ANTICLASTOGENIC AND HEPATOPROTECTIVE PROPERTIES OF GINGER (*ZINGIBER OFFICINALE*) EXTRACT AGAINST NITROBENZENE-INDUCED TOXICITY IN RATS

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Ginger or *Zingiber officinale* has been reported to possess antioxidant activities that can protect against free radical damage. We therefore carried out this study to investigate the possible ameliorative effects of ginger against the clastogenicity, hepatotoxicity and oxidative stress induced by nitrobenzene (NB) administration in wistar rats. Twenty male wistar rats were divided into four groups: group I (normal control group), fed with normal rat chow and clean drinking water only for seven days; group II, fed with 1000 mg/kg NB only for two days; group III, fed with 1000 mg/kg NB for two days, followed by treatment with 400 mg/kg *Z. officinale* ethanolic extract for seven days; and group IV, to which 400 mg/kg *Z. officinale* extract were administered only for seven days. The whole experiment lasted for seven days and animals were sacrificed 24 hours after the last dose to obtain the blood for plasma preparation. The following parameters were then determined: oxidative stress markers (catalase activity and extent of lipid peroxidation), hepatic function indices (aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and total bilirubin (TB). Total protein (TP) and lipid profile parameters – total cholesterol (TC) and triglyceride (TG) – were also measured. Femurs were harvested from the rats for micronucleus assay (clastogenicity) too. Comparing the normal control group with the NB only group, there was a significant increase ($p < 0.05$) in all the parameters investigated. Treatments with ethanolic extract of *Z. officinale* were able to significantly ($p < 0.05$) lower the number of micronucleated polychromatic erythrocytes (mPCEs) scored, the activities of enzymes and the concentrations of other parameters investigated to normalcy or near normalcy, when compared with the normal control. Histopathological examinations of the liver revealed no visible lesion in the normal control group, severe diffuse vacuolar degeneration of the hepatocytes in the NB treated group, mild diffuse vacuolar degeneration of the hepatocytes in the NB plus extract group, and slight diffuse vacuolar degenerations of the hepatocytes in the extract treated group. The findings of this study suggest that *Z. officinale* extract possesses anti-clastogenic and hepatoprotective properties against NB-induced toxicity

Keywords: *Zingiber officinale*, nitrobenzene, hepatoprotective, anti-clastogenicity.

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INTRODUCTION

Nitrobenzene (NB) is known as a hazardous air pollutant and has been proven to be a carcinogen in animals. According to the 1986 Cancer guidelines, it was classified as a group B2 chemical, a likely human carcinogen (1). It is a widely used industrial chemical, mostly in the production of aniline, but also in that of pesticides, shoe polishes, analgesics, dyes and pyroxylin compounds. During the metabolism of NB, intermediates such as nitrosobenzene (NOB) and phenylhydroxylamine (PH) – that play an important role in the process of NB carcinogenesis – are formed (2). It has been reported that the highest concentration of NB is usually found in the liver, brain, blood and stomach following accidental nitrobenzene poisoning in humans (3).

Ginger (*Zingiber officinale*) belongs to Zingiberaceae family and it is one of the world's best known spices, used since time immemorial for its health benefits. The commonly consumed part is the rhizome. Although native to Southern Asia, it is also cultivated in tropical areas, such as Jamaica, China, Nigeria and Haiti, wherein it is being used to produce substances such as curry powder, sauces, ginger bread, biscuits, ginger flavored carbonated drinks, pickles and confectionaries. Dry ginger is also reported to be used in the manufacture of oil, oleoresin, essence and processed meat (4). Ginger has been recommended for use as carminative, diaphoretic, antispasmodic, expectorant, circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic and digestive aid (5, 6). It is used to cure diarrhea, dysentery, fever, cough, ulcers, boils, and wounds (7). Ginger roots extracts were reported to possess polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity as well as hypolipidemic properties (8–11). Gingerol was found to inhibit lipid peroxidation induced by the $FeCl_3$-ascorbate system (12) as well as superoxide anions generated by xanthine oxidation (13). Ginger supplementation was reported not only to increase the concentrations of superoxide dismutase and catalase in the tissues and decrease the level of oxidized glutathione, but also to suppress colon carcinogenesis induced by a procarcinogen, dimethylhydrazine (DMH) (14, 15). The phytochemical screening results revealed the presence of alkaloids, carbohydrates, glucosides, proteins, saponins, steroids, flavanoids and terpenoids (16). The present study therefore investigated the potential anti-clastogenic and hepatoprotective properties of ginger (*Zingiber officinale*) extract against nitrobenzene-induced toxicity in rats.

