Modulatory effect of *Zingiber offinale* on chemical-induced gastric mucosal damage and hepatotoxicity in rats

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ABSTRACT

Evaluation of the hepatoprotective and anti-ulcerogenic properties of methanol extract of *Zingiber officinale* (MEZO) in rats was done. Twenty five adult male rats divided into five groups of five rats each were used for each of the studies. Groups, 2, 3 and 4 received varied dose levels of the extract (100, 200 and 400mg/kg), while groups 1 and 5 received normal saline and CCl_4 (5 ml/kg)/ranitidine (100 mg/kg) respectively. Gastric ulceration was induced in the rats by the administration of indomethacin 50 mg/kg and aspirin 200 mg/kg thirty minutes after extract treatments, and the animals sacrificed 8 h later. Hepatotoxicity was induced by a single dose administration of CCl_4 (5 ml/kg) after pre-treatment with MEZO for 8 days. Significant ulcer inhibition was produced in all the groups treated with MEZO. There was significant decreases ($p < 0.05$) in the CCl_4-induced elevations of liver enzymes, low density lipoprotein, total cholesterol and lipid peroxidation products in the serum of extract-treated rats when compared with the group treated with only CCl_4. This study showed that methanol extract of *Zingiber officinale* (MEZO) possesses potent anti-ulcerogenic and hepatoprotective properties and can be used as herbal remedy for the treatment of gatro-intestinal ulcers and liver damage.

Keywords: Carbontetrachloride, *Zingiber officinale*, liver enzymes, gastric ulcer, indomethacin, rats.

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INTRODUCTION

Ginger is a slender perennial plant that reaches a height of 2 feet and has greenish-yellow flowers resembling orchids. The underground rhizomes are the medicinally and culinary useful part of the plant. The rhizomes are irregular in shape and size with multiple lobes that are pale yellow and aromatic. Ginger has been thought to have originated in the tropical jungles of Asia and was among the first vegetative cultivated plants. It has been grown in India for thousands of years and today is widely cultivated in many tropical countries around the world [1]. Ginger (Zingiber officinale) has many reported pharmacological and antioxidant properties [2, 3]. Gingerols (the active phytonutrients in ginger) kills ovarian cancer cells by inducing apoptosis (programmed cell death) and autophagocytosis (self-digestion) [4]. Reports have shown that gingerols have the ability to inhibit the formation of pro- inflammatory compounds and exhibit direct anti -inflammatory effects. This explains why patients with osteoarthritis or rheumatoid arthritis experience reductions in their pain levels and immobility when they consume ginger regularly [5]. Other reported health benefits of ginger include alleviation of motion sickness, dizziness, nausea, vomiting during pregnancy and gastrointestinal disorders [6, 7]. Previous study reported the anti inflammatory effect of ginger on egg albumin-induced oedema in rats [8]. This study sought to evaluate the protective effect of ginger on experimentally induced gastric mucosal damage and CCl_4- induced hepatotoxicity in Wistar albino rats.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grade. They were products of Sigma Aldrich, Germany.

Plant material

The fresh ginger rhizomes (Zingiber offinale) were purchased from Nsukka local market and were identified by Mr. Ugwuozor, a taxonomist of Botany Department, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium unit of the Department of Botany, University of Nigeria, Nsukka. The ginger rhizomes were chopped into tiny bits, air-dried for two weeks and milled with a mechanical grinder. The ground plant (600 g) was macerated in methanol for 24 h, filtered with a white cloth and the filtrate concentrated using a rotary evaporator (IKA, Germany) at an optimum temperature of 40°-50^oC. The yield of the dried extract was 25.4 g.

Animals

Swiss albino mice (22-28 g) and adult Wistar rats (120-200 g) obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka were used. They were housed in metal steel cages and acclimatized in the laboratory for seven days before the

experiments. They were given free access to water and fed with growers mash (Niger Feeds, Nigeria) bought from the local market. The research was conducted in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985).

