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ASCORBIC ACID ATTENUATES HEMATOLOGICAL AND HEPATIC DERANGEMENTS IN RATS CO-TREATED WITH ATRAZINE AND SODIUM NITRATE

Fatoki John Olabode, Adeleke Gbadebo Emmanuel*, Raheem Olalekan Kazeem and Adedosu Olaniyi Temitope

Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

*Corresponding Author: geadeleke@lautech.edu.ng

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Abstract

The present study examined the roles of ascorbic acid (AA) on hematological indices, hepatic function and lipid profile, and liver histology in rats co-treated with Atrazine (ATZ) and sodium nitrate ($NaNO_3$). The hematological indices were determined using Azotta hematological auto-analyzer and Westergren method. Serum levels of alanine aminotransferase (ALT) and gamma-glutamyl transferase (γ-GT), and hepatic total cholesterol (TC), phospholipids (PL) and triglyceride (TG) were determined spectrophotometrically. Liver histology was performed using light microscopy. The [ATZ + $NaNO_3$] significantly ($p < 0.05$) reduced the levels of hemoglobin, red blood cells, hematocrit, and platelets, while erythrocyte sedimentation rate, white blood cells, lymphocytes, and granulocytes were elevated. Serum levels of ALT and GGT were significantly ($p < 0.05$) increased in [ATZ + $NaNO_3$] group. Hepatic TC, PL and TG were significantly elevated in [ATZ + $NaNO_3$] –treated rats compared with controls. Interestingly, these derangements were significantly ($p < 0.05$) improved in rats exposed to [ATZ + $NaNO_3$ + AA]. Liver histology revealed the presence of diffuse hepatic necrosis with cellular infiltration in [ATZ + $NaNO_3$] group, a feature not observed in rats exposed to [ATZ + $NaNO_3$ + AA]. The results of the present study suggest that ascorbic acid could potentially attenuate hematological and hepatic derangements induced by co-treatment with atrazine and sodium nitrate.

INTRODUCTION

Atrazine, (2-chloro-4-(ethylamino)-6-(isopropyl amino)-s-triazine), is an herbicide commonly used in controlling pre-emergent weeds in the plantations of cereals (such as rice, maize and sorghum), cotton and pineapple [1; 2; 3]. In soil, atrazine is degraded to form two major products, diethylatrazine and deisopropylatrazine, which are also persistent and can be nitrosated like the parent compound. When atrazine and its metabolites infiltrate into groundwater and farm crops, there is a serious health risk to humans and animals [4}. The maximum contaminant level of atrazine-based herbicide in drinking water is about 3 parts per billion (ppb), as documented by Costa Siva *et al* [5]. However, the U.S Environmental Protection Agency has set its maximum contaminant level in water to be 3µg/L, and its application has been prohibited in Europe [6; 7; 8]. A study by Pathak and Dikshit [9] showed a delayed body clearance of atrazine, putting its half-life value between 95 and 350 days. In humans and animals, atrazine has been found to interfere with the endocrine system, with adverse changes in the estrous cycle length, luteinizing hormone production, intrauterine growth and pubertal development [10]. Under aqueous condition, atrazine readily becomes nitrosated to form mononitrosoatrazine (mono-NNAT) and dinitrosoatrazine (di-NNAT) at a pH range of 2 to 4, similar to that of the stomach [4; 11]. *N*-nitrosoatrazine (NNAT) could cause substantial *in-vitro* chromosomal abnormalities in human lymphocytes, even at low concentrations [12]. The specific mechanism of toxicity of NNAT is not yet

elucidated, but most researchers have adopted the cytochrome-p450 –mediated metabolism of nitrosamines, involving CYP 2E1 enzyme, to explain the mechanism of its carcinogenicity [4]. Atrazine intoxication has been reported to induce oxidative changes in tissues of animal models [13; 14]. Furthermore, toxic reproductive effects of atrazine in animal models have been noted to include increases in estrogen and progesterone in female, while decrease in testosterone level was reported in male. Atrazine was also reported to induce hemorrhagic condition, sperm cell damage and vacuolation of seminiferous tubules. An incorporation of ascorbic acid was reported to reverse these derangements in the rats [15].

