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Amelioration of Isoniazid induced Oxidative Stress and Hematotoxicity by Vitamin C in Wistar Rats

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ABSTRACT

Background: This study was carried out to investigate the ameliorative effect of vitamin C against isoniazid-induced toxicity in some hematological and biochemical parameters in Wistar rats.

Methods: A total of seventy-two (72) adult Wistar rats of both sexes were used which were randomly distributed into 6 groups of 12 rats each. Isoniazid (INH) and Vitamin C were orally administered daily for 2 weeks and on the 7th and 14th day of the experiment, 6 rats from each group were humanely euthanized using ethyl ether and blood was collected for determination of antioxidant, and hematological parameters.

Results: This study revealed significant increase in the level of Malondialdehyde (MDA) in rats treated with INH only. These markers were significantly reduced following co-administration with vitamin C (10 mg/kg and 20 mg/kg) respectively. There was also significant reduction in activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH) in rats treated with INH only as compared to the control (distilled water) rats. These markers were significantly increased following co-administration with vitamin C (10 mg/kg & 20mg/kg) respectively. The study also showed that there was no significant difference between the test groups and the control group in hematological parameters.

Conclusion: This finding is indicative of hepatoprotective and antioxidant effect of vitamin C.

Key words— Catalase, Isoniazid, Malandyaldehyde,Superoxide dismutase, Vitamin C.

1. INTRODUCTION

The liver is the largest internal organ in the human body and it is the main organ for the metabolism and detoxification of drugs and environmental chemicals, thus vulnerable to injury by hepatotoxic agents [1]. Hepatotoxicity refers to liver dysfunction or damage that is associated with an overload of drugs or xenobiotics [2]. Certain drugs such as isoniazid (INH) may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity caused by the primary compounds but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature [3], [4]. Cytochrome P2E1 (CYP2E1) was proposed to play important roles in INH hepatotoxicity through formation of reactive oxidative species and other reactive metabolites [5], [6], [7]. Hepatotoxicity related symptoms may include a jaundice or yellowish discoloration of the skin, eyes and mucous membranes (due to high level of bilirubin in the extracellular fluid), pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool [8]. Vitamin C is the drugs that can ameliorate these side effects, hence its choice in this study. Vitamin C, also known as ascorbic acid, is a water-soluble compound, with a six-carbon structure related to glucose. It consists of two inter-convertible compounds: l-ascorbic acid, which is a strong reducing agent, and its oxidized derivative, l-dehydroascorbic acid [9]. Vitamin C exerts its antioxidant action by inhibiting lipid peroxidation and oxidative cell damage [10]. It has been used in various antioxidant studies such as Combine administration of silymarin and vitamin C in acetaminophen-mediated hepatic oxidative insults in Wistar rats, where vitamin C was found to ameliorate acetaminophen-mediated hepatic oxidative damage [11]. Therefore, this study was carried out to investigate the ameliorative effect of vitamin C on isoniazid-induced hepatotoxicity in rats.

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2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemical/Drugs and equipment
Isoniazid (Emzor Pharmaceutical Industries limited, Lagos, Nigeria), vitamin C (Sigma Aldrich Chemical Co., USA), distilled water, oral cannula, 5mls and 10mls syringes, mass spectrometer, automated hematology analyzer, centrifuge, microscope (Olympus)

2.1.2 Biological Materials
Seventy –two (72) adult Wistars rats of both sexes obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria, were used for the experiment. Animals were treated in accordance with approved institutional Animal Ethics Committee (ABUCAUC/2016/052). The animals were allowed to acclimatize in the experimental room for about two weeks before commencement of the study. Animals were placed on standard animal diet (Vital) feed and water ad libitum for the duration of the experiment. Wood shavings were used as beddings and these were regularly changed.

2.2 Methods

2.2.1 Isoniazid Dose Determination
The dose of isoniazid was determined using the method described by Jang-woo *et al*., [12].

