ascorbic acid can enter mitochondria through facilitated glucose diffusion via transporter and deliberates mitochondrial shield in contradiction of oxidative injury. Ascorbate has been concerned in various biological processes. It is considered a cofactor for various enzymatic phases in the monoamines', collagen, peptide hormones, amino acids, and carnitine synthesis. Moreover, it plays a significant role in the antioxidative defense at several stages [8].

The honeybee is a nutrient used for centuries as a disease fighter and health improver[9]. Honey consists of a mixture of carbohydrates, minerals, proteins, and water [10]. It has been reported that honey has antioxidant properties as it can decrease lipid peroxidation [11]. This goes back to its component of flavonoids and antioxidant trace elements [12].

Ascorbic acid and Honeybee exert benefits against liver, kidney and spleen injury in experimental impairment by exerting anti-inflammatory and antioxidant influences. Therefore, this study intended to estimate the possible positive effects of ascorbic acid and honey against Na nitrate-induced liver, kidney and

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spleen injuries through biochemical, histological and histochemical assessments.

MATERIALS AND METHODS

Chemicals

Sodium Nitrate ($NaNO_3$) (CAS number 7631-99-4, Merck Co., USA) and L-ascorbic acid (CAS number 50-81-7) were obtained from Sigma–Aldrich Corp (St. Louis, MO, USA). Honey was obtained from a local bee farm and kept within the Prophetic Medicine Foundation, Ismailia, Egypt with Commercial Registration code No (6332001/111330).

Animals

Thirty-six albino male rats were purchased from the animal house of Faculty of Veterinary Medicine and kept for 1 week for acclimatization under normal conditions, free access to water and standard ration with 50% humidity and a natural dark-light cycle.

Ethical approval:

This experiment was approved by the ethics committee of Suez Canal University, Faculty of Science, Ismailia, Egypt. All the experimental procedures were carried out according to the principles and guidelines of the Ethics Committee of the of Faculty of Science, Suez Canal University, Ismailia, Egypt conformed to "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996). The approval number was REC158/2022).

Rats were divided randomly into 6 groups (6 rats for each) and treated orally for one month. The control group was given saline, honey group was given (2.5g/kg body weight /day, orally) honey according to **Al-Seeni *et al.*** [13]. The ascorbic group was given (200mg/kg body weight /day, orally) L-ascorbic acid according to **Mohammed *et al.*** [14]. $NaNO_3$ group was given orally to rats at doses of 200mg/kg/day according to **Aly *et al.*** [15]. The honey/ $NaNO_3$ group rats were given (2.5g/kg body weight /day, orally) honey treatment 30 minutes before 200mg/kg/day NaNO3 and the Ascorbic/ $NaNO_3$ group rats were given (200mg/kg body weight /day, orally) L-ascorbic acid treatment 30 minutes before they received 200mg/kg/day NaNO3.

Blood and tissue sampling

Rats were fasted overnight 24 hrs. after the end of the experimental duration. Retro-orbital plexus blood samples were then drawn and sera were subsequently separated and then stored at -20°C for later use in biochemical analysis. Rats were weighed and then euthanized by cervical dislocation. Their liver, spleen, thymus and kidney were weighed in grams and the relative organ weights of each rat were then calculated. The liver, spleen and kidney were fixed and prepared for histopathological and histochemical examination.

Table (1): The relative kidney, liver, spleen and thymus weights of rats treated with sodium nitrate, honey and L-ascorbic acid

Biochemical analysis

Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were assayed using a commercial kit (SPIN-REACT company, SPAIN, REF: SP41274 and REF: MD41264) according to **Bergmeyer *et al.*** [16]**.** Creatinine level was detected using Diamond Diagnostic commercial kit according to **Heinegård and Tiderström** [17]. Serum interleukin 6 (IL-6) levels were determined using rats' commercial ELISA kits that were purchased from (IBL Co., Japan) Code No. 27194 according to **Jankord and Jentiolo** [18]. Serum tumor necrosis factor alpha (TNF-α) was determined using Kamiya Biomedical Co., USA with Cat. No. KT-19418 according to **Prince *et al.*** [19]**.**

Histopathological examination

Specimens (liver, kidneys and spleen) from all the experimental groups were harvested and then fixed in 10% neutral formalin. Following proper dehydration and clearing then the samples were implanted in paraffin wax. Five µm thickness sections were prepared and stained with H&E [20]**.** The liver and kidney sections were stained with periodic Schiff stain (PAS). The detected histopathological changes were examined blindly for the treatment. The PAS histochemical reactivity area percentage was estimated using Image J software, Japan from 9 random fields/slide. Five slides/ organ/ group were examined.

Statistical analysis

The analysis of variance test (One Way ANOVA), followed by the Duncan's test for intergroup comparisons, was used to calculate the mean ±SE of the examined groups' findings, with statistical significance set at $P \leq 0.05$. Post The Statistical Package for the Social Sciences Version 20 software was used to conduct all statistical analyses (SPSS Inc., Chicago, IL, USA).

RESULTS

Relative organs weights (kidney, liver and spleen)

The relative kidney, liver and spleen weights of $NaNO_3$ treated rats were significantly higher than those of rats in the control group ($P \leq 0.05$). The relative thymus weights of $NaNO_3$ treated rats were significantly lower than those of the control ones ($P \leq 0.05$). The honey/ $NaNO_3$ and the Ascorbic/ $NaNO_3$ group's liver, kidney and spleen relative weights were diminished significantly ($p \leq 0.05$) compared to the $NaNO_3$ group (Table 1).