Acute toxicity study

The acute toxicity test of the plant extract was carried out by the method of Lorke [9] to define the range of lethal dose and safe dose for the extract. Eighteen Swiss albino mice starved of food for 18 h but allowed access to water were used for the study. There were grouped into six groups (three mice per group) and treated intraperitoneally (i.p.) with the plant extract at varied dose levels (10, 100, 1000, 1600, 2900 and 5000 mg/kg). The animals were then observed for nervousness, dullness, in-coordination and or mortality for 24 h.

Anti-ulcer activity

Two experimental models of inducing experimental gastric ulcers were used to assess the anti-ulcer activity of Zingiber officinale. Twenty five adult male rats randomly divided into five groups of five rats each were used for each model. They were deprived of food for 18 hr (but allowed free access to water) and treated orally with normal saline and varying doses of MEZO. The extract and drugs used were freshly prepared as a suspension in normal saline and administered per oral (p. o.) to the animals in 5 ml/kg doses. Group 1 (control group) was administered per oral with normal saline (5 ml/kg). Groups 2, 3 and 4 received the plant extract at varied dose levels: 100, 200 and 400 mg/kg MEZO respectively. Group 5 (reference group) received the standard drug treatment of 100 mg/kg of ranitidine (Zantac®).

Indomethacin-induced ulcer

This assay was carried out using the method of Urushidani et al. [10]. The animals were deprived of food for 18 hr and treated orally with normal saline and varying doses of the plant extract. The extracts and drugs used were administered orally (p.o) to the animals as stated above. Thirty minutes later, 50 mg/kg of indomethacin was administered (p.o) to the rats. After 8 h, each animal in the groups was sacrificed by chloroform anaesthesia and the stomach removed and opened along the greater curvature, rinsed with copious volume of normal saline and pinned flat on a board. Erosions formed on the glandular portions of the stomach were counted and the ulcer index calculated as described in a study by Aguwa and Ukwe [11]. The ulcer was counted and scored as 0 = no ulcer; 1 = superficial ulcer; 2 = deep ulcer and 3 = perforations. The sum of all the lesions/ulcers in all the animals for each group (total ulcer score) was used to calculate the ulcer index. The percentage ulcer inhibition was calculated relative to control as follows:

$$\% \text{ ulcer inhibition (\% U.I)} = \left(1 - \frac{Ut}{Uc}\right) \times 100$$

Where Ut and Uc represents the ulcer index of the treated and control groups respectively.

Aspirin induced ulcer

Ulcer was induced according to the method of Willamson et al. [12] and also used in an earlier study [13]. The extracts, drug and vehicle were administered orally as stated above and one hour later, aqueous suspension of aspirin (generic, Nigeria) was given orally to the rats at a dose of 200 mg/kg. After 4 h, the animals were killed and the stomach removed and opened along the greater curvature. The stomach was rinsed in water, pinned flat on a board, examined with a hand lens (x10) and scored for ulcer. Erosions formed on the glandular portions of the stomach were counted and the ulcer index calculated as above.

Hepatotoxicity study

Hepatoprotective role of MEZO was evaluated in the serum of rats administered with and without carbontetrachloride (CCl_4). Twenty five adult rats divided into five groups of five rats each were used. Normal saline and olive oil served as the vehicle for the extract and CCl_4 respectively. The experiment was carried out for 10 days. Group 1 (vehicle control) received normal saline (p.o) for ten days and were administered with olive oil (i.p) on the 8th day. Groups 2, 3 and 4 were pretreated orally with varied doses of MEZO (100, 200 and 400 mg/kg) for 8 days and then challenged with CCl_4 (5 ml/kg). The administration of the extract continued for two more days. Group 5 (CCl_4 group) received normal saline for 10 days and was challenged with CCl_4 on the 8th day. All the animals were sacrificed on the 10th day.

Preparation of serum

Blood was collected from the rats by carotid bleeding into centrifuge tubes. The blood samples were centrifuged and the clear serum supernatant was used freshly for the assessment of some biochemical and liver function tests.

