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Histopathological Evidence of the Protective Effect of *Arachis hypogea* Seed on 1,2-Dimethylhydrazine-Dihydrochloride-Induced Liver Damage in Rats

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ABSTRACT: This study examined the effect of peanut-supplemented diet on the serum antioxidant status and histomorphological changes induced by 1,2-dimethylhydrazine-dihydrochloride on rat liver. Twenty-eight healthy albino Wistar rats used for this study were divided into seven groups of 4 rats each. The toxicant, 1,2-dimethylhydrazine-dihydrochloride (DMH), was administered subcutaneously at a dose of 25 mg/kg body weight. Group A (control) rats were maintained on normal rat feed. The Group B and C rats were maintained on normal feed, administered DMH for 12 and 24 weeks respectively. Incorporation of peanut into the diet was varied depending on the group, 12 weeks after DMH administration (Group D), concomitantly with DMH administration for 24 weeks (Group E) and 12 weeks before the administration of DMH (Group F). Group G rats were maintained on a peanut-supplemented diet only for 24 weeks. At the end of the treatment period all the animals were sacrificed under mild anaesthesia and blood collected by heart puncture. Portions of the liver were excised and fixed for histopathological examination. The result showed that DMH significantly ($p \leq 0.05$) increased serum MDA levels, this elevated MDA levels was reversed by the supplementation of the rat feed with peanut powder. There were also significant ($p \leq 0.05$) decreases in antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase activities in the groups that received DMH alone which were reversed in the groups exposed to DMH and peanut-supplemented diet. Histopathology results showed that DMH administration resulted in varying degrees of necrosis and fibrosis while the prophylactic administration of peanut incorporated diet to the rat resulted in normal liver architecture. Incorporation of peanut powder therefore has the ability to protect the liver from 1, 2-dimethylhydrazine-induced liver damage and also reduces oxidative stress markers while improving antioxidant enzyme activities.

Keywords: 1,2-dimethylhydrazine, Peanuts, Liver damage, Histology, Antioxidants.

Introduction

Although 1, 2- dimethylhydrazine is a known colon carcinogen producing tumour lesions in the colon; the liver is not spared the toxic effect of this carcinogen. Liver, an important organ of metabolism, is damaged when exposed to chemicals, toxins, infectious agents, drugs and food additives (Shimmi and Parash, 2016). Activation of DMH takes place in the liver and is mediated by a series of reactions involving azomethane (AOM) and methylazoxymethanol (MAM) as intermediates to the final carcinogenic compound, the extremely reactive methyldiazonium ion (Perše and Cerar, 2005). Metabolism of DMH is characterized by a series of oxidation steps; which involves the dehydrogenation to azomethane, followed by the *N*-oxidation of azomethane to azoxymethane and then the *C*-oxidation of azoxymethane to methylazoxymethanol catalysed by hepatic microsomes (Perše and Cerar, 2011). This last metabolite, methylazoxymethanol decomposes to yield the

extremely reactive methyldiazonium ion. The ultimate carcinogenic compound causes oxidative stress by methylating biomolecules present in epithelial cells of the colon, thus resulting to promutagenic events caused by inflammation (Abd-Elmoneim *et al*., 2013). The series of metabolic activation of the procarcinogen, 1, 2-dimethylhydrazine to the ultimate carcinogen, occurs mostly in the liver, and thus predisposes the organ to the oxidative damage.

Several health benefits has been linked with the consumption of peanuts notably weight management (Alper and Mattes, 2002), prevention of cardiovascular diseases (Feldman, 1999) as well as Alzheimer's disease prevention and cancer inhibition (Awad *et al*., 2000). These beneficial effects are largely due to the low levels of saturated fatty acids found in peanuts (Misra, 2004) and the absence of *trans*-fatty acids (Sanders, 2001), richness in mono- and poly-unsaturated fatty acids, micronutrients mainly vitamin E, folate, minerals (potassium, magnesium and zinc), fibre and phytochemicals, predominantly resveratrol (Sanders*et al*., 2000) and a host of several other phenolic compounds (Isanga and Zhang, 2007).