Groups	Relative liver weight	Relative kidney weight	Relative spleen weight	Relative thymus weight

Control group	2.350± 0.030^{cd}	0.310± 0.006^{b}	0.339± 0.012^{c}	0.144± 0.06^{ab}
Honey group	2.231± 0.060^{cd}	0.284± 0.008^{cd}	0.347± 0.004^{c}	0.152± 0.002^{ab}
Ascorbic group	2.161± 0.061^{d}	0.274± 0.005^{de}	0.3665± 0.024^{c}	0.1578± 0.006^{a}
NaNo3 group	3.386± 0.080^{a}	0.367± 0.007^{a}	0.830± 0.090^{a}	0.139± 0.006^{b}
Honey/ NaNo3 group	2.751± 0.050^{b}	0.304± 0.007^{bc}	0.507± 0.021^{b}	0.151± 0.004^{ab}
Ascorbic/ NaNo3 group	2.540± 0.071^{c}	0.260± 0.008^{e}	0.426± 0.010^{bc}	0.148± 0.004^{ab}

Information is displayed as means ±SE (n = 6). Following an ANOVA, a Duncan's multiple comparison test was used to determine if the mean values in the distinct superscript letters between the rows were statistically different at P ≤0.05.

Honey was administered to the Honey group (2.5 g/kg body weight/day, orally). The ascorbic group was given (200 mg/kg body weight /day, orally) L-ascorbic acid. $NaNO_3$ group was given orally to rats at doses of 200 mg/kg/day according to **Aly *et al.*** [15].

The honey/ $NaNO_3$ group rats were given (2.5 g/kg body weight /day, orally) honey treatment before 200 mg/kg/day NaNO3 and the Ascorbic/ $NaNO_3$ group rats given (200 mg/kg body weight /day, orally) L-ascorbic acid treatment before received 200 mg/kg/day NaNO3.

Biochemical Analysis

Table (2) demonstrated that the activities of serum ALT, AST, creatinine, TNF-α and IL-6 significantly upsurged ($P \leq 0.05$) in rats treated with $NaNO_3$ compared with the control. Pretreatment with honey or ascorbic acid before treatment with $NaNO_3$ significantly ($P \leq 0.05$) abridged the activities of ALT, AST, creatinine, TNF-α and IL-6.

Table (2): The biochemical analysis of rats treated with sodium nitrate, honey and L-ascorbic acid

Groups	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	TNF (pg/mL)	IL-6 (pg/mL)
Control group	28.63 ± 1.08^{bc}	24.9± 0.50^{c}	1.01± 0.06^{d}	16.89± 0.80^{cd}	15.76± 1.21^{c}
Honey group	25.81 ± 1.37^{cd}	22.5± 0.91^{d}	0.99± 0.06^{d}	14.92± 0.90^{d}	14.91± 0.50^{c}
Ascorbic group	23.34 ± 1.01^{d}	22.4± 0.80^{d}	0.88± 0.04^{d}	15.62± 0.20^{d}	13.28± 0.32^{c}
NaNo3 group	35.86 ± 0.87^{a}	36.27 ± 0.90^{a}	1.78±0. 06^{a}	33.74± 3.80^{a}	34.02± 1.80^{a}
Honey/ NaNo3 group	29.16 ± 0.32^{b}	29.43 ± 0.80^{b}	1.53± 0.30^{b}	24.21± 0.31^{b}	27.81± 1.10^{b}
Ascorbic/ NaNo3 group	28.47 ± 0.54^{bc}	29.93 ± 0.52^{b}	1.34± 0.04^{c}	21.11± 0.51^{bc}	26.14± 1.11^{b}

Information is displayed as means ±SE (n = 6). Following an ANOVA, a Duncan's multiple comparison test was used to determine if the mean values in the distinct superscript letters between the rows were statistically different at P ≤0.05. Tumor necrosis factor alpha, aspartate aminotransferase, alanine aminotransferase, TNF-α, and interleukin 6 are all abbreviations for different enzymes. The Honey group was given (2.5g/kg body weight /day, orally) honey.

The ascorbic group was given (200mg/kg body weight /day, orally) L-ascorbic acid. $NaNO_3$ group was given orally to rats at doses of 200mg/kg/day according to **Aly *et al.*** [15]. The honey/ $NaNO_3$ group rats were given (2.5 g/kg body weight /day, orally) honey treatment before 200mg/kg/day NaNO3 and the Ascorbic/ $NaNO_3$ group rats given (200mg/kg body weight/day, orally) L-ascorbic acid treatment before received 200 mg/kg/day NaNO3.

Histology of Liver, kidney and spleen

Histological examination of the livers of animals from all experimental groups is demonstrated in Figures (1 and 2). Hepatic tissue from the control, honey, and ascorbic acid groups revealed normal histological structure under optical microscopy.

The hepatocytes seemed to be typical big polygonal cells with conspicuous round nuclei and eosinophilic cytoplasm, and a few hepatic sinusoids were positioned between the hepatic cords where Kupffer cells were delicately positioned. The hepatic artery, bile duct, portal vein, and central vein were all encircled by healthy hepatocytes. The liver sections of $NaNo_3$-treated group exhibited histological perturbations. The most noticeable histological abnormality was the hydropic degeneration and focal necrosis of liver cells' with pyknotic nucleus. The hepatic tissues showed the presence of inflammatory cells infiltrations in the portal spaces.

The hepatic sections of honey/NaNo3 treated group revealed that histological damage induced by $NaNo_3$ was still persisted. The most distinct histological perturbation was the granuloma of liver cells and focal necrosis of hepatic cells.