2.2.2 Experimental Design
Wistar rats of both sexes were randomly distributed into 6 groups of 12 rats each; where Group 1; served as the control group and received normal saline 1ml/kg; group 2; received Isoniazid (INH) 30 mg/kg; group 3 and 4; received INH, 30 mg/kg + vitamin C, 10 mg/kg and INH, 30 mg/kg + vitamin C, 20 mg/kg, respectively, group 5 and 6; received Vitamin C 10 mg/kg and 20 mg/kg body weight respectively. INH and Vitamin C were orally administered daily for 2 weeks. On the 7th and 14th day of the experiment, 6 rats from each group were humanely euthanized using ethyl ether.

2.2.3 Sample Collection
Blood sample was collected from each rat through cardiac puncture into EDTA and plain bottles for hematological and biochemical analysis respectively. Liver tissues were also harvested and fixed in 10 % neutral buffered formalin for histopathological evaluation.

2.2.4 Determination of Oxidative Stress Parameters
Lipid peroxidation products (Malondialdehyde) was determined as Thiobarbituric Acid Reactive Substances (TBARS) according to Okhawa et al.,[13] with slight modification by Atawodi *et al*., [14] using trichloroacetic acid 15% (TCA) and thiobarbituric acid 0.67% (TBA). Catalase activity was measured by the method of Aebi [15]. Superoxide dismutase (SOD) was determine by the method describe by Fridovich [16] and reduced glutathione (GSH) concentration measurement was done according to the method of Ellman[17].

2.2.5 Determination of Hematological Parameters
Hematologic profiles such as red blood cell count, hemoglobin level, hematocrit (HCT), and total white blood cell count (WBC), WBC differential count, and Platelet count, were determined using Mindray automated hematology analyzer following the manufacturer's instruction.

2.3 Statistical Analysis
Data obtained were analyzed using SPSS version 20. Differences between means were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. Values were considered statistically significant at $p \leq 0.05$. The results obtained were presented in charts and tables.

3. RESULTS
3.1 Antioxidant Analysis
The CAT, SOD and GSH were significantly lowered ($p \leq 0.05$) for the rats treated with INH only as compared to those in the control group. While the MDA levels was significantly higher in INH treated group as compared to the control groups ($p \leq 0.05$). There was considerable decrease in the level of CAT, SOD and GSH in rats co-treated with INH + vitamin C (10 mg/kg and 20 mg/kg) respectively as compared to those in INH group (p

≤0.05). Also, lower concentrations of MDA were observed in rats co-treated with INH + vitamin C (10 mg/kg and 20 mg/kg) as compared to those in INH group with no significant difference. There was no significant difference for MDA and the enzymes (CAT, SOD and GSH) in the responses of the rats treated with vitamin C only and those in the control group at 7 and 14 days respectively (figures 1, 2, 3 & 4).

3.2 Haematological Analysis

The results of haematological parameters showed no statistically significant differences between groups when compared to the control group (Tables 1 and 2)

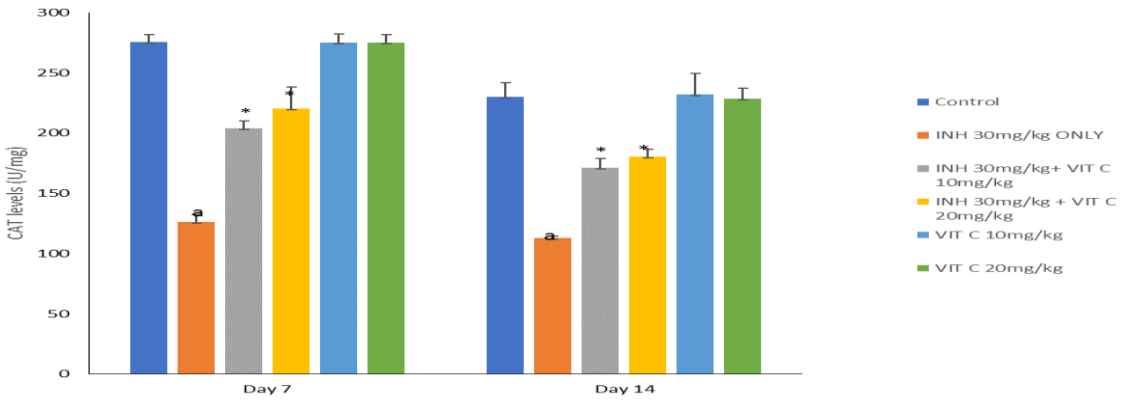

Figure 1: Effect of Co-administration of Vitamin C and Isoniazid on Catalase Levels in Wistar Rats (Day 7 & 14)

