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Toxicological Assessment of *Zingiber officinale* Roscoe (Ginger) Root Oil Extracts in Albino rats

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Abstract

Zingiber officinale Roscoe root is a medicinal plant popularly used to relieve symptoms of nausea/vomiting associated with motion sickness, surgery and pregnancy. Study was to screen the continuous use of the root oil extracts of ginger for any undesirable outcome in rodents. Acute toxicity was conducted in mice using OECD425 procedure. 50 male Wistar rats were randomly divided into five groups (10/group) and orally-treated daily, thus: Group I–Control (corn oil-0.5 mL/kg), Groups II-V received ZOF and ZOE (0.02, 0.002, 0.4 and 0.04) mL/kg body weight, respectively. Animals (6/group) were euthanized under diethyl ester and sacrificed. Blood samples were collected by cardiac-puncture for biochemical analysis. Vital organs were eviscerated, blotted/weighed and fixed in 10% formalin for histopathological assessment. Other animals (4/group) were retained for reversibility studies. Results presented significant(P<0.05) increased kidneys, lungs, liver and spleen weights at low doses of ZOF respectively. Increased ALT, decreased ALP and AST at low doses of ZOF and ZOE were respectively recorded. Malondialdehyde increased at low dose of ZOF. Histopathology of liver and spleen showed forms of pathology. Besides MDA, test effects were reversed on extract discontinuation. Results indicate that long-term use of ZOF may induce some undesirable effects like oxidative stress amongst others. Findings suggest caution on chronic use of ginger oils.

Keywords: *Ginger oils, histopathology, essential oils, oxidative stress, reversibility studies.*

Introduction

For centuries before the emergence of conventional medicine, various cultures around the world via traditional herbalism used medicinal plants as disease remedies. Mainly in developing countries, herbal medicine is still the mainstay of their primary healthcare. The use of herbs to treats disease is almost universal especially among non – industrialized societies, necessitating an estimate that about 80% of the World's population presently uses herbal medicine for some if not all aspects of their primary healthcare (WHO, 1997).

Zingiber officinale Roscoe (ginger; family: Zingerberaceae) is a tropical flowering plant, whose rhizome is widely used as a spice and a folk medicine. It is rich in oils that both kill micro-critters as well stimulate the immune system to do same. Ginger rhizome is claimed to be effective in the management of many ailments such as arthritis, colic diarrhoea, heat condition, common cold, flu-like symptoms, headache as well as dysmenorrhea (Altman and Marcussen, 2001). One of the most popular uses of ginger is to relief the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Gilani and Rahman, 2005). Also, it has been reported that ginger aids digestion, promote absorption, relieves constipation and flatulence in the GIT by increasing the activity of the smooth muscle (Eru *et al.*, 2014). Ginger extracts possesses a broad pharmacological spectrum including anti-inflammatory potentials (Young *et al*., 2005), antioxidant properties (Stoilova *et al*., 2007) and antinephrotoxic effects (Sakr *et al*., 2011). An earlier study suggests that ginger has antitumor effects on colon cancer cells by suppressing its growth, striking the Go/G1 phases, reducing DNA synthesis and inducing apoptosis (Abdullah *et al*., 2010).

Traditonal medicines are employed in the management of numerous disorders, underscoring the use of plant products and derivatives in the amelioration of common ailments. This is primarily due to the general belief or claim thereof that plant-based medicines or herbal drugs are completely safe without side effects, besides being cheap and easily accessible (Gupta and Raina, 1998). Therefore, it is imperative that the toxcity profile of such preparations be established, thus helping the public to make informed decisions as regard their use. The present day study was to screen the subchronic administration of essential and fixed oil extracts of Zingiber officinale roots for any undesirable outcome in rodents.