Dietary intake of plant-derived polyphenols could be a feasible way of preventing the onset of liver injuries. *Arachis hypogaea* (peanut) which is commonly consumed in most parts of the world is known to possess several polyphenolic compounds with free radical scavenging activity, which could protect the liver from oxidative damage. This study therefore aimed at examining the effect of *Arachis hypogaea* seed on the serum antioxidant status and histomorphological changes induced by 1, 2-dimethylhydrazine-dihydrochloride on rat liver.

Materials and methods

Chemicals: 1,2-Dimethylhydrazine dihydrochloride (DMH) was obtained from Sigma Aldrich, Germany. Halothane was a product of Piramal Healthcare Limited, India.

Plant material: Arachis hypogaea seeds were purchased at Uselu market, Benin City. The verification of the peanut seed and plant was carried out at the Department of Plant Biology and Biotechnology, University of Benin. The peanut plant was then deposited at the Hebarium and assigned a voucher number UBH_A352. The peanut seeds were sorted, cleaned and air dried for 48 h. The raw peanuts were then blended into fine coarse powder using an electric blender and then stored in air-tight containers in the refrigerator (4 °C). *Arachis hypogaea* seed powder was incorporated into the rat diet at 20 % level (20 g peanut powder to 80 g of rat feed; w/w) (Guyton *et al*., 2008).

Animal model and experimental design: Twenty-eight (28) healthy Wistar albino male rats were used. They were purchased from Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City Nigeria and housed in wooden framed/iron meshed cages in the Animal House of Biochemistry Department. The rats were divided into seven groups of 4 male rats each (labelled A to G). 1, 2-dimethylhydrazine-dihydrochloride, DMH was administered at a dose of 25 mg/kg body weight subcutaneously. Rats in each group were housed separately in a clean, disinfected cage in a room with a 12-hour light/dark cycle**.** The rats were maintained on growers mash (Bendel Feeds and Flour Mills Ltd, Ewu, Nigeria) and water *ad libitium* for two weeks before the start of the experiment. When the experiment started, Group A (control) rats were maintained on normal feed (growers mash) and a weekly subcutaneous injection of the vehicle (EDTA-Saline). Group B rats were provided normal rat feed and subcutaneous injection of DMH 25 mg/kg body weight (Veceric and Cerar, 2004) once weekly for 12 consecutive weeks. Group C rats were placed on normal feed and injected DMH 25 mg/kg body weight once weekly for 24 weeks. Group D rats received DMH and normal feed for 12 weeks followed by peanut-supplemented diet for the next 12 weeks. Group E rats received DMH injection and peanut diet concomitantly for 24 weeks. Group F rats were placed on peanut diet for 12 weeks prior to the administration of DMH for 12 weeks. Group G rats were placed on peanut diet throughout the experimental period of 24 weeks, but received once weekly injection of the vehicle (EDTA- saline). All groups were kept on their respective diet for 24 weeks.

Animal sacrifice, collection and preparation of samples for analyses: After 24 weeks, the animals were fasted overnight; each rat was anaesthetised using halothane saturated chamber. While under anaesthesia, the abdominal and thoracic region were opened and blood collected into plain sample tubes by heart puncture. The liver was excised and sections were obtained for histological examination. Sections for histology were fixed immediately in 10 % formol-saline. To obtain the sera, blood samples were centrifuged at 4,000 rpm for 10 min. Separated sera were kept at -20 °C until required for biochemical analyses. Liver histology was done by using standard laboratory procedure in the Department of Morbid Anatomy and Histopathology, UBTH, Benin. Preparation of colon sections for staining was carried out by the method described by Kiernan, (2008).