Nitrate, ingested through foods or drinking water, is reduced to nitrite, which in turn is converted to a nitrosating agent, capable of interacting with secondary amines, under gastric acidic condition to form *N*-nitrosocompounds [16]. In the United State, the maximum contaminant level for nitrate-nitrogen (NO_3-N) in drinking water has been documented to be between 10 mg/L and 11.3 mg/L, as reported by the World Health Organization [17]. In addition to drinking water, exposure to nitrate occurs through consumption of several foods, most especially green leafy and root vegetables, as reported by IARC [18] and Ward *et al*. [17]. However, in some African countries like Nigeria, Niger and Morocco, the median level of nitrate in ground water has been estimated to be 42.9 mg/L [18]. Studies have shown that many nitrosocompounds are deleterious to humans through induction of oxidative and nitrosative stresses and carcinogenicity [19]. Nitrites are also reported to adversely affect hematological parameters, including red blood cell (RBC) count, Hematocrit, Hemoglobin count and white blood cell (WBC) count [20]. Furthermore, nitrite oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) in blood, resulting in methemoglobinemia [21]. Studies have shown that neither atrazine nor nitrate could individually induce mutagenesis or carcinogenesis. However, when present together in drinking water, on a chronic basis, there is *in-vivo* formation of *N*-nitrosamine, causing carcinogenesis [4;22; 23] and non-Hodgkin lymphoma [4; 24].

Ascorbic Acid (Vitamin C) is a six-carbon compound naturally present in fruits and vegetables [25]. It is one of the water soluble vitamins required in adequate quantity in human diets, due to lack of an enzyme, L-gulono-γ-lactone oxidase, required in the biosynthesis of this vitamin [26]. Studies have shown that orally administered vitamin C is well absorbed in the gastrointestinal tract (GIT) via an active transport. The vitamin therefore becomes distributed to all body tissues, with higher levels in the adrenal and pituitary glands, as well as the retina of the eyes [27]. Vitamin C has been associated with several biological activities in the body, including intestinal absorption of trace metals, biosynthesis of serotonin and catecholamines, post-translational modification of certain proteins, and maintenance of antioxidant system [26; 28; 29]. Ascorbic acid is reported to improve Diabetes Mellitus induced by Streptozotocin, as well as, inhibit platelet aggregation in experimental rats [30; 31]. Mirvish [32] reported that endogenous nitrosation, by dietary nitrate, could be prevented by ascorbic acid, polyphenolics and many other compounds present in most vegetables, thereby inhibiting formation of nitrosocompounds in-vivo. The severally reported biological activities of ascorbic acid have shown the ameliorative potentials of this natural compound against toxic chemicals.

In the present study, we investigated the possible roles of ascorbic acid in attenuating the effects of co-treatment with atrazine and sodium nitrate on hematology, hepatic function and lipidemic indices in Wistar rats. The study aims at producing an alternative management of toxic co-exposure to atrazine and sodium nitrate.

MATERIALS AND METHODS

Chemicals

Ascorbic acid was purchased from Sigma-Aldrich Co. (St Louis, MO), USA. $NaNO_3$ (97.56% pure) was purchased from the British Drug House (BDH) Chemical Ltd., Poole, UK, while Atrazine (manufactured by Zhejiang Zhongshan Chemical Industry Group Co. Ltd. Changxing, China) was purchased from TJP Agrochemical store, Ogbomoso, Oyo State, Nigeria. Other chemicals were of good analytical grades and purest quality available.

Experimental Design

Experimental protocols were conducted in accord with the guidelines of the Institutional Animal Care and Use Committee and were approved (LAU/FBS/20/0015) by the Animal Ethical Committee of the Faculty of Basic Sciences, Ladoke Akintola University of Technology, Ogbomoso. Twenty-four male Wistar rats weighing 120–135 g were bought from Animal House of the Faculty of Basic Medical Sciences, University of Ibadan, Nigeria. The rats were kept inside plastic cages and fed with rat pellets and drinking water ad libitum. The rats were subjected to acclimatization for 7days under 12-h light/dark cycle and temperature of $29^0C \pm 2^0C$. The rats were assigned into four groups of six rats each. The first group (Control) was given rat pellet and drug vehicle (normal saline). Second group received oral intubations of ATZ (5 mg/kg) and $NaNO_3$ (3 mg/kg) three times in a week, third group received oral intubations of ATZ (5 mg/kg), $NaNO_3$ (3 mg/kg) three time per week and AA (15 mg/kg) daily, while the fourth group received oral intubation of AA (15 mg/kg) alone daily. The administrations were carried out for four weeks until sacrifice.