MATERIALS AND METHODS

Chemicals and kits

Nitrobenzene and other chemicals were of analytical grade, products of Sigma Chemical Co., Saint Louis, MO, USA or BDH Chemical Ltd, Poole, England. Alanine amino transferase (ALT), aspartate amino transferase (AST),

gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total protein, bilirubin, total cholesterol, and triglyceride kits were products of Cypress Diagnostics, Belgium.

Plant material

Zingiber officinale rhizomes were purchased from Kuto market, Abeokuta, Nigeria. Identification and authentication was done by Prof. D. A Agboola, Department of Biological Sciences, Federal University of Agriculture, Abeokuta, Nigeria. The identified and authenticated rhizomes were air-dried at room temperature under standard laboratory procedures.

Plant extracts preparation

The dried rhizomes were milled with a local miller to the powdery form. Three hundred grams (300 g) of the milled rhizomes were extracted in 95% ethanol for 72 hours. The mixture was then filtered and the filtrate was left to evaporate to dryness, under reduced pressure, in a rotary evaporator. The dried extract was stored at -4 ^{o}C until use. The ginger extract was administered to rats according to their body weights.

Animals

Twenty (20) wistar albino rats weighing 200-250 g each were purchased from the Department of Veterinary Anatomy, Federal University of Agriculture, Abeokuta, Nigeria. The animals were housed in metallic cages in the experimental small animal house of the Department. They were allowed to acclimatize for two weeks before the commencement of the experiment with 12 h light/dark cycle and a temperature of 28±2 ^{o}C, and were fed with normal rat chow diet (Ladokun feeds, Ibadan) and water *ad libitum*. The permission to use the animals was approved by the Animal Ethical Committee of the Institution.

Experimental design

The rats were randomly divided into four groups of five rats each:

- ***Group 1*****:** served as control group and received the vehicle (corn oil) for seven days.
- ***Group 2*****:** received intraperitoneal (i.p) injection of 1000 mg/kg nitrobenzene in corn oil, according to the method of Suganya *et al*. (17)
- ***Group 3*****:** received intraperitoneal (i.p) injection of 1000 mg/kg nitrobenzene in corn oil and 400 mg/kg ethanolic extract of *Z. officinale*.
- ***Group 4*****:** received 400 mg/kg ethanolic extract of *Z. officinale*.

Ginger was administered orally by gavage and administrations lasted for seven days.

Estimation of biochemical parameters

Twenty-four hours after the last administration, the animals were anaesthetized using diethyl ether. Blood was collected directly from the abdominal artery with heparinized syringes into clean heparinized tubes.

Preparation of plasma

Plasma was separated by centrifugation at 3000 rpm for 10 minutes and used for the estimation of various biochemical parameters: ALT, AST, ALP, GGT, total bilirubin, total protein, cholesterol and triglyceride, following the methods described in Cypress Diagnostics Kits, Belgium. The extent of plasma lipid peroxidation was determined based on the amount of malondialdehyde (MDA) formed according to the method of Buege and Aust (18), briefly described thus: 1.0 ml of the supernatant was added to 2 ml of tricarboxylic acid – thiobarbituric acid – hydrochloric acid (TCA-TBA-HCl) (1:1:1 ratio) reagent, boiled at 100^{o}C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against blank. MDA concentration was calculated using the molar extinction coefficient for MDA-TBA complex of $1.56 \times 10^{5}M^{-1}cm^{-1}$.

Catalase activity was determined according to the method of Sinha (19). The reaction mixture (1.5 ml) contained 0.01 M phosphate buffer, pH 7.0, tissue homogenate (0.1 ml) and 2M H_2O_2 (0.4 ml). The reaction was stopped by the addition of 2 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios), followed by heating in boiling water for 10 minutes, and then cooled at room temperature. The absorbance was read at 270 nm.

Preparation of bone marrow

The two femurs of the animals were also harvested and the bone marrow collected on microscopic slides for micronucleus assay following the method of Matter and Schmid (20). The slides were fixed in absolute methanol (BDH Chemical Ltd, Poole, England), air-dried, pre-treated with May-Grunwald solution (Sigma-Aldrich, procedure No GS-10) and air-dried. The dried slides were stained in 5% Giemsa solution and induced in 0.01M phosphate buffer, pH 6.8 for 30 seconds. Thereafter, they were rinsed in distilled water, air-dried and mounted. The slides were scored at ×400 magnification under light microscope for micronucleated polychromatic erythrocytes (mPCEs).