Serum biochemical assay: Malondialdehyde levels were measured based on its reaction with 2-thiobarbituric acid as described by Buege and Aust (1978). This method is based on the generation of MDA, an end product of the lipid peroxidation which reacts with thiobarbituric acid to yield thiobarbituric acid reactive substance

(TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm. Superoxide dismutase activity was assessed by the method of Misra and Fridovich (1972) which involved the auto-oxidation of adrenaline to adrenochrome at 420 nm. Catalase activity was examined by the method of Cohen *et al*., (1970), in which the decomposition of hydrogen peroxide was monitored at 480 nm. Glutathione peroxidase activity was determined by measuring the production of purpurogallin from pyrogallol at 420 nm as described by Nyman (1959).

Data analysis: The results obtained from the biochemical assays were expressed as mean ± standard deviation (SD). In order to establish whether the mean values were statistically significantly different from each other, analysis of variance (ANOVA) was done using SPSS software (Version 21.0).To know which means have differences that are significantly different, LSD multiple range test was done by employing the same SPSS computer software. Values were considered significant at $p \leq 0.05$.

Results

The results of Serum malonaldehyde, MDA levels, and the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities are presented in Table 1. Compared with control group, DMH treated groups showed significantly ($p \leq 0.05$) higher serum MDA levels. However incorporating peanuts into the diet of the rats either pre-, post-treatment or concomitantly was able to reverse the DMH-induced increase in serum malondialdehyde level. Incorporating peanuts into the diet of rats before the administration of DMH maintained the male colon MDA level to values close to that of the control. The activity of glutathione peroxidase in the serum of rats administered DMH only for 12 and 24 weeks respectively decreased significantly ($p \leq 0.05$) relative to the control group. Consumption of peanut diet significantly increased ($p \leq 0.05$) the activity of glutathione peroxidase relative to the groups given a subcutaneous injection of DMH for 12 weeks while being maintained on a normal diet. The incorporation of peanut into the feed 12 weeks after the first dose of DMH (group D) and incorporation of peanut 12 weeks before the administration of DMH for 12 weeks (group F) significantly ($p \leq 0.05$) increased the activity of the enzyme relative to the group maintained on normal diet after 12 weeks of DMH administration (group B). In the rats whose diet was supplemented with peanuts and administered DMH for 24 weeks concomitantly (group E), glutathione peroxidase activity significantly ($p \leq 0.05$) increased relative to the group that received DMH and normal rat feed for 24 weeks (group C). Serum catalase and superoxide dismutase activities were also significantly ($p \leq 0.05$) reduced in DMH only treated groups relative to control. Consumption of peanut supplemented diet significantly ($p \leq 0.05$) the activities of these enzymes relative to the group that received DMH for 12/24 weeks and maintained on normal rat chow (group B and C).

Table 1: Serum malondialdehyde levels, superoxide dismutase, catalase and glutathione peroxidase activities in Wistar rats

	Groups	MDA Level (mmol/ml) $\times 10^{-3}$	SOD Activity (Units/ml)	CAT Activity (K/min)	GPx Activity (Units/ml)
A	Control	16.90 ± 0.35	3.31 ± 0.38	1.74 ± 0.07	0.63 ± 0.07
B	DMH_{12}/NC*	$44.23 \pm 0.09^{a**}$	$1.46 \pm 0.87^{a**}$	$0.28 \pm 0.14^{a**}$	$0.40 \pm 0.08^{a**}$
C	DMH_{24}/NC	$67.89 \pm 0.21^{a,b}$	$0.70 \pm 0.45^{a,b}$	0.31 ± 0.12^{a}	0.34 ± 0.14^{a}
D	DMH_{12}/PNT_{12}	$40.5 \pm 0.22^{a,c}$	$2.18 \pm 0.37^{b,c}$	0.47 ± 0.22^{a}	$0.65 \pm 0.02^{b,c}$
E	$DMH_{24} + PNT_{24}$	$49.23 \pm 0.74^{a,c}$	$2.17 \pm 0.51^{b,c}$	$0.49 \pm 0.08^{a,b}$	$0.72 \pm 0.04^{b,c}$
F	PNT_{12}/DMH_{12}	$21.82 \pm 0.18^{b,c,d}$	$2.56 \pm 0.46^{b,c}$	$0.66 \pm 0.12^{b.c,d}$	$0.65 \pm 0.03^{b,c}$
G	PNT_{24}	$15.11 \pm 0.86^{b,c,d,e,f}$	$3.57 \pm 0.14^{b,c,d.e.f}$	$0.78 \pm 0.07^{b,c,d,e}$	$0.73 \pm 0.10^{b,c}$