Values are expressed as mean ± standard deviation, a= values are significantly different as compared to control (D/W) group at ($p \leq 0.05$) *= values are significantly different as compared to INH only (30mg/kg) at ($p \leq 0.05$); n=6, One-way ANOVA followed by Bonferroni post hoc test was used, D/W = Distilled water, INH = Isoniazid, Vit C = Vitamin C, CAT = Catalase.

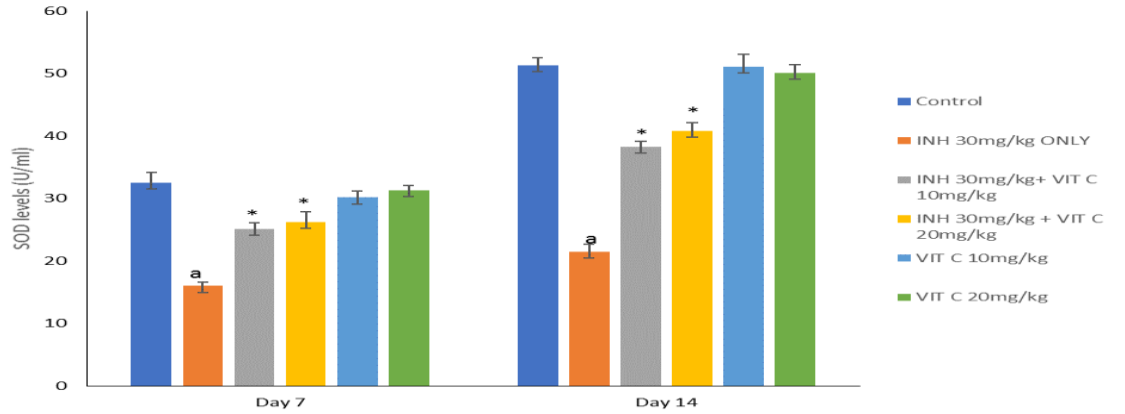

Figure 2: Effect of Co-administration of Vitamin C and Isoniazid on Superoxide Dismuatase Levels in Wistar Rats (Day 7 & 14)

Values are expressed as mean ± standard deviation, a= values are significantly different as compared to control (D/W) group; at ($p \leq 0.05$), *= values are significantly different as compared to INH only (30mg/kg) at ($p \leq 0.05$); n=6, One-way ANOVA followed by Bonferroni post hoc test was used, D/W = Distilled water, INH = Isoniazid, Vit C = Vitamin C, Superoxide dismutase.

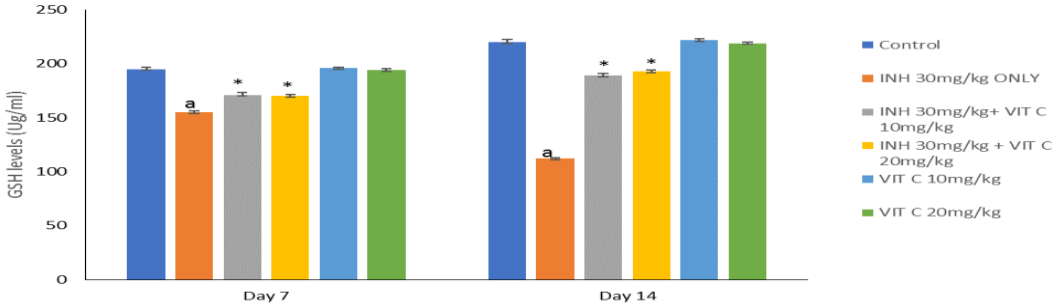

Figure 3: Effect of Co-administration of Vitamin C and Isoniazid on Glutathione Levels in Wistar Rats (Day 7 & 14)

