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AMELIORATIVE EFFECTS OF ASCORBIC ACID SUPPLEMENTATION ON MOULDY PEANUT DIET-INDUCED HISTOPATHOLOGICAL ALTERATIONS IN LIVER OF ALBINO RATS

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Abstract

Peanut meal contaminated with aflatoxins is well-known to cause deleterious effects on the liver. The current study was conducted to assess the effects of ascorbic acid (Vitamin C) supplementation in rats with mouldy peanut diet (MPD)-induced hepatic injury. Fifteen (15) male albino rats were divided into three groups (A - C) (n=5) namely: Control, MPD-control and MPD + Vit. C. respectively. Vit. C treatment was administered orally, and the investigation lasted for 28days. On Day 29, biochemical analyses to assess liver function was conducted. Relative organ weights and liver histopathological studies were also performed. Results revealed that treatment with Vit. C significantly prevented MPD-induced changes on ALT, AST, ALP and bilirubin, while marked reduction in relative liver weight of MPD-control rats was observed when compared with normal control ($p<0.05$). Liver histomorphology of MPD-control rats showed alterations consistent with hepatotoxicity, however, marked preservation of the hepatic parenchyma was observed in Vit. C-treated rats. In conclusion, the present study revealed that ascorbic acid (Vit. C) supplementation attenuates the hepatotoxicity induced by mouldy peanut diet in rats.

Keywords: *Mouldy Peanut Diet; Ascorbic Acid; Histopathology; Liver; hepatic injury; Rat*

Introduction

Peanut (*Arachis hypogaea* L), commonly known as groundnut, is a legume crop mainly grown for its edible seeds. It is widely grown in the tropics and subtropics, being one of the oldest oil crops (1). Peanuts are commonly eaten as snacks after roasting. Its consumption is associated with a reduced risk of cardiovascular diseases and promotion of weight loss (2). It has no specific dosage or toxicity, however, it has been shown to cause allergic reaction in few people (3).

Mouldy peanuts contain a toxin-producing fungus known as *Aspergillus flavus* (*A. flavus*). Mould can occur in nuts, seeds, corn, milk, wheat and legumes (4). Mould-contaminated peanuts appear shriveled or discolored and this mycotic contamination occurs when the peanuts are kept in a cool, dry place for preservation, to avoid spoilage (5). Susceptibility to mould contamination is increased by moist, humid storage conditions as well as extreme heat and drought before harvest (4,6).

Aspergillus flavus produces a class of potent mycotoxins known as aflatoxins (7). Aflatoxins occur widely in contaminated animal feeds and human foods thereby constituting a great threat to the health of animals and humans due to their highly toxic, hepatotoxicity, carcinogenic, mutagenic, teratogenic and immunosuppressive effects (8,9). The most biologically active component among the various types of aflatoxins is aflatoxin B (AFB) (10). The liver is the main target organ for aflatoxins and its hepatotoxicity is well established (11).

Vitamin C, a six-carbon compound structurally related to glucose, is always referred to as L-ascorbic acid (a strong reducing agent), and its oxidized derivative is L-dehydroascorbic acid. It is a well-known antioxidant, which has been reported to preserve hepatic microanatomy and normalize serum levels of liver marker enzymes in intoxicated animals (12-14). It potentiates the activities of free radical scavengers thus preventing microsomal lipid peroxidation, liver necrosis, inflammation and fibrosis (15). Vit. C is naturally found in plant-derived (citrus, soft fruits and leafy green vegetables) and animal-derived (kidney and liver) sources (16).

Agents with hepatotoxic potentials usually exert their harmful effects by the generation of reactive oxygen species (ROS) which leads to lipid peroxidation in the liver (17,18). This mechanism has propelled the continuous evaluation of the protective efficacy of antioxidants on the liver tissues of humans and animals. Previous animal studies have demonstrated the hepatoprotective efficacy of vit. C in experimentally induced hepatotoxicity (15). There is, however, paucity of information on the effect of Vit. C supplementation in animals placed on a mouldy peanut diet. The present study, therefore, assessed the hepatoprotective effect of ascorbic acid (vit. C) in mould peanut diet-induced hepatic damage in rats.

Methods

Experimental Location and Duration

The experimental studies were carried out at the College of Medicine, University of Nigeria. The entire research lasted for a period of 9.5 months [February – November, 2018].