Histological analysis

Liver sections were fixed in an aqueous 10% formaldehyde solution and washed in 10 mmol/L phosphate buffer pH 7.4 at 4^{o}C for 12 h. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye, and finally observed at ×400 magnification under a light microscope.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) to test for significant differences among the groups of rats using Statistical Package for Social Sciences program version 17.0. Data were expressed as mean ± standard deviation. P values less than 0.05 were considered statistically significant.

RESULTS

Induction of micronucleated polychromatic erythrocytes (mPCEs) formation in the bone marrow cells by NB and/or Z. officinale extract treatments

The mean number of micronucleated polychromatic erythrocytes (mPCEs) scored in the normal control group was 0.17 ± 0.06 (Fig. 1). This value was statistically significant ($p < 0.05$) when compared with nitrobenzene only administered group (84.4% increase). Treatment with *Z. officinale* extract significantly lowered ($p < 0.05$) the mean number of mPCEs from 1.25 ± 0.04 to 0.65 ± 0.02, which is about 48% decrease. Also, there was no significant difference ($p > 0.05$) between the normal control group and the *Z. officinale* extract only fed group (Fig. 1).

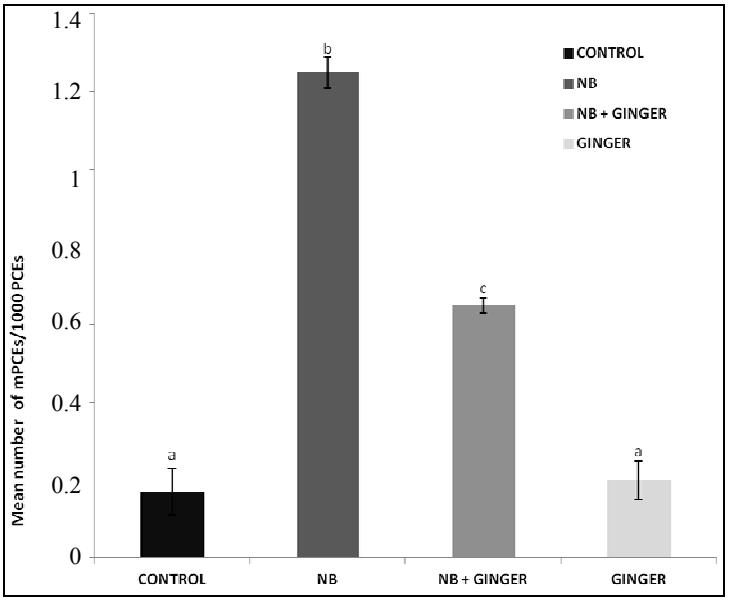

Fig. 1 – Mean number of micronucleated polychromatic erythrocytes (mPCEs) formed in the bone marrow cells. Values are expressed as mean ± standard deviation (SD); n = 5 in each group; a= significantly different from b and c; b = significantly different from a and c; c= significantly different from a and b.

Plasma malondialdehyde (MDA) levels as an index of the extent of lipid peroxidation in NB and/or *Z. officinale* extract treated rats

There was a significant ($p < 0.05$) increase (87%) in the mean MDA level of NB only administered group when compared with the normal control group (Fig. 2). This increase was significantly lowered ($p < 0.05$) by *Z. officinale* extract treatment to 0.50 ± 0.03 (79% decrease). There was no significant difference ($p > 0.05$) when the normal control group was compared with the ginger extract treated group (Fig. 2).