Values are expressed as mean ± standard deviation, n = 6, a= values are significantly different as compared to control (D/W) group; at ($p \leq 0.05$)
*= values are significantly different as compared to INH only (30mg/kg) at ($p \leq 0.05$);, One-way ANOVA followed by Bonferroni post hoc test was used, D/W = Distilled water, INH = Isoniazid, Vit C = Vitamin C, GSH = Glutathione.

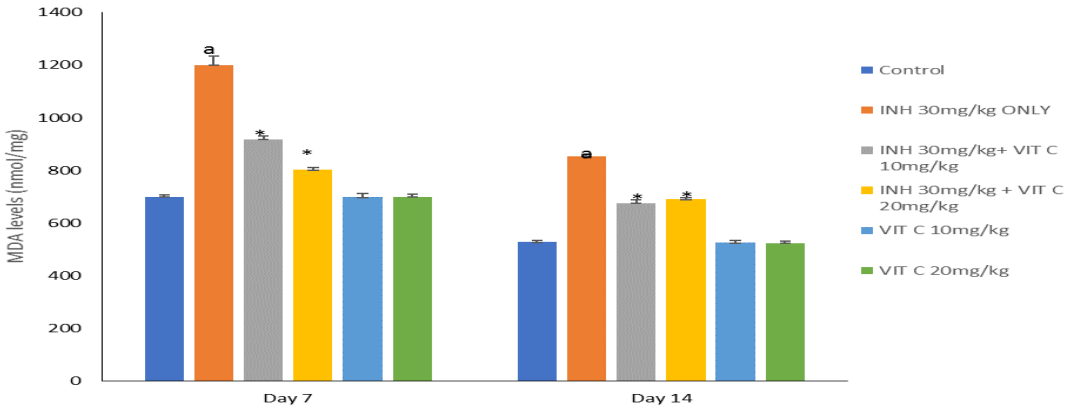

Figure 4: Effect of Co-administration of Vitamin C and Isoniazid on Malondialdehyde Levels in Wistar Rats (Day 7 & 14)

Values are expressed as mean ± standard deviation, n = 6, a= values are significantly different as compared to control (D/W) group; at ($p \leq 0.05$), *= values are significantly different as compared to INH only (30mg/kg) at ($p \leq 0.05$); One-way ANOVA followed by Bonferroni post hoc test was used, D/W = Distilled water, INH = Isoniazid, Vit C = Vitamin C, MDA = Malondialdyhide.

Table 1: Effect of Co-administration of Vitamin C and Isoniazid on Haematological Parameters of Rats at Day 7

Treatment	Parameters (Units)								
mg/kg	PCV (%)	HB (g/dl)	WBC × 10^3/ µL	RBC × 10^3/ µL	PTC × 10^3/ µL	N (%)	L (%)	M (%)	E (%)
D/W 1ml/kg)	34.00±1.34	11.25±0.53	3.48±0.32	6.08±0.05	7.13±0.06	12.67±0.56	84.50±0.56	1.50±0.22	1.33±0.33
INH 30	33.83±1.70	11.16±0.55	3.75±0.37	6.08±0.09	7.05±0.03	13.83±0.65	82.67±0.84	2.33±0.33	1.17±0.17
Vit C 10 + INH 30	35.17±2.06	11.82±0.68	3.82±0.17	6.95±0.10	6.95±0.05	13.00±0.37	84.00±0.26	1.67±0.33	1.33±0.21
Vit C 20 +INH 30	36.67±1.63	12.38±0.59	3.70±0.24	5.15±0.18	6.95±0.04	12.67±0.56	85.33±0.33	1.17±0.31	0.83±0.31
Vit C 10	34.17±0.60	11.23±0.33	3.62±0.34	6.02±0.07	6.95±0.06	12.00±0.05	85.17±0.65	1.83±0.31	1.00±0.26
Vit C 20	34.17±1.42	11.22±0.63	3.70±0.24	5.88±0.06	6.93±0.06	11.00±2.08	84.17±0.83	1.50±0.34	1.67±0.33