Determination of Body Weight

The body weights of the rats were determined on weekly basis using an electronic top-pan weighing balance (Songhai, China).

Animal Sacrifice and Sample Collection

After four weeks, the rats were fasted overnight and blood samples were collected by retro-ocular bleeding, separately into EDTA bottles and plain sample bottles. The blood in EDTA bottles was used for hematological studies, while the blood in plain sample bottles was allowed to coagulate and then centrifuged at 3000 *x g* for 10 minutes to obtain serum for enzyme assays. After blood collection, rats were sacrificed by cervical dislocation and liver was quickly excised and washed in ice cold 1.15% potassium chloride solution to remove blood stains. Liver (1.0 g) was homogenized in 4.0 ml of 0.1 M Phosphate buffer (pH 7.4) using Teflon homogenizer followed by cold centrifugation at 10,000 x g for 10 minutes, to obtain homogenates for determination of lipid parameters. The other portion of liver was fixed in 10% formalin for histological examination.

Hematological Studies

Determinations of the red blood cell, hemoglobin, hematocrit, total white blood cell count, and differential white blood cell (lymphocytes, granulocytes and platelets) counts were carried out with Azotta hematological auto-analyzer (China) and mean values calculated according to Agbasi *et al*. [33]. The erythrocyte sedimentation rate (ESR) was determined using the Westergren (Conventional) method [34; 35]. Briefly, 1.5 ml blood was mixed with 0.5 ml of 2.5 % sodium citrate. The blood mixture was sucked into a vertical glass Westergren tube for one hour. The sedimentation rate was estimated by using a ruler to measure the column of serum inside the tube, and values expressed in millimeter per hour.

Enzyme Assays

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (γ-GT) activities were determined using the LABKIT commercial kits purchased from Chemelex, SA. 08420 Canovelles, Barcelona (Spain) following the manufacturer's instructions.

Determination of Total Cholesterol, Triglyceride and Phospholipids Levels

Hepatic total cholesterol (TC), phospholipids (PL) and triglyceride (TG) levels were estimated according to the methods of Naito [36] and Buccolo and David [37].

Histopathology

Liver tissues were fixed in 10% formalin and then subjected to dehydration, first in 75% ethanol and finally in absolute alcohol. Clearing was carried with xylene, and paraffin wax was used for embedding. Ultra-thin sections (3 μm) were obtained, and the slides were treated with haematoxylin and eosin (H&E) stains [38]. The slides were then examined under a light microscope and interpreted by a Histopathologist.

Statistical Analysis

Values were obtained in duplicates and data expressed as Mean ± Standard deviation (n = 6). Student T-test and One-way analysis of variance (ANOVA) test were used for statistical comparison of treatments, taken significant values at $p < 0.05$. Data analysis was done using the Statistical Package for Social Sciences (SPSS) software for Windows version 10.0 (USA).

RESULTS

Effects of Ascorbic Acid on Body Weight Gain of Rats co-treated with Atrazine and Sodium Nitrate

The result in Figure 1 shows that [ATZ + $NaNO_3$] significantly ($p < 0.05$) reduced the body weight gain in the rats by 49.2 % relative to controls. However, a supplementation with ascorbic acid significantly increased the body weight gain by 44.5 % compared with [ATZ + $NaNO_3$] group of the experimental rats. In addition, treatment with ascorbic acid alone significantly improved ($p < 0.05$) the body weight gain comparable to control rats.