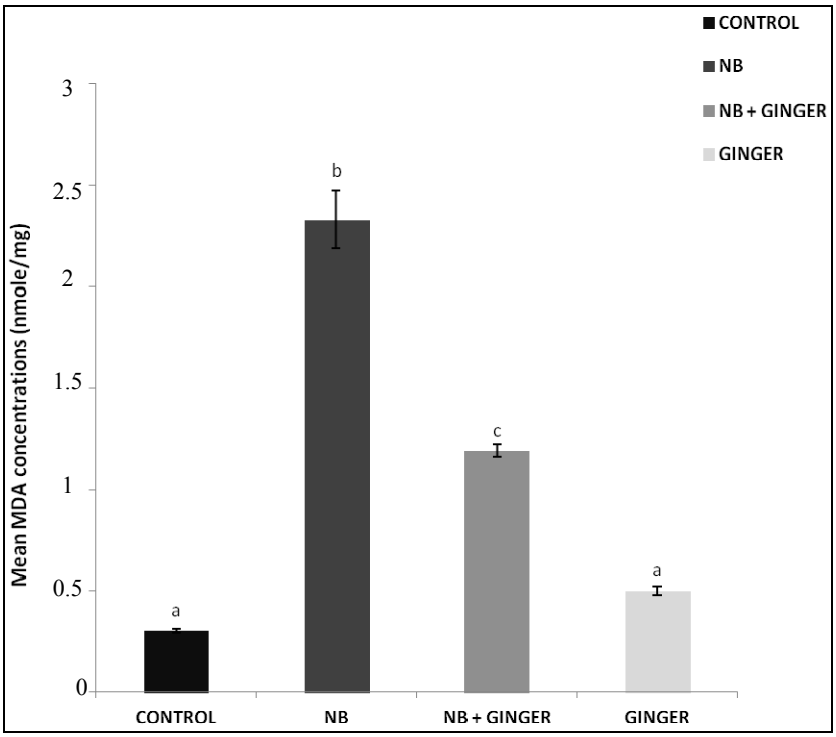

Fig. 2 – Malondialdehyde (MDA) concentrations in nitrobenzene and ginger treated rats. Values are expressed as mean ± standard deviation (SD); n=5 in each group; a= significantly different from b and c; b = significantly different from a and c; c= significantly different from a and b.

Plasma catalase (CAT) activity of NB and/or *Z. officinale* extract treated rats

Administration of NB to the rats significantly ($p < 0.05$) lowered the mean catalase activity (0.13 ± 0.05) when compared with the normal control group (0.28 ± 0.03). This decrease was about 54% (Fig. 3). Treatment of the NB toxicity by *Z. officinale* extract significantly increased ($p < 0.05$, 57% increase) the mean CAT activity to 0.30 ± 0.03.

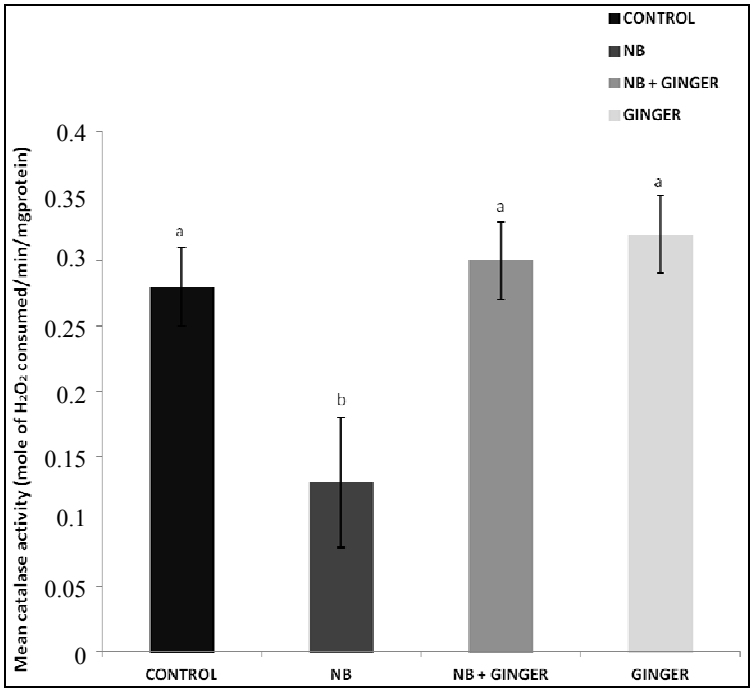

Fig. 3 – Mean catalase activity (expressed as specific activity) in nitrobenzene and ginger treated rats. Values are expressed as mean ± standard deviation (SD); n=5 in each group; a= significantly different from b and c; b = significantly different from a and c; c= significantly different from a and b.

Plasma activities of AST, ALT, ALP, GGT and total bilirubin levels of NB and/or *Z. officinale* extract treated rats

The mean plasma AST activity of NB only administered group (76.36 ± 3.82) increased significantly by 48% ($p < 0.05$) when compared with the normal control group (39.73 ± 2.04) (Table 1). However, there was no significant difference in the AST activities between control and the group with NB plus ginger extract.