The liver tissues revealed the existence of the mild proliferation of the bile duct in the portal area. In contrast, groups given L-ascorbic acid treatment before receiving $NaNO_3$ exhibited no damage with its normal histological appearance.

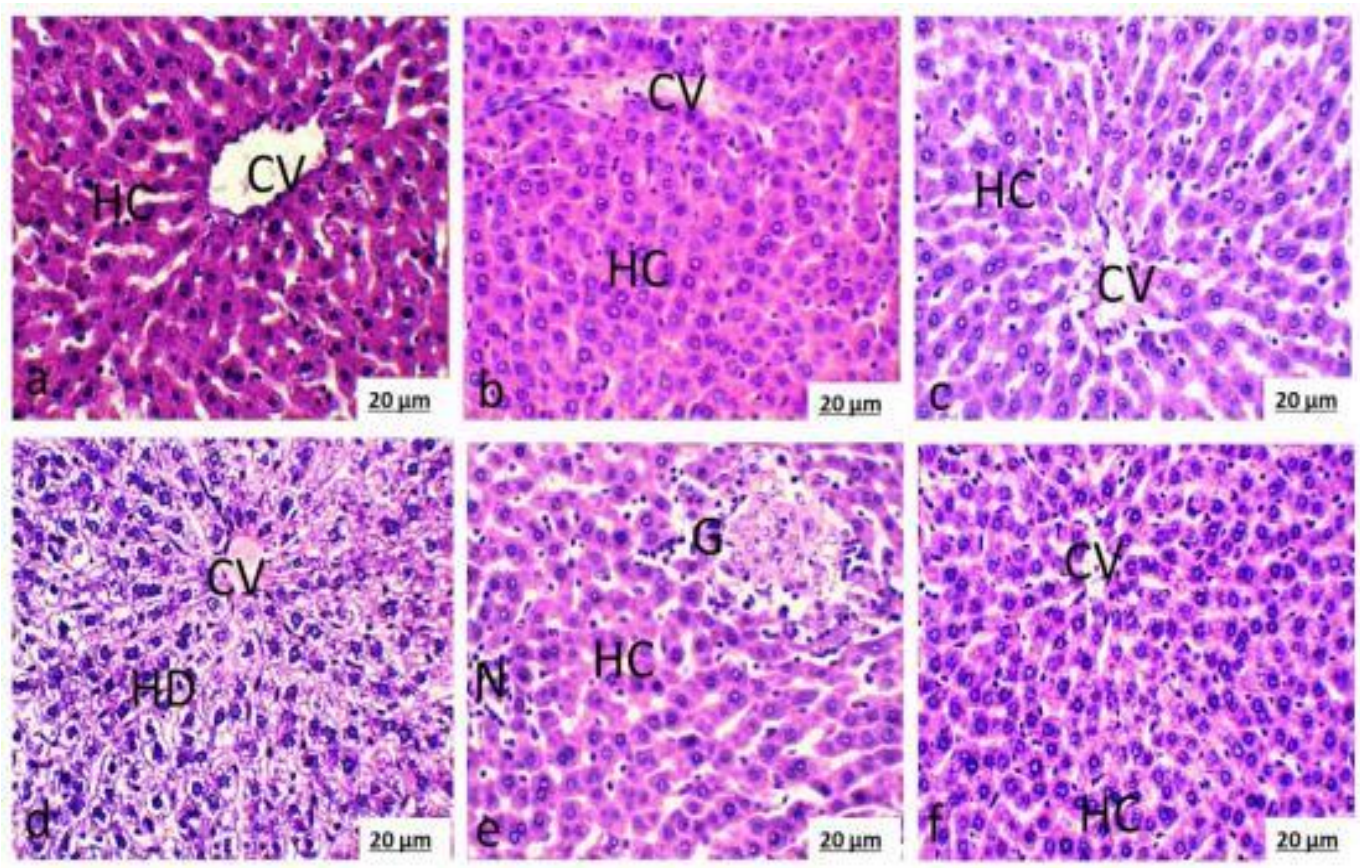

Figure (1): (**a**) Liver section of a control rat. Liver sections of rats treated with (**b**) honey and (**c**) L-Ascorbic acid showing normal histological structure of hepatocytes (HC) surrounding the central vein (CV). (**d**) Liver section of rats treated with $NaNo_3$ showing hydropic degeneration (HD) surrounding the central vein (CV). (**e**) Liver sections of rats treated with honey and $NaNo_3$ showing granuloma (G) and focal necrosis with pyknotic nucleus (N). (**f**) Liver sections of rats treated with ascorbic acid and $NaNo_3$ showing normal histological structure of hepatocytes (HC) surrounding the central vein (CV). (H&E, 400X).