Materials and Methods

Collection and Identification of Plant Material

The plant material with flowers was collected from the Faculty of Pharmacy Medicinal Plants Reserve, University of Uyo, Nigeria for botanical identification and voucher specimen referencing. It was identified and authenticated as *Zingiber officinale* by Prof (Mrs) Margret Bassey, a taxonomist in the Department Botany and Ecological studies, University of Uyo, Nigeria. Thereafter, the sample was conserved at the Faculty of Pharmacy Herbarium under the reference number UUPH 80©.

Extraction of Ginger oils

Fixed oil: About 4023.0 g of fresh ginger root was weighed, reduced to a paste using a laboratory mortar and macerated in n – hexane (Sigma, USA) for 72 h. It was vigorously shaken for 15 min prior to its filtration using Whatman filter paper. The vehicle (n – hexane) was evaporated off the filtrate by the use of a rotary evaporator until a dark – brown oily extract was obtained. The extract was allowed to cool and stored in a tight cap fitted container at 4 ^{0}C.

Essential oil: This was done using the steam hydro-distillation procedure. About 1000 g of fresh ginger root was grounded using an electric blender. The sample was placed in 1 L conical flask and connected to the Clevenger apparatus. Distilled water (500 mL) was added to the flask and heated to boiling point. The steam in combination with the essential oils was distilled into a graduated cylinder for 5 h and then separated from aqueous layer. The essential oil extracted was kept in a refrigerator until required for further analysis (Singh *et al*., 2008).

Experimental Animals

The animals used for the experiments were adult Swiss albino mice (23-26 g) and 50 adult Wistar rats (150-170 g), which were obtained from, and kept at the Department of Pharmacology & Toxicology Animal House of the University of Uyo, Nigeria. The animals were maintained under standard environmental conditions, given standard rodent feed and water *ad libitum*. The animals were acclimatized in the laboratory condition for 14 days prior to the experiment, during which they were given free access to food and water *ad libitum*. The care and use of animals were conducted in accordance with the National Institute of Health guide for the care and use of laboratory animals (NIH, 1996). Also, ethical approval for use of animals in research was obtained from the Experimental Ethics Committee on Animal Use of the Faculty of Pharmacy,

College of Health Sciences, University of Uyo, Nigeria.

Acute Toxicity Test

For this study, the acute toxicity test was determined using Up-and-Down testing approach (OECD 425). In line with this test guideline, the animals were dosed one at a time with *Z. officinale* oils after which cardinal signs of toxicity such as writhing, accelerated or decreased respiration, decrease motor function/suppressed movement, convulsion or paralysis were observed within 24 h as well as a 14-day further supervision for occurrence of toxic symptoms and mortality (OECD, 2001). The most widely accepted endpoint as measure of acute toxicity was mortality (death) within 24 h post-administration of ginger oils. Six (6) animals were treated with varying doses (0.02, 0.04, 0.06, 0.08 and 0.1 mL/kg body weight) of ginger fixed oil. While, 10 animals were respectively given 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4, 6, 8 and 10 mL/kg body weight of the essential or volatile oil. Thereafter, the dose at which mortality occurred for both oils was used to calculate the safe doses that were used for the study.

Study Design

After acclimatization, 50 adult male Wistar rats were randomly allotted to five groups (10 per group). These groups were respectively treated, daily with 0.5 mL/kg body weight of corn oil (group I-control), 0.02 and 0.002 mL/kg body weight of fixed oil (groups II and III), then 0.4 and 0.04 mL/kg body weight of essential oil (groups IV and V). The experimental animals were observed closely for behavioural changes, feeding habits, body weight and general morphological changes. After 60 days, the animals (6 per group) were euthanized under diethyl ether anaesthesia and sacrificed. Vital organs were eviscerated for internal macroscopic and histomorphological investigations. Blood samples were collected through cardiac puncture into different sample bottles for haematological and biochemical investigations. While the remaining animals (4 per group), were kept for reversibility of study effects.