Assessment of liver marker enzymes and biochemical parameters

Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were assayed by the Reitman-Frankel [14] colorimetric method using Quimica Clinica Applicada (QCA) test kits. Alkaline phosphatase (ALP) was measured by the method of Klein et al., [15] and total bilirubin was by the Jendrassik – Grof method [16] using Quimica Clinica Applicada (QCA) test kits. Serum cholesterol was measured by the method of Allain et al., [17] and low density lipoprotein (LDL) was by the method of Assmann et al. [18]. Lipid peroxidation was assayed as thiobarbituric acid reacting substances (TBARS) [19] while Vitamin C (ascorbic acid) was measured by the method of Goodhart and Shils, [20].

Statistical analysis

Data obtained were analyzed by one-way ANOVA using SPSS version 15.0 (SPSS Inc. Chicago, IL. USA). All values were expressed as mean ± SD. Difference between means was assessed by Duncan's new multiple range. $P<0.05$ was considered statistically significant between the groups.

RESULTS

Acute toxicity test

In the acute toxicity (LD50) test for the plant, no mortality or any significant change in the behaviour of the animals was recorded up to the dose of 5000mg/kg of the extract.

Effect of methanol extract of Zingiber officinale on indomethacin induced ulcer

Indomethacin induced gastric ulcer in nearly (23 of 25) all the rats in the groups. Ulcer produced in this model was seen as large black sores (Fig 1a). Treatment with the methanol extract of Zingiber officinale (MEZO) significantly reduced the ulcer lesions as seen with the reduced black spots on the gastric mucosa of the treated rats (Fig 1b). There was significantly reduced ulcer lesion indices (ULI) of 1.16 ± 0.36, 2.1 ± 0.25 and 1.9 ± 0.13 for the 100, 200 and 400 mg/kg b.w. respectively, as compared to the 4.08 ± 0.61 ulcer index obtained for the control. The percentage ulcer inhibitions obtained for the extract treated groups was non-dose dependent and lower than that obtained for the reference drug, ranitidine (Table 1).

Table 1: Effect of methanol extract of Zingiber officinale (MEZO) on indomethacin induced gastric ulcer in rats

Treatment	Dose (mg/kg)	Mean ulcer index	(%)UI
Normal saline	1 mL/kg	4.08 ± 0.61	-
MEZO	100	1.16 ± 0.36***	71.56
	200	2.1 ± 0.25**	48.53
MEZO	400	1.9 ± 0.13 **	53.4
Ranitidine	100	0.88 ± 0.12***	78.4

Values shown are mean ± SD (n=5). Level of Significance ***= $p<0.001$, ** = $p<0.01$, * = $p<0.05$

Effect of the methanol extract of Zingiber officinale (MEZO) on aspirin induced gastric ulcer in rats

Aspirin produced ulcer seen as dark red sores in all the animals used in this study (Fig. 2a). Treatment with the methanol extract of Zingiber officinale produced a reduction in the dark spots (Fig 2b) and a significant non-dose-dependent reduction ($p<0.001$) in the ulcer lesion indices of all the treated groups when compared to the control (Table 2). The ulcer reductions of 64, 51 and 69 % obtained for 100, 200 and 400 mg/kg b.w. of the extract respectively, were not as high as that obtained for ranitidine (76 %).

Table 2: Effect of the methanol extract of Zingiber officinale (MEZO) on aspirin induced gastric ulcer in rats

Treatment	Dose (mg/kg)	Mean index ± SEM	(%)UI
Normal saline	1mL/kg	2.70 ± 0.35 A	-
MEZO	100	0.95 ± 0.21*** A	64.8
MEZO	200	1.32 ± 0.31*** A	51.11
MEZO	400	0.82 ± 0.19*** A	69.62
Ranitidine	100	0.64 ± 0.17*** A	76.30

Level of Significance ***= $p<0.001$, ** = $p<0.01$, * = $p<0.05$

Fig 1a: Black ulcer sores induced by indomethacin (Control group) Fig 1b: Reduced ulcer sores (400mg/kg MEZO)