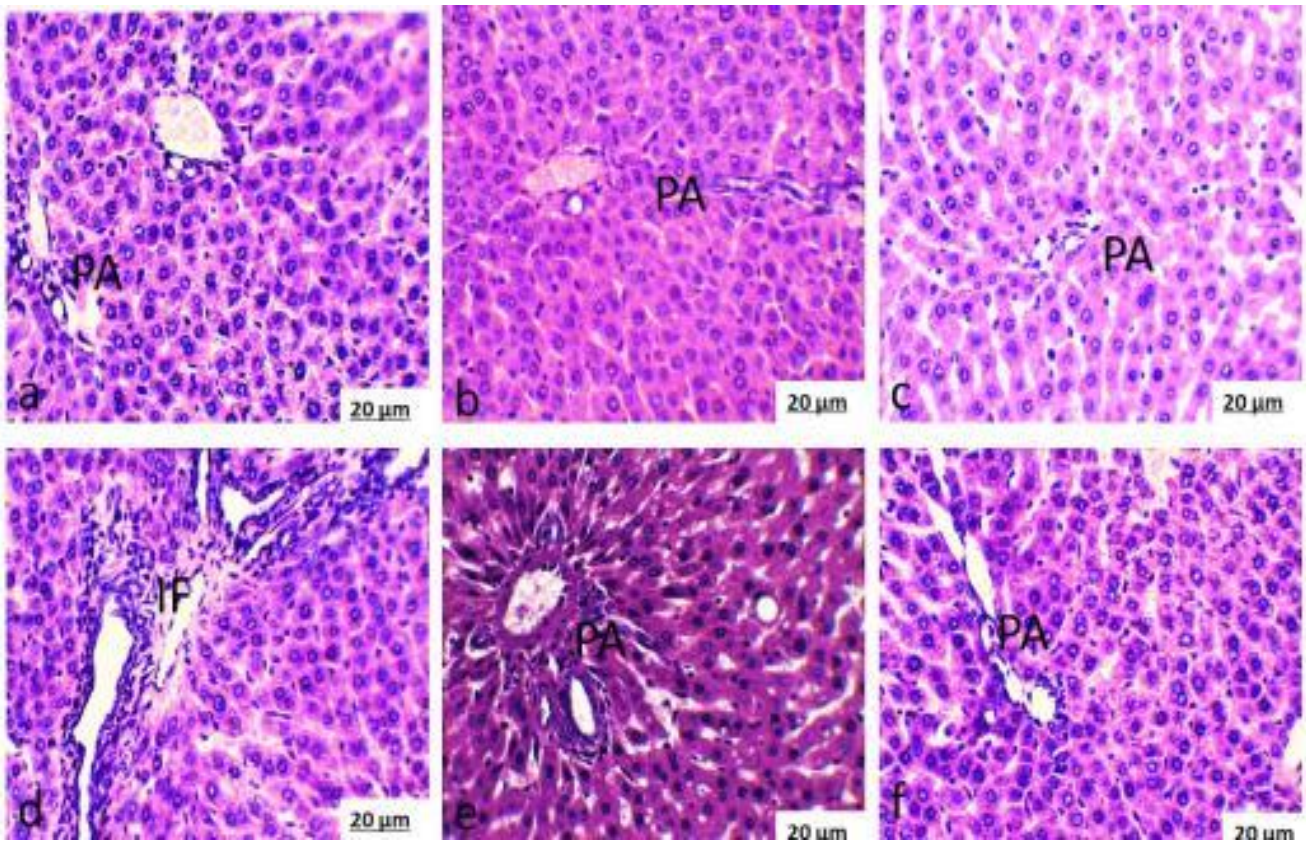

Figure (2): (**a**) Liver section of a control rat. Liver sections of rats treated with (**b**) honey and (**c**) L-Ascorbic acid showing normal histological structure of hepatocytes surrounding the portal area (PA). (**d**) Liver section of rat treated with $NaNo_3$ showing infiltration of inflammatory cells (IF) surrounding the portal area. (e) Liver sections of rats treated with honey and $NaNo_3$ showing normal histological structure of hepatocytes surrounding the portal area (PA) accompanied by a proliferation of the bile duct. (**f**) Liver sections of rats treated with ascorbic acid and $NaNo_3$ showing normal histological structure of hepatocytes surrounding the portal area (PA). (H&E, 400X).

Inspection of the hepatic sections of a control, honey and ascorbic acid treated rats showed a deep pink stain of a strong PAS reaction in the cytoplasm of hepatocytes while the nuclei did not exhibit any reaction (Figure 3; a, b &c). Inspection of liver of rats treated with $NaNo_3$ showed a significant decrease in the glycogen content (Figure 3 d). Examination of liver sections of treated rats with honey and $NaNo_3$ or ascorbic acid and $NaNo_3$ showed a deep pink stain of a strong PAS reaction in the pole of the cytoplasm of hepatic cells (glycogen flight) while the nuclei were negatively stained (Figure 3; e &f).