Values are presented as Mean ± S.E.M., a= values are significantly different as compared to control (D/W) group at ($p \leq 0.05$), *= values are significantly different as compared to INH only (30mg/kg) at ($p \leq 0.05$), One-way ANOVA followed by Bonferroni post hoc test. n=6, D/W = Distilled water, INH = Isoniazid, Vit C = Vitamin C, RBC=Red blood cells, WBC=White blood cells, PVC = Pack Cell Volume, HB = Haemoglobin, N = Neutrophils, M = Monocytes, E = Eosinphils and PTC = Platelets Count

Table 2: Effect of Co-administration of Vita min C and Isoniazid on Haematological Parameters of Rats at Day 14

Treatment	**Parameter**	**(Units)**							
mg/kg	PCV %	HB g/dl	WBC × 10^3/ µL	RBC× 10^3/ µL	PTC × 10^3/ µL	N%	L%	M%	E%
D/W (1ml/kg)	41.33±1.43	12.65±0.55	3.68±0.26	5.95±0.04	6.93±0.07	13.67±0.42	82.67±0.49	2.17±0.17	1.33±0.33
INH 30	38.00±0.73	12.80±0.24	2.90±0.42	5.95±0.07	6.90±0.07	12.50±0.56	83.33±0.95	2.17±0.40	1.33±0.33
Vit C 10 + INH 30	33.67±2.12	11.48±0.67	7.67±3.69	5.98±0.14	6.82±0.12	16.17±1.05	80.17±1.01	2.00±0.26	1.67±0.33
Vit C 20 +INH 30	36.17±1.35	12.15±0.53	3.62±0.44	5.85±0.06	7.02±0.07	14.83±0.87	81.50±0.85	2.00±0.37	1.50±0.22
Vit C 10	36.17±1.58	11.83±0.53	4.72±0.07	6.02±0.07	6.95±0.04	15.67±1.09	81.33±1.23	1.83±0.40	1.00±0.26
Vit C 20	35.33±0.88	11.73±0.32	4.02±0.07	5.87±0.06	7.07±0.14	14.00±0.58	82.00±0.77	2.33±0.33	1.50±0.22

Values are presented as Mean ± S.E.M., a= values are significantly different as compared to control (D/W) group at ($p \leq 0.05$), *= values are significantly different as compared to INH only (30mg/kg) at ($p \leq 0.05$), One-way ANOVA followed by Bonferroni post hoc test used. n=6, D/W = Distilled water, INH = Isoniazid, Vit C = Vitamin C, RBC=Red blood cells, WBC = White blood cells, PVC = Pack Cell Volume, HB = Haemoglobin, N = Neutrophils, M = Monocytes, E = Eosinphils and PTC = Platelets Count

4. DISCUSSION

Isoniazid is a potent antituberculous agent which acts by inhibiting lipid and DNA synthesis of mycobacterium tuberculosis thus inhibiting cell wall synthesis in the organism [18], [19]. One of the commonest adverse effect of antituberculous agent is hepatotoxicity [20]. The side effects of INH have been associated with induction of oxidative stress via free radical formation and reactive oxygen species (ROS) [21]. The oxidative stress induced by INH causes hepatocellular damage or necrosis leading to elevation of serum marker enzymes which are released from the liver into the blood [22], [23]. Antioxidants (such as vitamin C) alleviate the tissue damage by inhibition of free radical chain reaction and resultant prevention of peroxidative deterioration of structural lipid membrane organelles [10]. A significant increase in serum malondyaldehyde concentration in the rats treated with INH only was obtained in this study. This finding could be due to the oxidative stress-induced by INH leading to lipid peroxidation of the cellular membranes whose toxic product causes damage to macromolecules. This is in accordance with the report of Xavier *et al.*, [10] and Osama *et al.*,[24]. Conversely, the significant decrease in MDA concentration in the rats co-treated with vitamin C (10 mg/kg and 20 mg/kg) indicated the antioxidant potentials of vitamin C and its ability to alleviate the lipid peroxidative cellular membrane injury. This is in conformity with the previous studies by Ajiboye *et al.*, [19]; Gini and Muraleedhara, [23]; Santos *et al.*, [25]. This study also revealed a significant reduction in plasma activities of endogenous antioxidants (SOD, CAT and GSH)