Fig 2a: Reddish ulcerative sores on the gastric epithelial walls induced by aspirin (Control group)

Fig 2b: Reduced ulcerative sores on the gastric epithelial walls (100mg/kg MEZO)

Effect of the methanol extract of Zingiber officinale (MEZO) on liver marker enzymes

Table 3 show that of CCl_4 administration significantly increased ($p<0.01$) the serum alanine amino transferase activities of rats when compared to that of the control rats that received only normal saline (23.83 ± 2.75). Treatment of the animals with methanol extract of Zingiber officinale (MEZO) produced significant non-dose dependent decreases ($p<0.05$) in the serum alanine amino transferase (ALT) activities, with values of 37.00 ± 3.35, 37.33 ± 3.73 and 34.83 ± 3.37 for the 100, 200 and 400 mg/kg b.w of the extract respectively, when compared

with that of the rats treated with only CCl_4 (52.00 ± 4.00). Serum AST activities of the CCl_4 treated rats (54.33 ± 4.23) were significantly increased ($p<0.01$) when compared to that of the control rats that received only normal saline (25.67 ± 3.62). The AST activities of all the groups treated with MEZO (100, 200 and 400mg/kg) after CCl_4 administration showed significant dose-dependent reductions (39.67 ± 4.32, 37.67 ± 2.89 and 28.67 ± 2.27 respectively) when compared to that of the CCl_4 treated group (54.33 ± 4.23). Compared to control group, CCl_4 treated group showed significant increases ($p<0.05$) in their serum alkaline phosphatase (ALP) activities (106.66 ± 1.06). Treatment with MEZO after CCl_4 administration produced a significant decrease ($p<0.05$) in the serum alkaline phosphatase (ALP) activities, with values of 87.63 ± 4.10, 93.69 ± 1.64 and 90.0 ± 4.81 for the 100, 200 and 400 mg/kg b.w of the extract respectively, when compared with the CCl_4 treated group (106.66 ± 1.06).

Effect of the methanol extract of Zingiber officinale on some biochemical parameters

Table 4 show that serum conjugated and total bilirubin concentrations of the CCl_4 treated group were significantly higher ($p<0.05$) than that of the control that received only normal saline. Treatment of the animals with both CCl_4 and different doses of the MEZO produced significant non-dose dependent decreases ($p<0.05$) in their conjugated and total bilirubin concentrations when compared to that of the CCl_4 treated group.

The concentration of vitamin C in the serum of rats treated with CCl_4 were decreased (0.77 ± 0.06) when compared with that of control (1.12 ± 0.03), Treatment of the animals with MEZO (100, 200 and 400 mg/kg b.w) after CCl_4 administration produced a dose dependent significant increase ($p<0.05$) in vitamin C concentration with values of 1.20 ± 0.07, 1.23 ± 0.08 and 1.30 ± 0.1 respectively, when compared with that of the CCl_4 treated group.

There was significant increase ($p<0.05$) in total cholesterol, low density lipoprotein (LDL) and MDA concentrations of the CCl_4 treated group when compared with that of the control. Treatment of the groups with MEZO significantly decreased the total cholesterol, low density lipoprotein (LDL) and MDA concentrations when compared to that of the CCl_4 treated group (table 4).

Table 3: Effect of the methanol extract of Zingiber officinale liver marker enzymes

Treatment groups / Parameters	Control	CCl_4 + 100 mg/kg	CCl_4 + 200 mg/kg	CCl_4 + 400 mg/kg	CCl4
Alanine amino transferase (U/L)	23.83 ± 2.75 a	37.00 ± 3.35 b	37.33 ± 3.73 b	34.83 ± 3.37 b	52.00 ± 4.00 c
Aspartate amino transferase (U/L)	25.67 ±3.62 a	39.67 ± 4.32 b	37.67 ± 2.89 b	28.67 ± 2.27 a, b	54.33 ± 4.23 c
Alkaline phosphatase (U/L)	87.63 ± 4.10 a	87.63 ± 4.10 a	93.69 ± 1.64 a	90.00 ± 4.81 a	106.66 ± 1.06 b

Means with different lower case letters (a, b, c) across the row, between groups i.e. between control and test groups are significantly different ($p<0.05$).