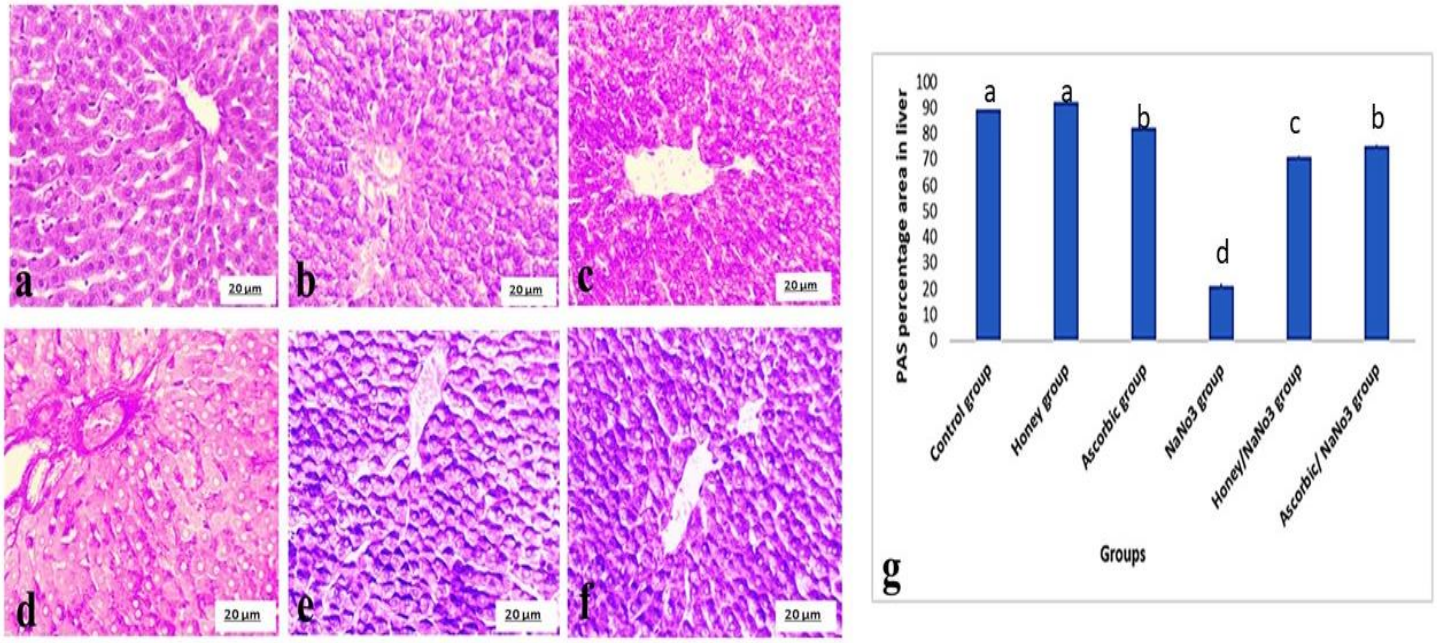

Figure (3): (a) Liver section of a control rat. Liver sections of rats treated with (b) honey and (c) L-Ascorbic acid showing strong PAS reaction in the cytoplasm of hepatocytes with negative stained nuclei (d) Liver sections of rats treated with $NaNo_3$ showing decreased glycogen content. Liver sections of rats treated with (e) honey and $NaNo_3$ and (f) ascorbic acid and $NaNo_3$ showing strong PAS reaction in the pole of the cytoplasm of hepatocytes (glycogen flight) with negative stained nuclei. (PAS, 400X). (g) Histogram of the PAS percentage area between different groups. Different superscript letters denoted significant differences at $P \leq 0.05$.

Figure (4) displayed the results of a histological analysis of the kidney of rats from various groups. Kidney tissue from the control, honey, and ascorbic groups showed a normal shape of the cortex including glomerulus with surrounding renal tubules under light microscopy (proximal and distal convoluted tubules). The NaNo3 group's kidney showed histological alterations that included the glomerulus shrinking, tubules enlarging and becoming foamy, and the brush boundaries on the tubules disappearing. The glomerulus and renal tubules were protected, but these renal alterations were significantly reduced by treatment with honey or L-ascorbic acid prior to NaNo3.

Bowman's capsules and the basement membranes of the proximal and distal convoluted tubules of the treated and control rats' treated kidney sections stained with PAS revealed a strong positive reactivity. Additionally, a strong response was seen in the proximal convoluted tubules' brush boundaries (Fig. 5). When Bowman's capsules and tubules were examined in renal sections from mice treated with NaNo3 and stained with PAS, there was a minimally favorable result. The proximal convoluted tubules' brush border showed signs of weak and partial loss (Figure 5d). A favorable response was seen in the tubules and Bowman's capsules when the kidney sections of rats fed with honey and ascorbic acid before NaNo3 were stained with PAS. Additionally, a significant reactivity was seen in the proximal convoluted tubules' brush boundaries (Figure 5; e & f).