Vital Organs Measurement

At the end of the study, qualitative data on the weights of vital organs (heart, lungs, liver, kidneys, and spleen) were assessed by a careful dissection of each organ from the euthanized animals. Isolated organs were blotted dry and weighed fresh on a sensitive balance. Then the organs were then fixed in 10% formalin for histopathological examination. Each weighed organ was standardized for 100 g

body weight of each rat using the formula below:

$$\text{Standardized organ weight} = \frac{\text{Weight of each organ}}{\text{Body Weight of rat}} \times 100\ \text{g}$$

Haematological Parameters

Blood samples were collected through cardiac puncture from each diethyl-ether anaesthetized rat into different EDTA-coated sample bottles. The blood samples were analysed for red blood cells count (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cells count (WBC) and differential WBC (neutrophil, eosinophil, basophil, lymphocyte and monocytes). These parameters were analysed within 24 h post-collection using automated haematology analyser according to manufacturer's protocols (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan) at the Department of Haematology, University of Uyo Teaching Hospital, Nigeria.

Biochemical Parameters

Whole blood of each rat was centrifuged at 2500 rpm for 20 min at 10 ^{0}C to separate the serum. The activities of serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined at 340 nm using the method described by Reitman and Frankel (1957), while serum alkaline phosphatase (ALP) was determined at 405 nm using the method of Bessey *et al*. (1946). Serum creatinine (SCr) level was assayed according to the method of Newman and Price (1999), based on the reaction of creatinine with an alkaline solution of sodium picrate to form a red complex. Serum urea level was estimated by endpoint colorimetric test using diagnostic kits. These determinations were done spectrophotometrically at the University of Uyo Teaching Hospital, Nigeria using Randox™ analytical kits according to standard procedures of manufacturer's protocols.

Serum catalase (CAT) activity was assayed according to the method of Cowell *et al*. (1994), by measuring catalase degradation of H_2O_2 using redox dye (ELISA Kit: QuantiChrom^{TM}, BioAssay Systems, USA). Serum superoxide dismutase (SOD) activity was measured by the xanthine oxidase method (ELISA Kit: Cayman Chemical Company, USA), that monitors the inhibition of nitro blue tetrazolium reduction by the sample (Sun *et al*., 1988). While, serum MDA level was assayed by determining thiobarbituric acid reactive substances (TBARS) (ELISA Kit: QuantiChrom^{TM}, BioAssay Systems, USA) produced during peroxidation following the method of Ohkawa *et al*. (1979).

Histopathological Assessment

The liver, kidneys, lungs, heart and spleen were immediately excised from the euthanized animals, freed from adventitia, blotted dry, weighed, sectioned and fixed in 10% formalin for histological studies. Fixed sections were passed through xylene, alcohol and water to ensure that the tissue was totally free of wax and alcohol. Each section was then stained with haematoxylin and eosin for photo-microscopic assessment using light microscope at a magnification of 400. In order to minimize bias, the pathologist had no knowledge of the doses and treatments given to the different groups of experimental rats (Yemitan *et al*., 2015).

Reversibility Studies

Administration of both fixed and essential oils of *Z. officinale* was discontinued among the remaining animals (4/group) for a 14-day period. However, during this period, the experimental animals were given access to food and water *ad libitum*. Their weights were measured and recorded. After the 14-day period, the animals were euthanized/sacrificed under diethyl ether anaesthesia, dissected and blood samples were collected through cardiac puncture into different sample bottles for haematological and biochemical analysis. Organs were eviscerated for internal macroscopic and histopathological assessments (using the same procedures employed in the main test).

Statistical Analysis

Obtained data from this study were statistically analysed using SPSS (version 20). Statistical significance between the experimental groups was evaluated by means of one – way analysis of variance (ANOVA) at 5% confidence interval. Results of the study are presented as Mean ± S.E.M. *P* values less than 0.05 ($P<0.05$) were considered significant.

Results

Acute Toxicity

Five (5) out of the six treated animals at varying doses (0.02, 0.04, 0.06, 0.08 and 0.1 mL/kg body weight) of the fixed oil survived. However, at 0.2 mL/kg, mortality was recorded (Table 1).