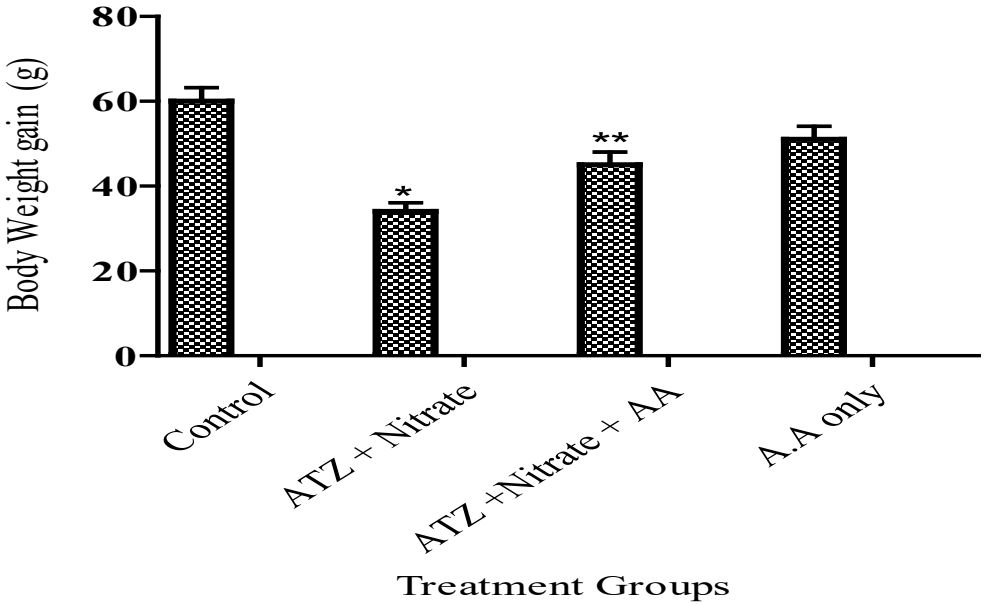

Figure 1: Effect of ascorbic acid (AA) on body weight gain of rats co-treated with atrazine (ATZ) and sodium nitrate ($NaNO_3$)
Data expressed in mean ± SD; n = 6
*-Body weight gain statistically lower compared with control ($p < 0.05$)
**- Body weight gain statistically higher compared with [ATZ + $NaNO_3$] group ($p < 0.05$)

Effects of Ascorbic Acid on Hematological Indices in Rats co-treated with Atrazine and Sodium Nitrate

The results in Table 1 show that [ATZ + $NaNO_3$] significantly ($p < 0.05$) lowered the levels of hemoglobin, red blood cell and hematocrit, while Erythrocyte sedimentation rate was significantly increased relative to controls. A co-treatment with ascorbic acid significantly ($p < 0.05$) improved the levels of hemoglobin, red blood cells and Erythrocyte sedimentation rate compared with the ATZ + $NaNO_3$ group of rats. Table 2 shows that ATZ + $NaNO_3$ significantly ($p < 0.05$) elevated the levels of white blood cells, lymphocytes and granulocytes, and reduced that of platelets compared with controls. However, supplementation with ascorbic acid during [ATZ + $NaNO_3$] treatment significantly reduced the level of white blood cells. No significant effects ($p > 0.05$) were noticed on the levels of lymphocytes, granulocytes and platelets in the experimental rats.

Table 1: Effects of ascorbic acid (AA) on hemoglobin (Hb) concentration, red blood cell (RBC) count, hematocrit (Hct) and erythrocyte sedimentation rate (ESR) in rats co-treated with atrazine (ATZ) and sodium nitrate ($NaNO_3$)

Treatments	Hb (g/dL)	RBC x10 ^{6} (mm^3)	Hct (%)	ESR (mm/hr)
Control	11.95 ± 2.14	6.39 ± 1.08	43.2 ± 4.67	1.35 ± 0.00
ATZ + $NaNO_3$	6.84 ± 1.87^a	3.43 ± 0.83^a	32.4 ± 3.82^a	3.89 ± 1.01^b
ATZ + $NaNO_3$ + AA	9.94 ± 2.01^c	7.92 ± 1.03^c	31.8 ± 5.20^a	2.03 ± 0.28^d
AA	13.13 ± 2.11^{bc}	5.29 ± 1.31^c	41.9 ± 4.51	1.45 ± 0.04^d

Data expressed in mean ± SD; n = 6
^{a} - Statistically lower compared with control ($p < 0.05$)
^{b} - Statistically higher compared with control ($p < 0.05$)
^{c} - Statistically higher compared with [ATZ + $NaNO_3$] group ($p < 0.05$)
^{d} - Statistically lower compared with [ATZ + $NaNO_3$] group ($p < 0.05$)