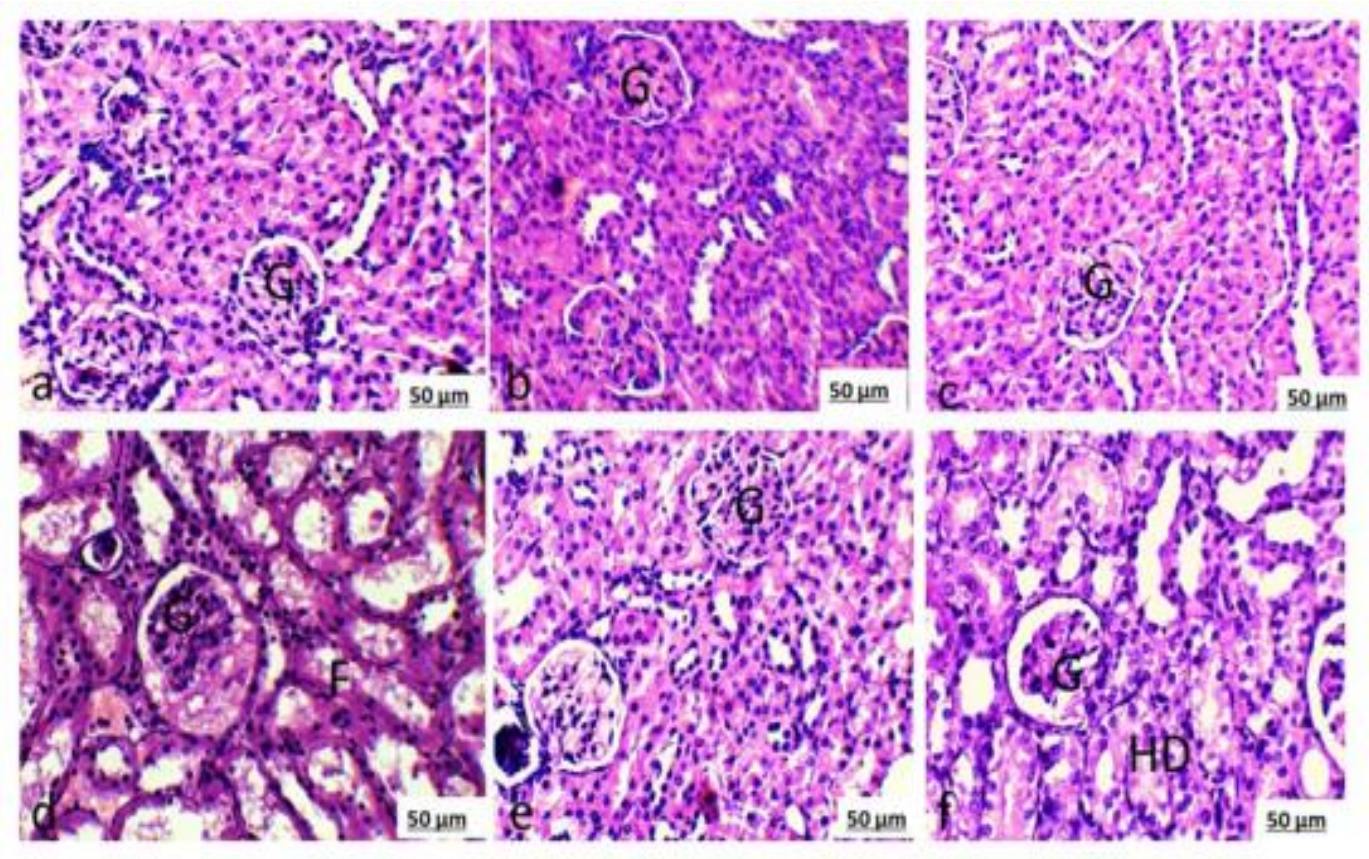

Figure (4): (a) Transverse kidney section of (**a**) control rat. Transverse kidney sections of rats treated with (**b**) honey and (**c**) L-Ascorbic acid showed a normal glomerulus (G) and renal tubule structure. (**d**) kidney sections of rats treated with $NaNo_3$ showed a foamy appearance of renal tubules (F) and shrinkage glomerulus (G). (**e**) Kidney sections of rats treated with honey and $NaNo_3$ showed a low number of atrophic glomeruli, normal glomerulus (G) and normal renal tubules. (**f**) Kidney sections of rats treated with ascorbic acid and $NaNo_3$ showed a normal glomerulus (G) and hydropic degenerated (HD) of the renal tubules. (H&E, 200X).

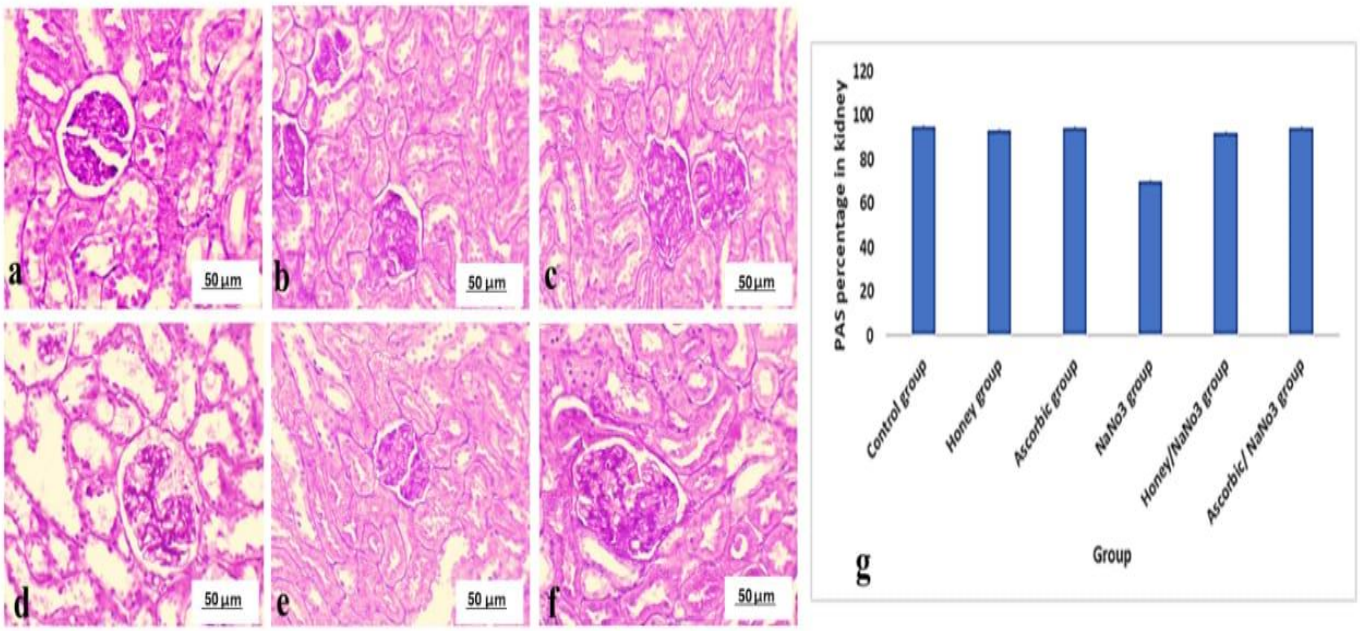

Figure (5): (a) Transverse Kidney section of a control rat. Kidney sections of rats treated with (b) honey and (c) L-Ascorbic acid showed strong PAS reaction in the Bowman's capsules and the tubules (d) Kidney sections of rats treated with $NaNo_3$ showed weak PAS stain reaction in the brush borders of proximal convoluted tubules and the Bowman's capsules are noticed. Kidney sections of rats treated with (e) honey and $NaNo_3$ and (f) ascorbic acid and $NaNo_3$ showed strong PAS reactions in the Bowman's capsules and the tubules. (PAS, 200X). (g) Histogram of the PAS percentage area between different groups. Different superscript letters denote significant differences at $P \leq 0.05$.