Laboratory animals

Fifteen (15) male albino rats of the Wistar strain weighing between 100 – 110g were obtained from the Animal house of the Department of Physiology, University of Nigeria. The animals were kept in clean cages in the Animal House of the Department of Anatomy, University of Nigeria, Enugu Campus. The animals were kept under standard environmental conditions and a 12:12 hr light/dark cycle. Water and commercially available rat chow (Top Feed® limited, Ibadan, Nigeria) were provided for the animals *ad libitum*. The animals were allowed to acclimatize for one week at the laboratory condition prior to the experimentation.

Ethics statement

Animal handling was in accordance with Institutional and International guidelines for care and use of Animals in Scientific Research (19). The experimental procedure was approved by the Animal Welfare and Ethics Committee, Department of Animal Science, University of Nigeria, Nsukka.

Mouldy peanut diet preparation

Fresh Peanuts were purchased from Ogbete Main market in Enugu State and stored in moist and humid

storage condition which led to development of moulds. The Mouldy peanut diet (MPD) was prepared daily by mixing 25% of the mouldy peanuts with 75% of the standard rat chow (Top Feeds®).

Study design

The rats were randomly divided into three (3) groups (A - C) (n=5). Group A served as the normal control and was fed standard rat diet and clean water only. MPD was given to rats in groups B and C for 28 days (4 weeks). However, oral administration of Vitamin C (100mg/kg b.wt.) was also given to all rats in group C only, once daily via oral gavage from Day 15 till the end the study period (2 weeks).

Body weight measurements

The body weights (in grams) of each rat were recorded on Day 0 and at four-day intervals (Days 4, 8, 12, 16, 20, 24, and 28) throughout the course of the study and the average body weights for the groups determined.

Blood collection and biochemical Analyses

The animals received the last treatment dose on Day 28. On Day 29, after a 12 hour fast, each animal was anaesthesized and blood sample (4 ml) was obtained via retro-orbital puncture of the media canthus of the eye. The blood sample was placed in plain bottle, centrifuged at 3000rpm for 15 minutes and sera obtained were stored frozen as preparatory to further biochemical assays. Serum levels of the liver marker enzymes (alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST), total bilirubin (TB) and conjugated bilirubin (CB) were determined using commercial reagent kits. ALT and AST were analyzed using the endpoint techniques of Reitman and Frankel (20) provided by Randox Laboratories Ltd, United Kingdom. Alkaline phosphatase was estimated using the method of Roy (21) provided by Teco diagnostics, USA. Total and conjugated bilirubin were estimated using the method described by Tietz (22).

Relative Organ weight measurements

The rats were sacrificed under mild chloroform anaesthesia. The liver, kidneys, heart, spleen and stomach of each rat were isolated, blotted with filter paper and weighed on a balance immediately. The relative organ weight (ROW) for each of the samples was calculated as the ratio of organ weight and the animal's body weight (at the end of experiment) x 100) of each rat.

Gross and Histopathological studies

The excised liver tissues were necropsied to assess for macroscopical changes. The tissues were further fixed in 10% formal saline prior to histological processing for light microscopical examination (23). Haematoxylin and Eosin (H&E) staining procedure was employed to stain the liver sections cut at 5-µm using a rotary microtome. The liver sections were examined using an Olympus™ binocular microscope with in-built lighting system.

Statistical Analysis

Data obtained were expressed as mean ± S.E.M. of five rats per group. Hypothesis testing was conducted using one-way analysis of variance (ANOVA). This was followed by Tukey-highest significant difference (HSD) post-hoc test and Student's t *test* to determine the statistical significance of the differences in the parameters among the groups. SPSS software package program (SPSS, Chicago, IL; version 20.0) was used for the analyses. The level of significance at $p<0.05$ was considered to indicate statistical significance.

Results

Effect on body weights

Data in Table 1 summarized the effects of treatment with Vitamin C on four-day interval body weight differences on rats fed with MPD for 28 days. Feeding the rats with MPD-only (Group B) caused marked increase in body weight similar to the weight gain observed in normal diet and Vit-C treated groups after 8 days of feeding. However, further feeding with MPD caused a marked reduction in body weight gain by the 28th day when compared with groups A and C ($p<0.05$). No obvious body weight change was observed with Vit. C treatment (Group C) when compared with the normal diet-fed group. However, a significantly improved body weight gain of Vit. C-treated group was observed by the 24th day better than that of the normal diet-fed rats ($p<0.05$). Regardless of the significant changes in the body weight gain of the rats in different groups, it was observed that all live weights of the animals

improved from the start of the experiment to the end.