in rats treated with INH only which indicates the presence of ROS produced by INH resulting in imbalance between free radicals and antioxidant defense system due to consumption of these endogenous antioxidants in the process of neutralizing them. This finding is in conformity with the reports by Osama *et al*., [24] and Swamy *et al*., [26] where reduction in the levels of (SOD, CAT and GSH) was observed in rats treated with INH alone. The significant increase in enzymatic antioxidants (SOD, CAT and GSH) in rats co-treated with INH and vitamin C (10 mg/kg and 20 mg/kg) respectively is supported by the report of Sabiu *et al*., [27] where vitamin C improved serum enzymatic and non-enzymatic antioxidant levels in rats, probably due to its intrinsic ability to scavenge free radicals [28]. Vitamin C as water soluble antioxidant directly scavenges superoxide anion and hydroxyl radicals while SOD an enzymatic mitochondrial and or cytosolic enzyme catalyzes the conversion of superoxide anion to hydrogen peroxide and water [29]. Osama and co- workers, [24] revealed that administration of isoniazid causes normocytic normochromic anemia in rats. Another adverse reaction of isoniazid administration in humans is pure red cell aplasia (PRCA) [30], [31], which is a syndrome characterized by a normocytic normochromic anemia with severe reticulocytopenia and marked reduction or absence of erythroid precursors from the bone marrow [32]. Isoniazid can also cause sideroblastic anemia, characterized by deficient heme synthesis and an increase of ring sideroblasts in bone marrow [33]. However, this is not in line with the present study which revealed no significant difference in all the hematological parameters measured. The reason for this is not known but it may be due differences in doses used. Vitamin C a reducing agent which releases iron from reticuloendothelial system to ferritin; reduces ferric iron to ferrous iron which bind less strongly with polyphenol and phytic acid to form insoluble complex. [34]. Vitamin C also enhances the availability and absorption of iron from non-heme iron sources [35]. Vitamin C reduces harmful oxidants in the stomach and promotes iron absorption elevating blood cell formation [34]. However, in this study, administration of vitamin C alone was found not to improve the level of hematological parameters as compared to the control. This is in contrast to the study by Santhrani *et al*., [36] which reported that Vitamin C at a dose of 50 mg/kg produced slight increase in total RBC count and at higher doses (100 and 200 mg/kg) produced a significant increase in total RBC count. The lower doses (10 mg/kg and 20 mg/kg) of vitamin C used in this study may be insufficient to affect haemopoiesis and could be the reason why no significant changes were discovered in the haematological parameters measured. Furthermore, in this study similar findings were observed both at day 7 and 14 in all parameters (Oxidative stress markers and hematological parameters).

5. CONCLUSION

This study showed that isoniazid causes oxidative stress. However, co-administration of isoniazid and Vitamin C ameliorated this effect. It could be concluded that vitamin C ameliorated the toxic effect caused by isoniazid on oxidative parameters in Wistar rats

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Conflict of Interest

I declare that there was no conflict of interest amongst the Authors.

Contribution of the Authors

AN Bako, BB Maiha, SB Anafi conceived the research; Amelioration of isoniazid induced oxidative stress and hematotoxicity by Vitamin C in Wistar Rats. Laboratory preparation and data analysis were conducted by AN Bako and supervised by BB Maiha and SB Anafi. The Manuscript was written by AN Bako, BB Maiha and SB Anafi. The other authors MS Yusuf and AM Kabiru also reviewed and approved the submitted manuscript. The paper was finalized and submitted by AN Bako.

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