Histological examination of the spleen of rats from the different groups is shown in figure (6). The spleen tissue from the control, honey, and ascorbic acid groups showed normal splenic histo-architecture, including normal lymphoid white pulp with lymphoid follicle having periarteriolar lymphoid sheath around the central artery, and normal hematogenous red pulp. Rats given NaNo3 nevertheless had histopathological changes in the spleen tissue. The most protuberant lesions were many apoptotic lymphocytes detected in the white pulp. Lymphocyte apoptosis is characterized by the condensation of nuclear chromatin, shrinkage of individual lymphocytes and fragmentation of apoptotic cells into membrane-bound bodies. Whereas the spleen of rats treated with honey or ascorbic acid before $NaNo_3$ showed normal white pulp with decreased apoptotic lymphocytes and normal red pulp.

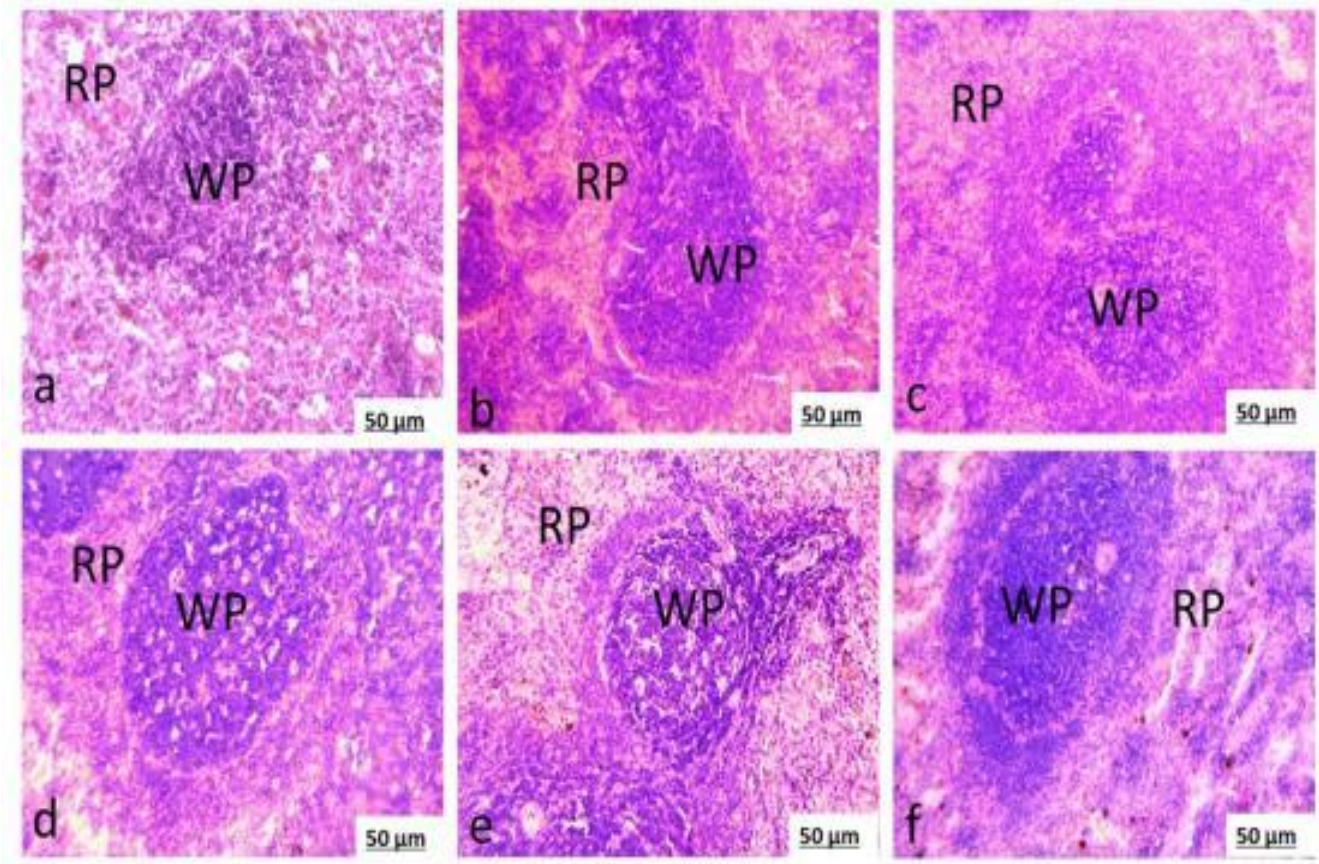

Figure (6): (a) Spleen section of (a) control rat. Spleen sections of rats treated with (b) honey and (c) L-Ascorbic acid showed a normal white pulp (WP) and red pulp (RP). (d) Spleen sections of rats treated with $NaNo_3$ showed increasing of apoptotic lymphocytes in white pulp (WP) and normal red pulp (RP) (e) Spleen sections of rats treated with honey and $NaNo_3$ showed decreasing in an apoptotic lymphocyte in white pulp (WP) and normal red pulp (RP). (f) Spleen sections of rats treated with ascorbic acid and $NaNo_3$ showed normal white pulp (WP) and normal red pulp (RP). (H&E, 200X).