For the essential oil administered at varying doses (0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4, 6, 8 and 10 mL/kg body weight), six out of the ten treated animals all survived (Table 2). Observed cardinal signs of toxicity for both oils were decreased motor activity, convulsion and paralysis.

Body Weight of Treated Animals

The result presented significant ($P<0.05$) increase and decrease in mean weight gain. The mean weight gain per week was

recorded to be 15.01 ± 0.40 g in group II that was treated with 0.02 mL/kg body weight of ZOF (Table 3). Whereas, the mean weight loss per week was recorded to be 9.60 ± 0.60 g and and 7.16 ± 0.44 g in groups IV and V that were administered 0.4 and 0.04 mL/kg body weight of ZOE respectively (Table 3). The decrease in body weights were observed after the first week of admnistration and continue throughout the duration of the study (Table 3).

Effect of Ginger oils on Organ Weights

Significant (P<0.05) increase in organ weight of the kidneys, lungs, liver and spleen were recorded at 0.02 mL/kg and 0.002 mL/kg body weight of ZOF compared to control. However, there was no significant difference in the weight of the heart compared to control. At 0.4 and 0.04 mL/kg body weight of ZOE, there were no significant differences in the weights of all eviscerated organs compared to control (Table 4).

Effect of Ginger oils on Biochemical Parameters of Treated Animals

Significant (P< 0.05) decrease in ALP (165.64 ± 5.16 U/L) but an increase ALT (42.33 ± 1.63 U/L) was recorded at 0.002 mL/kg body weight of ZOF. However, there was no significant difference in the value of AST at this dose compared to control (Table 5). AST (66.45 ± 7.05 U/L) was significantly reduced at 0.04 mL/kg body weight of ZOE. There were no significant differences in the renal metabolites measured, compared to control (Table 5).

Effect of Ginger oils on Oxidative Stress Markers in Rat Serum

The result showed a significant (P<0.05) elevation in the MDA level at 0.002 mL/kg body weight of ZOF (0.36 ± 0.01) compared to the control. There was no significant difference in the concentrations of CAT and SOD at all levels tested (Table 6).

Effect of Ginger oils on Haematological Parameters

At the doses of ZOF and ZOE tested, there was no difference observed in all the haematological parameters (Table 7).

Effect of Ginger oils on Histopathology of Vital Organs

At the end of the 60 days sub chronic toxicity study, histopathological examinations of the liver, heart, lungs, kidney and spleen of rats in the control group presented normal/preserved cellular architecture. Some forms of pathologies (vascular congestion, vacuolated nuclei, congested sinusoids and moderate scar of necrosis) were observed in the liver and spleen of the experimental groups that were administered fixed oil of *Z. officinale*

(Fig. 1 - 2). There were no observable differences in the histology of the heart, lung and kidney compared to the control group. Also, there were no observable differences in the histology of the organs treated with essential oil of *Z. officinale*.

Reversibility of Test Effects

Reversibility of test effects on organ weight showed no difference in liver, lungs, kidneys and spleen weights (Table 8). Also, there were no significant differences in AST, ALP and ALT that were tested. However, reversibility of test effects on oxidative stress analysis showed significant ($P< 0.05$) increase in MDA level at 0.002 mL/kg body weight of ZOF.

Discussion

Conventionally, significant changes in body and internal organ weights are considered sensitive indices of toxicity after exposure to toxic substance (Farah *et al*., 2013). The recorded increase in weights of the spleen, lungs, kidneys and liver could indicate hypertrophy, and hypertrophy of organs is a first-hand indication of toxicity of chemical or biological substance (Ping *et al*., 2013). In this study, the observed increase in liver weight in proportion to the body weights of the control rats and ZOF – treated rats may be indicative of an underlying pathology of the liver. This finding is in agreement with that of Eleazu *et al.* (2013). However, this observed effect was reversed during the 14-day reversibility study.