Table 2: Effects of ascorbic acid (AA) on white blood cell, lymphocytes, granulocytes and platelets counts in rats co-treated with atrazine (ATZ) and sodium nitrate ($NaNO_3$)

Treatments	White blood cells x 10^3 (mm^3)	Lymphocytes (%)	Granulocytes (%)	Platelets x10^3 (mm^3)
Control	8.65 ± 2.10	10.12 ± 2.21	2.31 ± 0.91	307 ± 16.42
ATZ + $NaNO_3$	15.27 ± 1.79[b]	14.98 ± 2.11[b]	3.05 ± 0.77	196 ± 13.67[a]
ATZ + $NaNO_3$ + AA	10.18 ± 2.52[d]	13.17 ± 3.10	3.85 ± 1.01	197 ± 10.60[a]
AA	9.93 ± 2.03	9.15 ± 2.01[d]	2.91± 0.11	419 ± 31.11[c]

Data expressed in mean ± SD; n = 6
[a] - Statistically lower compared with control ($p < 0.05$)
[b] - Statistically higher compared with control ($p < 0.05$)
[c] - Statistically higher compared with [ATZ + $NaNO_3$] group ($p < 0.05$)
[d] - Statistically lower compared with [ATZ + $NaNO_3$] group ($p < 0.05$)

Effects of Ascorbic Acid on Hepatic Function and Lipid Profile in Rats co-treated with Atrazine and Sodium Nitrate

The results in Figure 2 show the effects of the treatment on the activities of alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma-glutamyl transferase (GGT) enzymes in the rats. The activities of ALT and GGT enzymes were significantly ($p < 0.05$) increased in [ATZ + $NaNO_3$] group by 45.5% and 84.%, respectively, while that of AST was not significantly ($p > 0.05$) affected, compared with the control rats. However, ascorbic acid significantly ($p < 0.05$) reversed the effects on the activities of ALT and GGT by 25% and 54.3%, respectively, compared with [ATZ + $NaNO_3$] group. The effect ascorbic acid alone was observed to be comparable to control treatment. Table 3 shows that [ATZ + $NaNO_3$] significantly elevated the hepatic levels of total cholesterol, triglycerides and phospholipids compared with the controls rats. Interestingly, the effects were significantly ($p < 0.05$) attenuated in [ATZ + $NaNO_3$ + AA] co-treated rats.

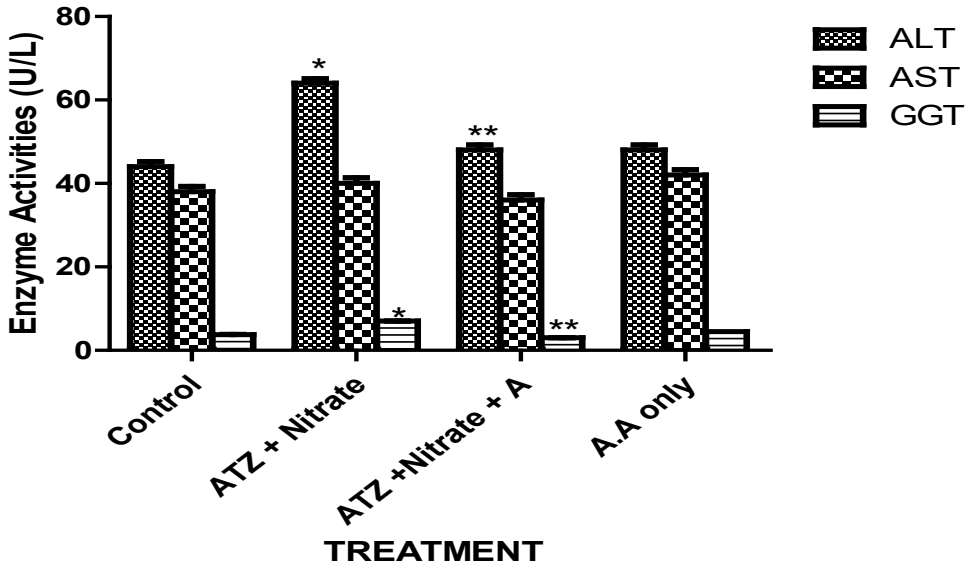

Figure 2: Effects of ascorbic acid (AA) on alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma glutamyl transferase (GGT) activities in rats co-treated with atrazine (ATZ) and sodium nitrate ($NaNO_3$)
Data expressed in mean ± SD; n = 6
*-Activity statistically higher compared with control ($p < 0.05$)
**- Activity statistically lower compared with [ATZ + $NaNO_3$] group ($p < 0.05$)