Values are given in mean ± SD. Mean values (n=4)

PNT = Peanut, NC: Normal rat chow, DMH: 1, 2-dimethylhydrazine, Control = NC and water with weekly subcutaneous injection of EDTA-saline solution, DMH_{12}/NC = Maintained on NC while treating with DMH for 12 weeks, DMH_{24}/NC = Maintained on NC but treated with DMH for 24 weeks, DMH_{12}/PNT_{12} = Treated with DMH for 12 weeks while on NC and later maintained on peanut diet (PNT) for12 weeks, $DMH_{24}+PNT_{24}$ = Treated with DMH and peanut simultaneously for 24 weeks, PNT_{12}/DMH_{12}= Maintained on peanut diet for 12 weeks, followed with DMH treatment for 12 weeks, PNT_{24} = Maintained on peanut diet for 24weeks with weekly subcutaneous injection of EDTA- Saline solution.

**Values with superscripts a, b, c, d, e or f are significantly different from the value of the group with the corresponding upper case letter A, B, C, D, E or F ($p \leq 0.05$).

The results of the histopathological examination are shown in Plates 1 – 7. Group A, the Control animals (Plate 1) presented normal hepatocytes, sinusoid, bile duct and portal vein. Rats administered DMH showed varying

degrees of vascular ulceration, congestion, periportal infiltrates of inflammatory cells and fibrosis, depending on the length of administration. The rats maintained on DMH for 12 weeks and peanut diet for the next 12 weeks and peanut-supplemented diet concomitantly for 24 weeks (Plate 4 and 5, respectively) revealed heavy infiltrates of inflammatory cells, hepatocyte necrosis and congestion. However, the rats maintained on peanut-supplemented diet for 12 weeks prior to DMH administration (Plate 6) showed normal hepatocyte and vascular architecture of the liver.

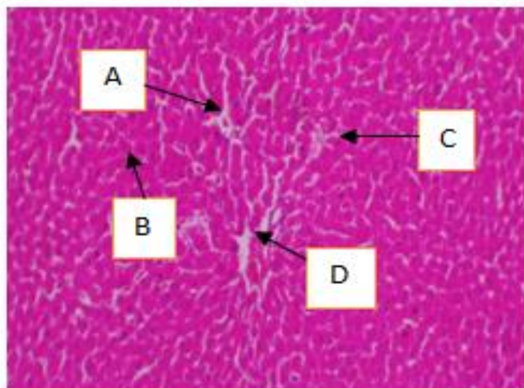

Plate 1: Photomicrograph of Group A (control) rat liver: A, hepatocytes; B, sinusoids; C, bile duct; and D, portal vein (H&E x 100)

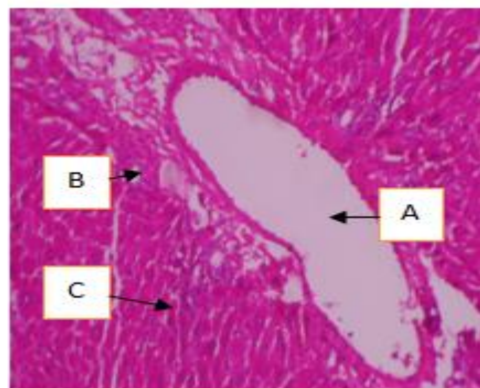

Plate 2: Photomicrograph of Group B rat liver maintained on normal feed and administered DMH for 12 weeks: A, patchy vascular ulceration B, congestion; and C, mild periportal infiltrates of inflammatory cells (H&E x100)