Effect on organ weights

The effects of the treatments in the present study on relative organ weights is represented in Table 2 and Figure 1 (I-V). As shown in Table 2, statistically significant decrease in liver weight and liver index was found in Group B (MPD-control) when compared with the normal control (group A) ($p<0.05$). Similarly, a decrease ($p<0.05$) in relative spleen (Fig. 1-III) and stomach (Fig. 1-IV) weights were observed in group B when compared with the normal control. However, increased and decreased weights of the kidneys (Figs. 1-I&II) and spleen (Fig. 1-III) respectively in Vit. C- treated rats were observed when compared with the control ($p<0.05$). Conversely, no significant change was observed in relative heart weights of both MPD control and Vit. C-treated rats when compared with the normal control (Fig. 1-V).

Effect on serum biochemistry

The effects of Vit. C supplementation on some serum biochemical parameters (ALT, AST, ALP, TB and CB) in MPD-induced hepatotoxicity are shown in Table 3. Data showed markedly increased levels of ALT, AST and ALP and decreased levels of TB and CB in MPD-only fed rats when compared with values from normal control ($p<0.05$). Treatment of rats with Vit. C (100mg/kg b.wt.) showed increased levels of ALP only ($p<0.05$) whereas the other parameters remained unchanged when compared with normal control. Comparison of values from Vit. C-treated rats with MPD-only fed rats showed statistically significant changes in all parameters ($p<0.05$) except in ALP and TB.

Histopathological findings

The light microscopical findings obtained in the present study are shown in the photomicrographs in Figure 2. Features from the liver of control rat (Figure 2-I) showing the pan-lobular [I(A)] and periportal [I(B)] regions reveal normal hepatic histoarchitecture consisting of prominent central vein (Cv), normal portal tracts (Pt), and sinusoidal spaces (S) which are flanked by plates of normal hepatocytes (H). In the MPD-only fed group, markedly disrupted histoarchitecture of the hepatic parenchyma is evident [Figure 2-II(A)]. Hepatocytes necrosis (n), cellular infiltration (arrows) and sinusoidal dilation are observed [Fig. 2-III(B)]. However, the severity of the lesions associated with hepatotoxicity induced by MPD was markedly reduced upon treatment with 100mg/kg b.wt. of Vit. C (Figures 2-III). Few pericentral hepatocytes appear mildly ballooned (bH) and show some wispy cytoplasmic content [Fig. 2-III(B)].

Discussion

Animal models of hepatic injury have long been used to assess the efficacy of hepatoprotective agents. The liver plays a vital role in the detoxification of xenobiotics and toxins (24). In the present study, a mouldy peanut diet (MPD) was used to induce hepatic damage in albino rats and the hepatoprotective activity of ascorbic acid (vitamin C) against the liver injury was investigated. Histopathological lesions on the liver, elevated levels of serum ALT, AST, ALP and decreased TB and CB levels specified that hepatic injury has occurred as a result of feeding with the MPD. However, as observed, supplementation with Vit. C preserved the histoarchitecture of treated rats from MPD-induced damage.

Mould-contaminated peanuts contain *Aspergillus flavus* which is well known to produce secondary metabolites, known as aflatoxins, which cause liver damage (25-27). Aflatoxins elicit hepatotoxicity via a number of mechanisms which include accumulation of reactive oxygen species, apoptosis, cytotoxicity and genotoxicity (28,29). Hepatocytes affected by aflatoxin-induced hepatotoxicity become damaged and release their constituents into the blood circulation. Histopathological findings in the current work revealed profound deleterious effects on the liver of the rats following feeding with mouldy peanut diet (MPD). These lesions were found to be consistent with effects caused by aflatoxin B1-induced hepatotoxicity (29-31). The extensive hepatocellular necrosis observed is similar to reports from previous studies (26,31-34) and this finding may have resulted from the binding of the toxic metabolite of the mycotoxin which invariably would lead to oxidative stress.