Table 3: Effects of ascorbic acid (AA) on hepatic levels of total cholesterol, triglycerides and phospholipids in rats administered with atrazine (ATZ) and sodium nitrate ($NaNO_3$)

Treatments	Total cholesterol (mM/L)	Triglycerides (mM/L)	Phospholipids (mM/L)
Control	3.73 ± 0.15	1.24 ± 0.56	1.25 ± 0.14
ATZ + $NaNO_3$	5.42 ± 1.51[b]	2.64 ± 0.71[b]	2.75 ± 1.00[b]
ATZ + $NaNO_3$ + AA	2.98 ± 1.01[d]	1.95 ± 0.63[d]	1.16 ± 0.55[d]
AA	2.48 ± 1.24[d]	1.19 ± 0.32[d]	1.03 ± 0.21[d]

Data expressed in mean ± SD; n = 6
[a] - Statistically lower compared with control ($p < 0.05$)
[b] - Statistically higher compared with control ($p < 0.05$)
[c] - Statistically higher compared with [ATZ + $NaNO_3$] group ($p < 0.05$)
[d] - Statistically lower compared with [ATZ + $NaNO_3$] group ($p < 0.05$)

Histopathology of Rat Liver

The photomicrographs of the histopathological examination of the rat liver (Figure 3) show that [ATZ + $NaNO_3$] treatment induced diffuse hepatocyte necrosis and cellular infiltration compared with the control rats. However, on supplementation with ascorbic acid, no visible lesions were noticed, comparable to both controls and rats treated with ascorbic acid alone, as also shown in Figure 3.