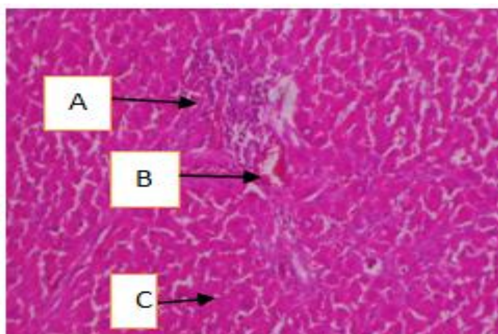

Plate 3a: Photomicrograph of group C maintained on normal feed and administered DMH for 24 weeks Rat liver: A, heavy periportal infiltrates of inflammatory cells; B, congestion; C, fibrosis (H&E x 100)

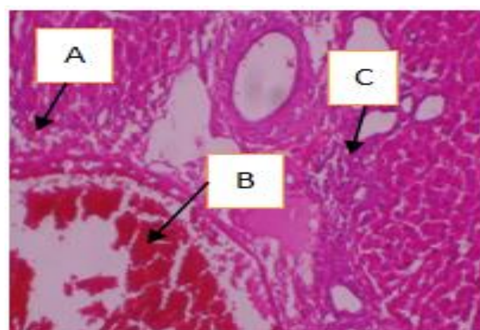

Plate 3b: Photomicrograph of Group C rat liver maintained on normal feed and administered DMH for 24 weeks. Rat liver: A, patchy necrosis; B, congestion; and C, heavy periportal infiltrates of inflammatory cells (H&E x 100)

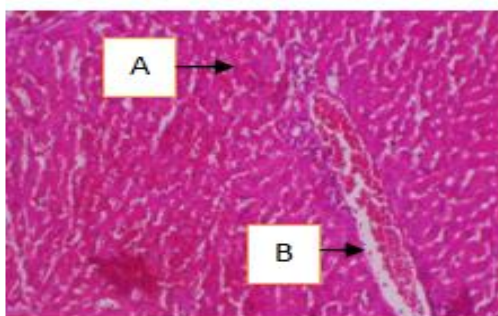

Plate 4: Liver photomicrograph of Group D administered DMH and normal feed for 12 weeks followed by a peanut-supplemented diet for the next 12 weeks. Rat Liver: A, mild periportal infiltrates of inflammatory cells; and B, vascular congestion (H&E x 100)

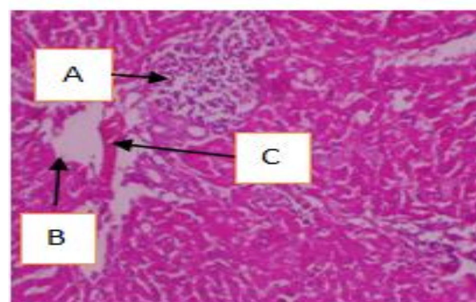

Plate 5: Photomicrograph of Group E maintained on DMH and peanut-supplemented diet concomitantly for 24 weeks. Rat Liver: A, heavy infiltrates of inflammatory cells; B, hepatocyte necrosis; C, congestion (H&E x 100)

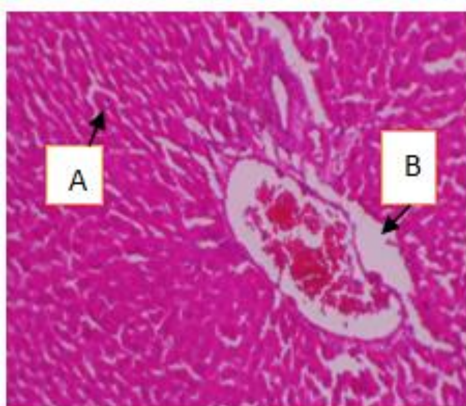

Plate 6: Rat liver given peanut diet for 12 weeks then DMH for 12 weeks: A, normal Hepatocyte; and B, vascular architecture (H&E x 100)