Sinusoidal dilation of the hepatic tissue as observed in the current study has also been reported by other researchers (32), although a contraction has

been documented contrarily (31). The cellular infiltration within the tissue parenchyma, as observed in the present study, occurred in response to the degenerating hepatocytes. This feature is in agreement with that reported by other researchers (29,30). Previous works have also documented vascular congestion, engorged portal areas and hyperemia (26,35) in the liver of animals following aflatoxin-induced hepatoxicity. However, these features were not observed in the current study. Consistent with the microscopical features, markedly altered levels of serum biochemical parameters were observed in the present study and these alterations provide a useful information on the type and extent of hepatic injury (24,36).

In this study, treatment with the Vit. C counteracted the effect of MPD-induced hepatic injury by reversing the serum levels of the liver marker enzymes and bilirubin to as close to those observed in the normal (control) rats. A concurrent better preservation of hepatic histoarchitecture further buttressed the hepatoprotective efficacy. The antioxidant role of Vit. C is well-established. Its hepatoprotective efficacy against drugs, chemical, insecticides, organophosphate insecticides and heavy metals is attributed to its antioxidant property (15). Thus, the hepatoprotection offered in the present study would be attributed to the ability of Vit. C to inhibit lipid peroxidation in the liver by its potentiating action on the activities of free radical scavengers. A comprehensive review of various research works which have demonstrated the hepatoprotective efficacy of Vit. C via antioxidative means in experimental animal models of hepatotoxicity has been documented (15).

The marked body and organ weight decrease associated with feeding with MPD in this study suggests an impairment of growth in the animals. Previous studies have documented on the reduced weight gain and absolute body weight loss associated with dietary exposure of animals to aflatoxins (37-40). Possible mechanisms to this growth impairment may be due to decreased feed intake or reduced efficiency of nutrient usage (25,40). Treatment with Vit. C was observed to have improved the body weight gain of treated rats even better than the untreated (control) group.

In conclusion, the present study has revealed that adverse effects exerted by a mouldy peanut diet on the histological structure of the liver could be attenuated by ascorbic acid (Vitamin C) supplementation. Hence, Vit. C is an agent which may be used successfully to prevent hepatic injury from mould-contaminated feeds.

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Conflict of interest

None declared.

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Table 1: Effect of treatment with Ascorbic acid (Vit. C) on body weight changes.

Day	GROUPS		
	A	B	C
4	**29.44±1.97#**	**22.38±1.23***	25.78±3.14
8	**9.00±1.20#**	**19.64±0.75***	14.34±2.47
12	10.22±0.78	7.22±1.30	6.64±1.90
16	11.50±2.36	9.82±1.64	14.86±3.32
20	**9.84±1.83#**	**5.00±1.02***	5.84±0.95
24	8.80±0.45	5.64±1.59	**17.78±2.38*#**
28	**9.66±0.47#**	**1.78±0.13***	**8.76±1.19#**

A: Control rats fed a standard diet; B: Mouldy peanut diet (MPD) control; C: Vitamin C + MPD. Data expressed in mean ± SEM; *p<0.05 when compared to the normal control (Group A) and #p<0.05 in comparison to the MPD control (Group B).

Table 2: Effect of treatment with Ascorbic acid (Vit. C) on final body weight and liver index of rat fed a mouldy peanut diet.

GROUPS	Parameters			
	Initial Body weight (g)	**Final Body weight (BW*) (g)**	**Liver weight (LW) (g)**	**Liver index (LW/BW*) (%)**
A	102.44±1.54	**190.90±5.84#**	**10.93±1.76#**	**5.71±0.82#**
B	103.48±1.30	**174.96±2.74***	**7.56±0.84***	**4.32±0.50***
C	101.10±1.79	**195.10±1.67#**	9.61±1.04	4.92±0.52
F-ratio	0.588	38.183	8.864	6.140
Sig.	0.571	0.000	0.004	0.015

A: Control rats fed a standard diet; B: Mouldy peanut diet (MPD) control; C: Vitamin C + MPD. Data expressed in mean ± SEM; *p<0.05 when compared to the normal control (Group A) and #p<0.05 in comparison to the MPD control (Group B).

Table 3: Effect of treatment with Ascorbic acid (Vit. C) on some biochemical parameters (AST, ALT, ALP, Total bilirubin and Conjugated Bilirubin) upon MPD-induced hepatic injury.