Table 1

Effects of nitrobenzene and ginger treatment on liver function markers

Group	AST (U/l)	ALT (U/l)	ALP (U/l)	GGT (U/l)	Total bilirubin (mg/dl)
I (Control)	39.73 ± 2.04^{a}	4.67 ± 0.96^{a}	2.20 ± 0.08^{a}	3.57 ± 0.14^{a}	1.68 ± 0.20^{a}
II (NB only)	76.36 ± 3.82^{b}	9.63 ± 2.59^{b}	4.84 ± 0.23^{b}	7.93 ± 0.62^{b}	4.86 ± 0.19^{b}
III (NB + Ginger)	42.17 ± 1.66^{a}	5.25 ± 0.21^{a}	3.30 ± 0.16^{a}	4.59 ± 0.98^{a}	3.71 ± 0.13^{c}
IV (Ginger)	52.79 ± 2.73^{c}	6.27 ± 0.25^{a}	2.86 ± 0.15^{a}	2.86 ± 0.78^{a}	2.08 ± 0.17^{a}

Values are expressed as mean ± standard deviation (SD); n=5 in each group. Values with different superscripts (a, b, c) along the same column are significantly different ($p < 0.05$) – *i.e*, a= significantly different from b and c; b = significantly different from a and c; c= significantly different from a and b.

There was a significant ($p < 0.05$) increase in the mean activity of ALT of NB only group (9.63 ± 2.59) when compared with the other groups. However, no significant difference was observed among the control, NB plus ginger, and ginger alone group. A similar trend was seen in the ALP activities. There was a significant ($p < 0.05$) decrease in the mean activity of the enzyme by 32% in the group treated with *Z. officinale* extract after NB administration. This decrease is close to the value of control.

Mean GGT activity of the NB only group (7.93 ± 0.62) was also significantly ($p < 0.05$) elevated by 55% when compared with the normal control group (3.57 ± 0.14) (Table 1). Ginger extract treatment significantly ($p < 0.05$) lowered the observed increase by 42%.

Mean total bilirubin concentration of the NB only group (4.86 ± 0.19) was significantly ($p < 0.05$) higher than the mean concentration of the normal control group (1.68 ± 0.20) by 65% (Table 1). Treatment of the toxicity by ginger extract also significantly lowered the mean concentration of total bilirubin, but not close to the mean concentration obtained for the normal control group.

Plasma total cholesterol, triglyceride (TG), and total protein of NB and/or *Z. officinale* extract treated rats

There was a significant ($p < 0.05$) difference (34% increase) in the mean concentration of total cholesterol of the normal control group (30.30 ± 1.35) when compared with the toxicant only group (45.79 ± 1.67) (Table 2). This increase in mean concentration seen in the NB only group was significantly ($p < 0.05$) lowered by the *Z. officinale* extract treatment (40% decrease) to 27.61 ± 0.91 (Table 2). The decrease is comparable to the concentration obtained in the normal control group.

Table 2

Effects of nitrobenzene and ginger treatment on plasma total cholesterol, triglyceride, and total protein

Group	TC (mg/dl)	TG (mg/dl)	TP (mg/dl)
I (Control)	30.30 ± 1.35^{a}	75.21 ± 13.39^{ac}	6.74 ± 0.98^{a}
II (NB only)	45.79 ± 1.67^{b}	106.67 ± 26.82^{b}	3.30 ± 0.66^{b}
III (NB + Ginger)	27.61 ± 0.91^{a}	65.64 ± 11.31^{a}	6.69 ± 1.68^{a}
IV (Ginger)	33.27 ± 1.23^{a}	84.87 ± 10.06^{c}	7.22 ± 1.29^{a}

Values are expressed as mean ± standard deviation (SD); n=5 in each group. Values with different superscripts (a, b, c) along the same column are significantly different ($p < 0.05$) – *i.e.*, a= significantly different from b and c; b = significantly different from a and c; c= significantly different from a and b.

A similar trend was observed for TG, as there was a significant ($p < 0.05$) difference (29% increase) in the mean concentration of TG of the normal control group (75.21 ± 13.39) when compared with the NB only group (106.67 ± 26.82). Also, the increase in mean concentration seen in the toxicant only group was significantly ($p < 0.05$) lowered by the ginger extract treatment (38% decrease) to 65.64 ± 11.31 (Table 2).