The commonest enzymes usually employed as indicators of hepatocellular damage are the transaminases (i.e. ALP, ALT and AST). Increase or decrease in the activity of liver enzymes parameters might be suggestive of liver injury (Abdelhalim and Moussa, 2013). Generally, observed increase in serum enzyme activities are roughly proportional to the extent of tissue damage. Since the liver contains high concentrations of ALT when working properly, liver damage causes the release of high concentrations of ALT into the bloodstream. Thus, the increase in the specific activity of ALT following ZOF administration suggests that there may be a leakage thereof, which may be interpreted to mean damage to the liver. ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are superabundantly found in the liver and thus, in the event of cell membrane leakage and/or complete rupture, these nonspecific biomarkers are measurable in the serum (Tiedge *et al*., 1997).

The histopathological assessment of the liver presented some forms of pathologies (vacuolated nuclei, vascular congestion

and moderate scars of necrosis). Vascular congestion within the liver is usually followed by edema – which is defined as the abnormal accumulation of fluid in the extravascular portion of the extracellular space. As reported by Riede and Werner (2004), pathologically, edema is the result of an "exudation process". Most often than none, prolonged or chronic congestion leads to fibrosis of the necrotic parenchyma that produces congestive cirrhosis (Riede and Werner, 2004). There is a correlation of the result of the biochemical analysis of the liver to its histopathological examination. Such correlation is suggestive or indicative of a possible liver damage following sub chronic administration of ZOF to the experimental animals.

The histopathological assessment of the spleen revealed some pathology like inflamed splenic cells etc. The spleen is the largest lymphoid organ in the body and is highly vascular. The primary functions of the spleen include haematopoiesis, destruction of blood cells, blood reservoir and antibody production site. Owing to its essential functions, increase in the weight of the spleen as well as its distorted histology may constitute hindrance to its normal physiology. Therefore, this result is suggestive of possible toxic effects of ZOF on the spleen. However, these toxic effects were reversed on discontinuation of ZOF.

Furthermore, lipid peroxidation is one of the major outcomes of free radical-mediated injury that directly damages cell membranes and generates a number of secondary products including aldehydes, such as malondialdehyde (MDA). Thus, MDA is a highly reactive compound and the most abundant aldehyde produced during polyunsaturated fatty acids peroxidation (Davey *et al*., 2005). MDA is one of the many electrophile species that causes toxic stress in cells and form covalent protein adducts (Farmer and Davoine, 2007) known to be advanced lipoxidation end-products (ALE). There has been report of high MDA-7 expression promoting malignant cell survival in chronic lymphatic leukaemia (Sainz-Perez *et al*., 2006) especially as MDA is known to be potentially mutagenic. Owing to it overly reactive nature, MDA is known to react with DNA to form DNA adducts such as the mutagenic M_1G_1. This mutagenic DNA adduct is found in edible oils like palm oil, sunflower oil etc. (Douredjou and Koner, 2008). Therefore, since MDA-mediated DNA adduct is found in heated edible oils and spices like ginger, cautionary use of these substances becomes necessary. The production of MDA is indicative of high oxidative stress in tissues/organisms (Goudah *et al.*, 2015). Thus, the persistent increase in serum MDA levels even after 14 days of

reversibility study indicates oxidative stress from lipid peroxidation.

Conclusions

Following the 60-day sub chronic toxicity studies, the results obtained in this study indicates that fixed oil of *Zingiber officinale* (ZOF) have the inherent ability to induce an array of toxicities such as organs' toxicities (hypertrophy of the liver, kidneys, lungs and spleen), cellular toxicity and oxidative stress. These findings therefore necessitate caution on the chronic and/or indiscriminate use of ginger root as well as it derivatives.

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Declaration of interest

None

Contributors

This work was carried out in collaboration between all authors. Authors OKY, HOCM and IE designed the work. Author EOI wrote the protocol. Author GJU wrote the first draft of the manuscript, managed the literature searches, edited and wrote the final manuscript. Authors EOO and JAU reviewed and vetted the first draft. Author OKY performed the statistical analysis. Authors OKY and EOI managed the

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