GROUPS	Biochemical Parameters				
	ALT(iu/l)	AST(iu/l)	ALP(iu/l)	TB(mg/dl)	CB(mg/dl)
A (Control - SD)	**33.60±5.60#**	**112.80±2.94#**	**355.32±12.46#**	**4.26±0.88#**	**2.22±0.32#**
B (MPD only)	**166.60±47.45***	**161.60±18.96***	**398.33±0.03***	**1.54±0.23***	**0.70±0.13***
C (MPD + 100mg/kg Vit. C)	**28.00±1.41#**	**118.00±2.00#**	**385.21±0.84***	3.48±1.08	**1.90±0.42#**
F-ratio	8.083	5.792	9.345	2.950	5.301
Sig.	0.004	0.006	0.017	0.091	0.022

Data expressed in mean ± SEM; *p<0.05 when compared to the control (Group A) and #p<0.05 in comparison to the negative control (Group B). ALT – Alanine transaminase; AST – Aspartate transaminase; ALP – Alkaline phosphatase; TB – Total bilirubin; CB – Conjugated bilirubin; SD – Standard diet; MPD – Mouldy peanut diet.

Figure 1: Graphs showing relative organ weights of rats in normal control and treatment groups. I: Right kidney; II: Left Kidney; III: Spleen; IV: Stomach; and V: Heart. * and ¥ indicate p<0.05 when compared to normal control (Group A) and MPD control (Group B) respectively. [Groups A: Normal control; B: MPD-control; C: MPD +Vit. C]

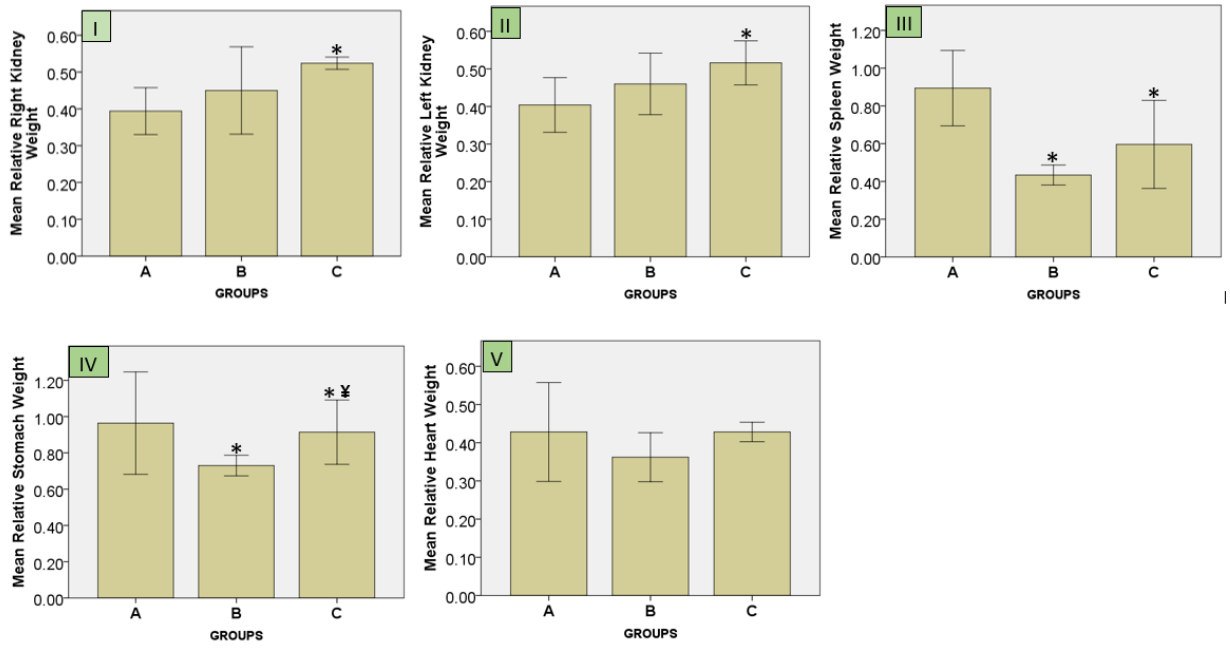

Figure 2.(I-III) Effect of Vitamin C administration on the Liver histomorphology of rats with Mouldy Peanut Diet-Induced Hepatic Damage. [Stain: H&E; (I): Normal control rat *(Mag: A-x100; B-x200)*; (II) MPD-only fed rat *(Mag: A-x100; B-x400)*; (III) MPD-fed rat + Vitamin C] *(Mag: A-x100; B-x400)*