DISCUSSION

Biochemical analysis and histology were investigated to assess the protective impact of honey or ascorbic acid on the indicated toxicity caused by exposure to sodium nitrate relative to organ weight. Alterations in the weight of organs are one of the key parameters in toxicology studies [20-21]. In the present work, rats treated with sodium nitrate demonstrated a highly significant rise in the relative weights of the liver, spleen and kidney while the thymus relative weight was decreased significantly than the control rats at the end of experimental periods. This increased organ relative weight and decreased thymus relative weight may be due to the increased level of sodium nitrate in the body leading to cytotoxic and inflammation in the body organ [22].

Therefore, a decline in the thymus relative weight ratio may be utilized as a sole indicator of immunosuppression. Thymic weight decreased as a direct result of immunosuppression or a nonspecific reaction to NaNo3 stress [23]. The results of the current study agree with **Rajini *et al.*** [21]**.** On the other hand, the relative weight of the spleen, liver and kidney significantly decreased in the treated sodium nitrate group with honey or ascorbic acid compared to the sodium nitrate group to weights near to the control ones.

The liver performs a critical physiological role in toxic situations because it eliminates the toxicants once they have been digested and destroyed [24]. The liver detoxification process subsequently compromises the integrity of the cell membrane, increasing the level of enzymes in the blood [25]. The current research showed that sodium nitrate-treated rats had considerably higher serum AST and ALT activity. The histological study has supported the use of increased transferase activity in the blood as a marker for liver tissue damage. **Delgado *et al.*** [26] found that rats administered sodium nitrate showed a significant increase in blood AST and ALT activities. Additionally, it is plausible to presume that ascorbic acid might play a preventive role against the toxicity brought on by the $NaNo_3$ than honey. In NaNO3-treated rats, the liver showed hydropic degeneration, hepatocytes necrosis and infiltration of inflammatory cells occurring in the portal area. These changes could be a consequence of membrane disruption induced by $NaNO_3$ according to

Bouaziz-Ketata *et al.* [27]. Honey and ascorbic acid could amend hepatic damage to a high degree, as revealed by the reduction of ALT and AST activities. Evidence of this view in the current results reflected the ability of ascorbic acid to protect the liver from $NaNo_3$-induced liver damage. This might be due to the antioxidant capacity of honey [28], and ascorbic acid [29] that assisted to prevent membrane fragility and subsequently declined the leakage of ALT and AST enzymes into circulation. Moreover, the giving the ascorbic acid to the $NaNO_3$-treated rats upgraded the nitrate induced liver histological perturbations nearly to its normal appearance.

Results of the herein study revealed a marked increase in serum urea and creatinine levels following $NaNO_3$ administration. Furthermore, $NaNO_3$ encouraged toxic injuries to the kidney tubules and loss of renal functional integrity as demonstrated by histological examination of the present study. Pre-treatment with honey or ascorbic acid significantly abridged the elevated serum urea and creatinine levels and hasten the renal histological picture. These results match those were got before by **Ridzuan *et al.* [30]** and **Osama *et al.* [31]** who revealed the protecting role of honey in counteracting nephrotoxicity in experimental animals and humans. Moreover, **Moreira *et al.* [32]** declared the protective role of L-ascorbic acid against nephrotoxicity.

The present study showed a decrease in glycogen content as appeared in the decreased percentage of PAS stained in rat treated with $NaNo_3$ that result agreed with **Helal *et al.* [33]** stated that the existence of nitrate ions leads to the release of glucose from glycogen, so blood glucose rises while hepatic glycogen diminishes. The administration of honey and ascorbic acid in the NaNO3-treated group increased the glycogen content as appeared by the increased percentage of PAS stained. This confirmed the glycogen storage property of the honey and ascorbic acid.

Rats treated with NaNo3 showed many apoptotic lymphocytes were detected in the white pulp of the spleen that decreased when rats were treated with honey and ascorbic acid. Lymphocyte apoptosis normally befalls in the splenic B-cell-rich follicular germinal centers of rodents but may also be increased in T-cell and/or B-cell (periarteriolar lymphatic sheaths) sections with experimental exposures to viruses, radiation, chemicals, or endotoxin **[34].**

Another explanation of liver, kidney and spleen damage tempted by NaNo3 is the mediation of the inflammation development as demonstrated by increased pro-inflammatory cytokine IL-6 content and TNF-α. These were publicized previously by **Yang *et al.* [35]** who described that NaNo3 could promote inflammatory pathways by the increase of the secretion of cytokines, including IL-6 and TNF-α. Pre-treatment with honey or ascorbic acid significantly reduced NaNo3 elevation in TNF-α and Il-6. There is emerging evidence to propose that honey and ascorbic acid exert ameliorative and anti-inflammatory effects by suppressing the production of inflammatory intermediaries, such as TNF-α and IL-6.

CONCLUSIONS

Sodium nitrate impaired the liver, kidney and spleen structure. We can conclude that L-ascorbic acid is better than honeybee extract in its advantageous role in overwhelming the arisen adverse influences of sodium nitrate exposure. It is recommended that the use of sodium nitrate as a food additive must be limited, also, the usage of L-ascorbic acid as an anti-inflammatory and antioxidant agent to prevent the induced toxic effect of NaNo3.

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Conflict of interest: Nil.

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