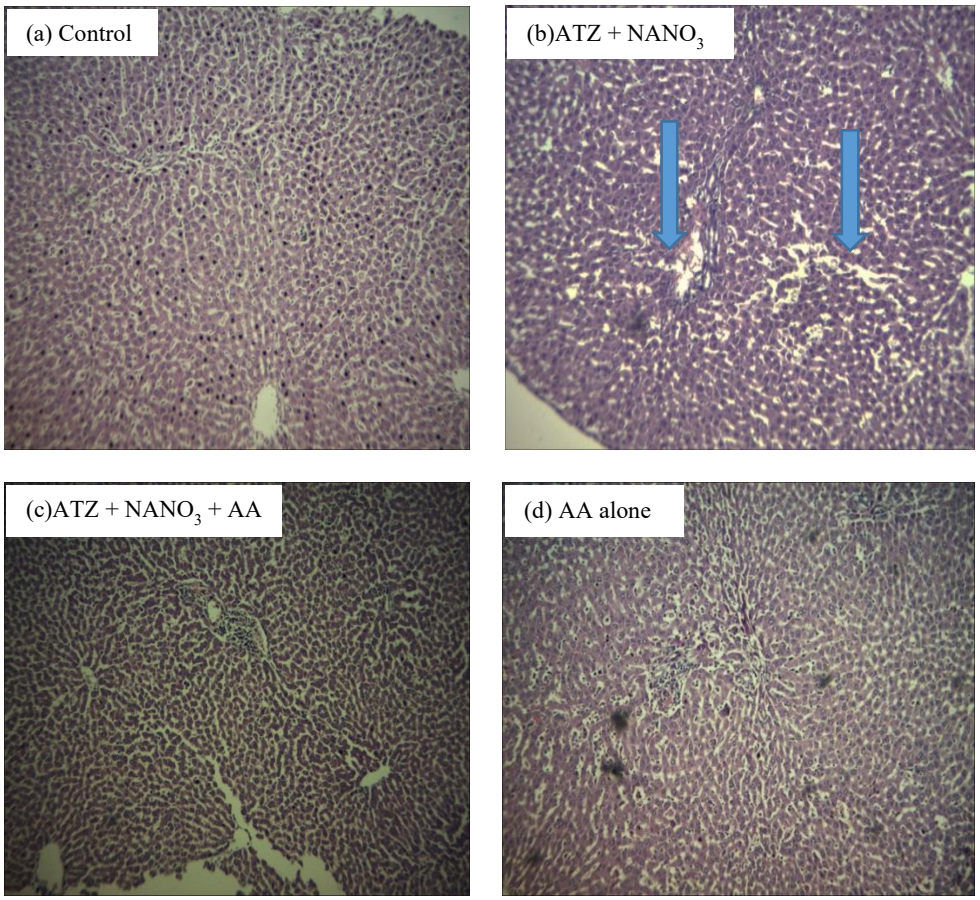

Figure 3: Micrographs of liver tissues: (a) control- no visible lesion. (b) [ATZ + $NaNO_3$] -diffuse hepatocyte necrosis and cellular infiltration (shown with blue arrows). (c) [ATZ + $NaNO_3$ + AA] -no visible lesion. (d) AA alone- no visible lesion (x 100)

DISCUSSION

The present study examined the effects of co-administration of atrazine (ATZ) and sodium nitrate, and the possible attenuation of these effects by ascorbic acid in experimental rats. The results of the present study revealed that a co-treatment with atrazine (5 mg/kg) and sodium nitrate (3 mg/kg) significantly reduced body weight gain in the experimental rats. An earlier study conducted by Adeleke *et al*. [13] has also shown that ATZ caused a significant reduction in body weight gain of rats. Interestingly, the present study revealed the potential of ascorbic acid supplementation to increase body weight gain similar to control rats. Irfan *et al*. [39] reported that constant breakdown of structural proteins in the body is often associated with loss in body weight. Thus, the reduction in weight gain observed in the [ATZ + $NaNO_3$] – treated rats may be due to breakdown of body structural proteins in the rats. However, supplementation with ascorbic acid was able to improve the weight gain of the rats. In human studies, low plasma ascorbic acid has been associated with both body mass index (BMI) and waist circumference [40; 41]. Furthermore, ascorbic acid functions as a co-factor in the biosynthesis of carnitine, which is a metabolite needed in the oxidation of fatty acids. A high level of ascorbic acid will therefore result in the biosynthesis of carnitine, with increased breakdown of fatty acids, which could have been stored up, contributing to weight gain [42; 43].

A combined treatment with ATZ and sodium nitrate significantly ($p < 0.05$) reduced the levels of hemoglobin (Hb), red blood cell (RBC), hematocrit (Hct) and platelets, while erythrocyte sedimentation rate (ESR) was increased. The percentage of blood volume occupied by the RBC has been described as the packed cell volume (PCV), while the level of RBC is an indirect measure of both the hemoglobin and hematocrit levels. Studies by Ramesh *et al*. [44] and Naji *et al*. [45] revealed that ATZ reduced the RBC and hemoglobin levels, while the hematocrit level was increased. A decrease in RBC has been linked with inhibition of erythropoiesis and a high level of erythrocyte destruction in the hematopoietic organs of animals [46]. Furthermore, a reduction in RBC formation has been associated with reduced oxygen-carrying capacity of blood in to body tissues [47]. However, a study by Pulgdollar *et al*. [48] has revealed that ATZ significantly increased the hematocrit count in Atlantic salmon fish. The ESR is a less-specific marker usually elevated in many acute and chronic conditions associated with tissue necrosis and inflammation. The increase in ESR during inflammation is due to the entry of a large amount of fibrinogen into the blood, causing red blood cells to stick together [35]. An increased ESR has been connected with certain metabolic problems such as diabetes and its related disorders [49; 50]. Furthermore, we noticed that [ATZ + $NaNO_3$] elevated white blood cells, lymphocytes and granulocytes counts, whereas a reduction was observed in platelets count of the experimental rats. Naji *et al*. [45] reported that ATZ caused a reduction in WBC, while lymphocyte level was elevated in *Acipenser nudiventris*. A condition in which WBC level increases has been described as leukocytosis, which could result from tissue necrosis and inflammation [51]. White blood cells are central to the initiation and maintenance of body defense mechanisms against pathogenic infections. For instance, the activities of lymphocytes and neutrophils have been linked with phagocytotic attacks against body infections [52; 53]. An increase in the level of WBC due to ATZ-induced toxicity has been suggested to be associated with increased proliferation of pluripotential hematopoietic cells [54]. The increased ESR and white blood cells count, being noticed in the [ATZ + $NaNO_3$] group in the present study, may indicate inflammation and related disorders in the experimental rats. Interestingly, ascorbic acid supplementation was able to reverse the effects. A study by Patel and co-researcher [55] showed that ascorbic acid could potentially improve bacterial clearance through increased macrophage phagocytotic function in the respiratory system of mice model under ROS-induced hyperoxia. Ignatius *et al*. [31] noted that ascorbic acid could reduce the platelet count in experimental animals.