The mean concentration of total protein in the normal control group was 6.74 ± 0.97 (Table 2). This mean concentration was significantly lowered ($p < 0.05$) by 51% when compared with the NB only group (3.30 ± 0.66). Treatment of the toxicity by *Z. officinale* extract was able to significantly ($p < 0.05$) raise the mean concentration also by 51% from 3.29 ± 0.66 to 6.69 ± 1.68 (Table 2).

Histopathological studies in liver of NB and/or *Z. officinale* extract treated rats

Results of histopathological examinations were presented in Fig. 4. Histopathological examination revealed that the normal control group showed no

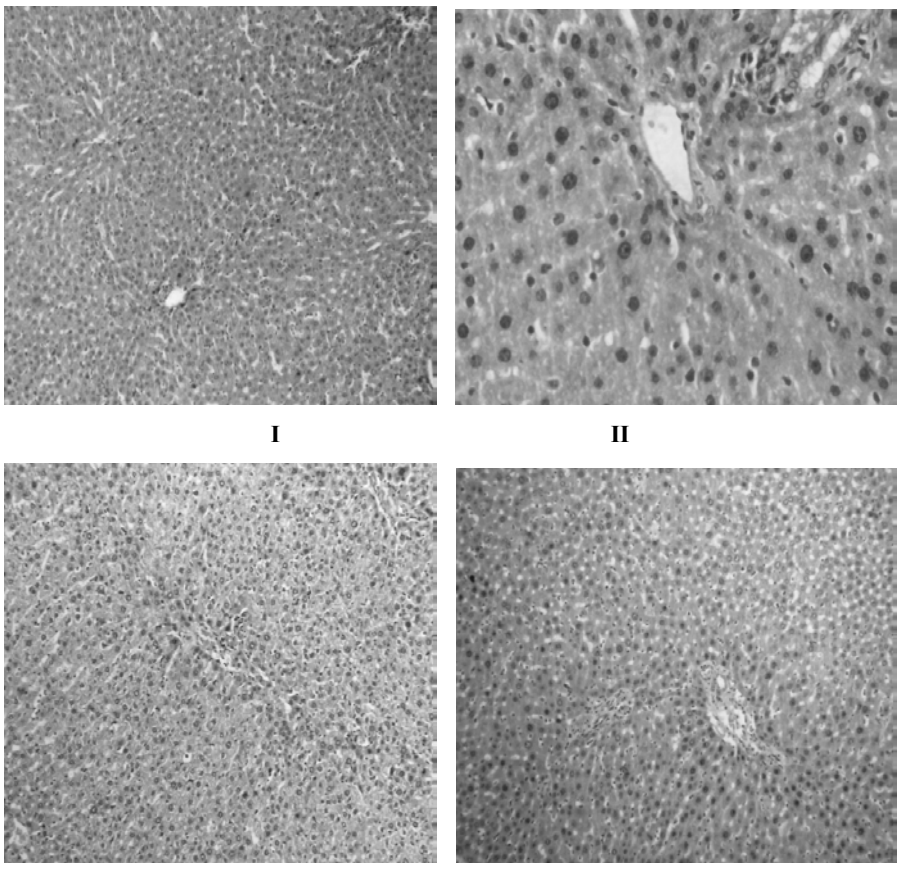

Fig. 4 – Selected photomicrographs of the liver sections (× 400) of nitrobenzene and ginger treated rats. There were no visible lesions in the liver of rats in the control group (Plate I), a diffuse vacuolar degeneration of the hepatocytes in the NB only administered group (Plate II), mild diffuse vacuolar degeneration of the hepatocytes in the NB plus *Z. officinale* extract treated group (Plate III), while a slight diffuse vacuolar degeneration of the hepatocytes was also observed in the *Z. officinale* extract only (Plate IV).

visible lesion (Plate I). There was a diffuse vacuolar degeneration of the hepatocytes in the NB only administered group (Plate II), mild diffuse vacuolar degeneration of the hepatocytes in the NB plus *Z. officinale* extract treated group (Plate III), while slight diffuse vacuolar degeneration of the hepatocytes was also observed in the group treated with *Z. officinale* extract only (Plate IV).

DISCUSSION

Ginger (*Zinginber officinale*) is one of the most popular spices and also one of the top five antioxidant foods, and reports have suggested that it shows considerable anti-inflammatory, antioxidant, anti-platelet, hypotensive and hypolipidemic effects (21).