Table 4: Effect of the methanol extract of Zingiber officinale (MEZO) on some biochemical parameters

Treatment groups Parameters	Control	CCl_4 + 100 mg/kg	CCl_4 + 200 mg/kg	CCl_4 + 400 mg/kg	CCl_4
Conjugated bilirubin (mg/dl)	0.11 ± 0.00 a	0.18 ± 0.02 a, b	0.15 ± 0.02 a	0.18 ± 0.03 a, b	0.29 ± 0.04 b
Total bilirubin (mg/dl)	0.24 ± 0.05 a	0.38 ± 0.08 a	0.34 ± 0.08 a	0.38 ± 0.06 a	0.67 ± 0.07 b
Vitamin C (mg/100ml)	1.12 ± 0.03 b	1.20 ± 0.07 b	1.23 ± 0.08 b	1.30 ± 0.1 b	0.77 ± 0.06 a
Total cholesterol (mg/dl)	137.78 ± 3.85 a	151.11 ± 10.18 b, c	157.78 ± 3.85 c	144.22 ± 3.67 a, b	179.67 ± 5.86 d
Low Density Lipoprotein (mg/dl)	55.37 ± 8.77 a	67.72 ± 4.37 a	66.90 ± 1.88 a	66.49 ± 2.13 a	94.28 ± 4.17 b
Malondialdehyde conc (µg/dl)	0.40 ± 0.02 a	0.49 ± 0.08 a	0.45 ± 0.08 a	0.50 ± 0.16 a	0.89 ± 0.03 b

Means with different lower case letters (a, b, c) across the row, between groups i.e. between control and test groups are significantly different ($p < 0.05$).

DISCUSSION

The methanol extracts of ginger (Zingiber officinale) exhibited strong anti-ulcerogenic effect against both indomethacin and aspirin induced gastric ulcers with percentage ulcer inhibition that were slightly lower than that obtained for ranitidine, the reference anti-ulcer drug used. Prolonged use of aspirin and other non-steroidal anti-inflammatory drugs are associated with gastrointestinal bleeding and ulcer. The ulcer formation can occur either by direct mucosal injury which involves the breaking of the mucosal barrier and exposure of the underlying tissue to the corrosive action of excess acid and pepsin or by a decrease in endogenous gastric prostaglandin production and release through COX-1 and COX- 2 inhibition [21]. These naturally occurring prostaglandins are important for the production of gastric bicarbonate and mucous which are the key components of the stomach protective barrier and in the maintenance of submucosal blood flow. Previous reports on ginger showed that it exerts its anti-ulcerogenic activity through a mechanism of acid and pepsin inhibition, augumentaion of mucin secretion and decrease in cell shedding [7, 22].

Most non-steroidal anti-inflammatory drugs including indomethacin and aspirin, used in the control of inflammatory pain and disorders are known to cause gastric erosions and abdominal ulcers after prolonged use [23]. Reduction of the indomethacin and aspirin induced ulcers shown by MEZO could also be attributed to the high flavonoid content of ginger plant. Phytochemical analysis of ginger showed that the plant contain flavonoids in abundance [8], this could be the active constituent exerting the anti-ulcerogenic effect. Halliwell et al. [24] proposed that the antioxidant and other protective effects of plant flavonoids could occur before absorption, within the gastrointestinal tract and could account for the ability of flavonoid-rich foods to protect against gastric and colonic ulcers.