A co-treatment with Atrazine and sodium nitrate significantly ($p < 0.05$) increased the serum activities of ALT and GGT, with AST unaffected. These three enzymes are commonly employed as indicators of hepatocellular integrity. The release of ALT, AST and GGT enzymes into the blood circulation is an indication that the structural integrity of the liver cells has been compromised [56; 57]. The increased activities of both ALT and GGT in the serum of experimental rats, as observed in the present study, has thus shown that a co-treatment with atrazine and sodium nitrate induced damage of the hepatocytes, causing leakages of the three enzymes into to the serum. Interestingly, a supplementation with ascorbic acid significantly improved the serum activities of both ALT and GGT in the rats.

The results on the hepatic levels of total cholesterol (TC), triglycerides (TGs) and phospholipids (PL) show that co-treatment with ATZ and sodium nitrate significantly ($p < 0.05$) elevated the levels of TC, TG and PL in the rats. However, a study carried out by Naji *et al*. [45], using *Acipenser nudiventris*, revealed that ATZ reduced the levels of both TC and TGs. In hypercholesterolemia, cholesterol becomes deposited in the walls of blood vessels, leading to atherosclerotic development in the body [58]. Triglycerides (TGs) are synthesized from free fatty acids and glycerol. Foulds *et al*. [59] reported that fatty liver occurs when the hepatic uptake or *de-novo* synthesis of free fatty acids is greater than their oxidative breakdown in the body. Derangement in lipid metabolism has been reported as a key risk factor in etiology of cardiovascular diseases [60]. Studies by Cave *et al*. [61] and Olsvik *et al*. [62] have suggested that chemical intoxication could result in hepatic lipid accumulation and pathology. Atrazine intoxication, which majorly occurs in liver, has been reported to disrupt

lipid metabolism in experimental animals [63; 64]. However, ascorbic acid supplementation was found to significantly ameliorate the effects of atrazine and sodium nitrate on the lipid parameters in the rats.

The photomicrographs of hepatic histology reveal that the rats in [ATZ + $NaNO_3$] group show evidence of diffuse hepatocyte necrosis and cellular infiltration. However, the rats supplemented with ascorbic acid, and those treated with ascorbic acid alone showed no visible lesions, as also observed in the control rats. An earlier study indicated that ATZ could induce mild hepatocyte degeneration and periportal cellular infiltration, as well as oxidative stress in rats [13]. Studies by Wei *et al*. [11] and Rhodes *et al*. [4] have observed gastric formation of toxic *N*-nitrosoatrazine from atrazine and nitrate. It could thus be suggested that hepatic derangements, noticed in the present study, may be due to formation of *N*-nitrosoatrazine in the rats. Separate treatment with ATZ or nitrate has not been observed to induce mutagenesis or carcinogenesis in animal models, but on combined chronic exposure through ground water, they could induce tumour occurrence [22; 4]. Several studies have revealed the potential of ascorbic acid to inhibit endogenous nitrosation, by scavenging nitrosating agents [17; 65; 66]. The hepatic derangement observed through the histological examination, in the present study, may be associated with the disruption of lipid metabolism in the rats. This is in accordance with previous studies by Cave *et al*. [61] and Olsvik *et al*. [62], which noted a link between the pathologic state of liver and abnormal lipid metabolism in experimental animals. In the present study, ascorbic acid supplementation showed evidence of restoration in the hepatic tissues of the rats.

CONCLUSION

The present study has shown that a co-treatment of atrazine and sodium nitrate could potentially induce hematological disorders, hepatic injury and disruption of lipid metabolism in rats. However, ascorbic acid supplementation was able to reverse these deleterious changes in the animals.

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CONFLICTS OF INTEREST

No conflicts of interest among the authors.

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