The present study focused on the anti-clastogenic and hepatoprotective effects of *Z. officinale* against NB-induced toxicity in rats. Known as a predictive index for evaluating the carcinogenic potential of occupational and environmental chemical exposure, the bone marrow micronucleus assay was used to assess the clastogenicity of the NB (22, 23). Clastogens stimulate the formation of micronuclei by causing chromosomal breaks and interfering with spindle formation (19, 24). Our findings showed that NB significantly increased ($p < 0.05$) the mean number of mPCEs in the bone marrow cells of rats when compared with the normal control group, confirming the potential carcinogenic property of NB. Treatment with the extract of *Z. officinale* significantly ($p < 0.05$) lowered the scored mPCEs. It was therefore suggested that ginger was likely to be a good candidate for protection against cancer. The significant ($p < 0.05$) reduction in the micronuclei counts by *Z. officinale* could be adduced to its anti-tumorigenic and anti-oxidant properties, since such properties have been reported to be responsible for conferring some levels of protection against certain types of cancer (25, 26).

Reactive oxygen species are continuously formed in the body, which cause cell damage. It is therefore necessary for tissues to be protected against this oxidative injury through intracellular and extracellular antioxidants (27). An antioxidant is a substance that delays or inhibits oxidative damage to target molecules (28, 29). The result of the oxidative stress and antioxidant enzyme markers in this work revealed a significant increase in the mean levels of malondialdhyde (MDA), an index of lipid peroxidation in the NB only group. Treatment with the *Z. officinale* extract significantly ($p < 0.05$) decreased the mean MDA levels. This is similar to the report of Ahmad *et al*. (30) that ginger extract successfully lowered the lipid peroxidation induced by ethionine, a carcinogen in rats. Also, Chang *et al*. (13) reported that gingerol, a bioactive component of ginger, possessed an antioxidative effect by inhibiting phospholipid peroxidation induced by xanthine oxidase activity. However, catalase activity in NB-treated rats was significantly decreased ($p < 0.05$) when compared with the control, but the

enzyme activity was significantly improved ($p < 0.05$) by the ginger extract intervention. This probably suggested that ginger induced the activity of this enzyme, such that the rats would be able to scavenge free radicals being generated *via* NB activity.

AST, ALT, ALP and GGT activities are used in the diagnosis of hepatic injuries and diseases. The activities of these enzymes are known to increase in blood as a result of hepatic damage or injury (31). The observed significant increase in the activities of ALT, AST and ALP in the group of rats with NB alone is not surprising and agrees very well with the report of Rajalakshmy *et al*. (32). However, *Z. officinale* administration was able to decrease the activities of these enzymes, thereby suggesting possible hepatoprotection against NB toxicity. Kazeem *et al*. (33) have also reported a hepatoprotective effect of ginger against carbon tetrachloride in rats.

High levels of cholesterol, triglycerides, LDL-C, VLDL-C have been associated with heart disease, insulin resistance and diabetic mellitus (34). In this study, there was a significant increase ($p < 0.05$) in the mean concentrations of total cholesterol and triglyceride, but a significant decrease in the mean concentration of total protein in NB intoxicated rats, when compared with the normal control. The ginger extract was able to significantly restore the mean concentrations to close normalcy. This is in agreement with the report that ginger acts as a hypolipidemic agent in cholesterol-fed rabbits (34, 11). Also reported was that ginger stimulated the conversion of cholesterol to bile acids by significantly increasing the activity of hepatic cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acids biosynthesis resulting in the elimination of cholesterol from the body (35), thereby causing decreased serum cholesterol and triglycerides in the body (36). Histopathological studies showed no visible lesion in the normal control rats, diffuse vacuolar degeneration of the hepatocytes in the NB only administered group, mild diffuse vacuolar degeneration of the hepatocytes in the NB plus *Z. officinale* extract treated group, suggesting potential hepatoprotection, while a slight diffuse vacuolar degeneration of the hepatocytes was observed in the *Z. officinale* extract only group too.

CONCLUSION

In conclusion, the ethanolic extract of *Z. officinale* showed good anticlastogenic, hepatoprotective and hypolipidemic activities in NB-induced toxicity. Thus, consumption of *Z. officinale* should be encouraged. However, further work is in progress aiming to explore the effect of this plant extract on other enzymatic and non-enzymatic antioxidants, such as superoxide dismutase and glutathione peroxidase activities as well as reduces glutathione and vitamin C levels.

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