Carbon tetracloride (CCl_4) challenge caused a marked rise in the serum levels of the liver enzymes; alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP), of the rats used in this study, demonstrating severe hepatic damage. It also caused elevated levels of total and conjugated bilirubin, serum low density lipoprotein (LDL), total cholesterol and lipid peroxidation products (MDA), and decreased levels of vitamin C, demonstrating oxidative stress and reduced antioxidant levels in the CCl_4 treated groups. Alanine amino transferase (ALT) and aspartate amino transferase (AST) are important in the diagnosis of heart and liver diseases. Damage to the liver typically results in a leakage of ALT and AST into the blood stream. Elevations of the plasma levels of both enzymes are indicative of inflammation or injury to the liver cells. Drug induced liver damage has been reported to correlate with an increase in the activity of these enzymes [25, 26]. In this work, treatment of the animals with methanol extract of Zingiber officinale (MEZO) decreased the CCl_4 induced elevated levels of the liver enzymes, total and conjugated bilirubin in the serum, suggesting that ginger possesses anti-hepatotoxic and liver protective activities. Bilirubin, a major breakdown product of haemoglobin rises when there is liver injury or damage leading to the discolouration of the skin known as jaundice. Reduction of the CCl_4 induced elevations of total and conjugated bilirubin by MEZO further shows its protective effect against CCl_4 induced liver toxicity. Ginger perhaps, protects the liver by enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts. MEZO also attenuated the CCl_4- induced elevated levels of low density lipoprotein, total cholesterol and lipid peroxidation products (MDA) and ameliorated the induced depletion of vitamin C. CCl_4 induced liver inflammation and damage can result in locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators. Studies have shown that certain natural extracts containing antioxidants protect against the CCl_4 induced inflammation and impairment in hepatic function [27, 28] and that the efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects of a hepatotoxin or of maintaining the normal physiological mechanism that are unbalanced by a hepatotoxin [29].

The protective effect of ginger extracts on CCl_4 induced liver damage can be correlated to its direct antioxidant effect. In normal tissues, there is a balance between the production of reactive oxygen metabolites (ROMS) and their scavenging by cellular antioxidants. Oxidative stress occurs when the rate of free radical production exceeds its rate of removal by antioxidants or when the rate of depletion of cellular antioxidants exceeds its rate of replacement [30]. Antioxidants in plants scavenge these free radicals and thus reduce oxidative damage. Ginger is a source of a large number of antioxidants that reduce lipid oxidation by enhancing the activities of internally produced antioxidants. It contains active constituents such as zingerone which function as an effective scavenger of free radical ions [31].

The reduction in MDA concentration and increase in vitamin C concentration in the ginger extract treated groups observed in this study could also be attributed to the antioxidant and free radical scavenging properties of the plant constituents. MDA is a metabolic product of lipid peroxidation, the level of which is increased in oxidative stress. Therefore, the reduction of CCl_4 induced oxidative stress by anti-lipid peroxidative activity might be a mechanism of anti-oxidative action of ginger. The ability of MEZO to mitigate the CCl_4 induced depletion of vitamin

C concentration suggests that some of the constituents of ginger have the capacity to boost antioxidant status and immune system levels in the body. Report by Ray [32] showed that ginger increased glutathione level and reduced lipid peroxidation by scavenging free radicals in rat blood and maintaining the activities of the antioxidant enzymes- SOD, catalase and glutathione peroxidase in the rats.

In this study, MEZO attenuated the CCl_4- induced elevated levels of low density lipoprotein and total cholesterol. Tanabe et al. [33] reported that ginger lowered plasma cholesterol in experimentally induced hypercholesterolemia in mice. Reduction of low density lipoprotein concentration by the extract decreases the risk of DNA oxidative damage through lipid peroxidation. LDL oxidation causes accumulation of fat within the artery walls, thereby clogging up the arteries and increasing the risk of atherosclerosis and cardiovascular diseases [34]. Balanced cholesterol level reduces the incidence of LDL oxidation and the associated risk of atherosclerosis and other related heart diseases.

This investigation supports the report of other studies on the anti-oxidative activity of ginger and suggests that it also possesses and anti-ulcerogenic and anti-hepatotoxic properties.

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