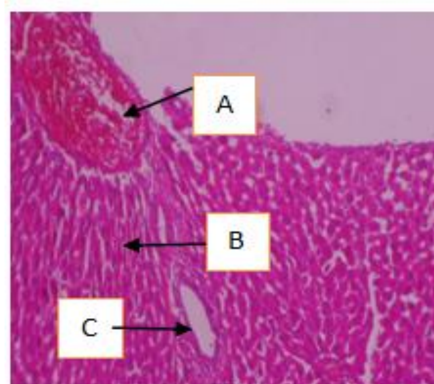

Plate 7: Photomicrograph of Group G given peanut diet only for 24 weeks: A, active portal congestion; B, normal hepatocyte; and C, biliary architecture (H&E x 100)

Discussion

Oxidative stress is defined as an imbalance between oxidants and antioxidants accompanied by overproduction of reactive oxygen species (ROS) (Sies et *al*., 2017). Oxidative stress results in the production of oxidation products and the depletion of endogenous antioxidants. Excessive ROS damage cellular structures and macromolecules, leading to cellular dysfunction and ultimately cell death (Finkel and Holbrook, 2000).
Malondialdehyde (MDA) is a product of lipid peroxidation and a sensitive and reliable biomarker of oxidative tissue damage (Gutteridge, 1995). Several studies have reported high levels of MDA have been detected in blood samples from cirrhotic patients and animals models (Wolter *et al*., 1984, Aboutwerat *et al*., 2003, Lee *et al*., 2010; Galicia-Moreno *et al*., 2016, Eboh *et al*., 2016, Zheng *et al*., 2019, Zelber-Sagi *et al.,* 2020). Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) act as free radical scavengers. These antioxidant enzymes as electron donors react with the reactive oxygen species (ROS) to form innocuous products and inactivate the free radicals (Ghanbari *et al*., 2016). Therefore, antioxidants protect cells against oxidative damage (Valko *et al*., 2006). The cellular metabolism of a chemical carcinogen is crucial for the initiation of carcinogenesis (Maru *et al*., 2014). Verceric and Cerar (2004) reported that DMH is activated in the liver through chain reactions involving the intermediates, azomethane and methylazoxymethanol (MAM), to the ultimate carcinogenic metabolite, methyldizonium ion, which is very reactive. Liver of rats exposed to DMH showed vascular ulceration as well as periportal infiltrates of inflammatory cells. Furthermore, at an extended period of DMH administration, fibrosis was observed. Fibrosis refers to an excessive accumulation of extracellular matrix proteins including collagen. This occurs in most chronic cancer cases. Liver fibrosis is as a result of chronic liver damage (Bataller and Brenner, 2005). From the result of the histological examination of the liver, it was evident that rats administered peanut-supplemented diet prior to DMH administration showed normal microarchitecture of the liver. This is in agreement with earlier reports by Xiu-Fen *et al*. (2011) and Shimini & Parash (2015). This protective action of *Arachis hypogea* seed-supplemented diet is attributed to the fact that peanuts contain a wide array of polyphenolic compounds notably resveratrol, isoflavones and phenolic substances, which are potent in inhibiting the onset of cancer (Guyton *et al*., 2008). In addition, polyphenols have been shown to inhibit the formation of procarcinogen molecules *in vivo* and also enhances the isozyme P_{450}, which in-turn modulates phase II biotransformation enzymes, scavenge electrophilic molecules and enhances repairs *in vivo* (Maru *et al*., 2014). Peanuts also contain polyunsaturated fatty acids (PUFA) such as omega-3, which have been shown to reduce chemically-induced tumorigenesis in rodents due to its anti-proliferative and anti-inflammatory abilities (Dwivedi *et al*., 2003).

Conclusion

The result obtained from this study showed that 1, 2 –dimethylhydrazine significantly increased oxidative stress and caused liver damage, but peanut supplementation reversed these effects by reducing oxidative stress

markers and improving antioxidant enzyme activities. In addition peanut diet protected the liver against DMH-induced necrosis and fibrosis. Conclusively, this therefore study suggests that incorporation of peanut to the diet of the experimental animals prior to DMH administration effectively protected the liver from DMH-induced damage.

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