

Hepatoprotective Effects of Ginger (*Zingiber officinale*) on Mercury-Induced Hepatotoxicity in Adult Female Wistar Rats

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ABSTRACT

Ginger (*Zingiber officinale*) has over the years been discovered to possess so many therapeutic solutions to certain health problems and this has being of great help to the health sector. In this study, the hepatoprotective and curative effects of stock solution ginger on the liver of mercury induced adult female wistar rats were evaluated in varying doses. 24 adult female wistar rats were used in this study and allocated into 4 groups of 6 animals each. Groups 2, 3 and 4 served as the test groups receiving 500mg/kg of ginger, 2ml/kg of mercury and a combination of 500mg/kg ginger and 2ml/kg mercury respectively, while the group 1 was the control group, all these within a period of 28 days after 14 days of acclimatization. 24 hours after the last administration of the extract was done, the animals were sacrificed in a chloroform chamber and each of them was placed in the anatomical position for organ harvesting proper before processing the tissues by histological techniques and then analysis. The results obtained showed that ginger had the ability to cure and protect the liver against damage already caused by the mercury. This study therefore suggests that ginger be added as a supplementary diet to individuals already exposed to mercury.

Keywords: Curative, Ginger, Hepatoprotective, Liver, Mercury

INTRODUCTION

So many times man has been exposed to so many toxic harmful substances which have caused a great deal of mortality but in the quest to survive all, he has also sought for materials that could either minimize or eradicate the effects of these toxic substances.

Ginger, a member of the family, *Zingiberaceae*, is a herbaceous perennial plant that grows stems about 1m tall, bearing yellow flowers and green leaves. In 2012, Nigeria was ranked as one of the top five largest producers although India is the largest of all producers (FAO of UN, 2014). Its characteristic odour and flavor is caused by a mixture zingerone, shogaols and gingerols which are volatile oils that compose 1-3% of the weight of a freshly acquired ginger. Gingerols are known to possess sedative, antipyretic, anagelsi and antibacterial properties and in laboratory animals are found to increase motility in the gastrointestinal tract (O'Hara *et al.*, 1998).

Ginger (*Zingiber officinale*) has been found to possess a lot of phytochemicals that contribute to its medicinal properties. These phytochemicals are zingerone, shogaols, gingerols, pardols, β -phellandrene, curcumene, cineole, geranyl acetate, terpenes, borneol, geraniol, limonene, β -elemene, zingiberol, linalool, α -zingiberene, β -bisabolene, zingiberenol and α -farnesene (Baliga *et al.*, 2011).

Scientific studies have shown that ginger reduces the oxidative stress induced by various hepatotoxins by increasing the activity or levels of the antioxidant enzymes (Afshari *et al.*, 2007), and this mechanism would have played a cardinal role in the observed hepatoprotective effects of ginger against diverse class of toxicants.

Other studies have shown that the extracts of ginger and some of its phytochemicals modulate the activity of both phase I and II enzymes, and to mediate their hepatoprotective effects, in at least a part. With respect to the phase I enzymes, studies have shown that feeding ginger causes an increase in the levels of microsomal cytochrome p450-dependent aryl hydroxylase, cytochrome p450 and cytochrome b5, this effect therefore would have increased the polarity of non-polar xenobiotic compounds (Sambaiah and Srinivasan, 1989). Additionally, studies have also shown that the oral feeding of ginger oil increases the activity of aryl hydrocarbon hydroxylase and GST in mice along with increases the activities of UDPGT, aryl hydrocarbon and

quinone reductase, and also to fasten the elimination of the partially metabolized hepatotoxins from the liver (Banerjee *et al.*, 1994).

Lindane, an anti-lice and anti-scabies agent, is a potent toxin and damages the nervous system, liver and kidneys and when experimentally induced into the liver causes hepatotoxicity of the liver from increased lipid peroxidation but ginger provided in diets (1%w/w) was found very effective in reducing this hepatotoxicity as well as concomitantly decreasing the levels of the lindane-induced lipid peroxidation (Ahmed *et al.*, 2008).

Mercury (chemical symbol Hg and atomic number 80) a naturally occurring heavy metal occurs in several forms, all of which except elemental liquid mercury (which does not produce toxicity even after injection) produce toxicity or death with less than a gram when induced or exposed to (Clarkson and Magos, 2006). Its zero oxidation state (Hg^0) exists as vapour or as liquid metal while its mercurous state Hg_2^{2+} exists as inorganic salts, and its mercuric state Hg^{2+} may exist in form of either inorganic salts or organomercury compounds, these three forms or groups vary in their toxic effects which include damage to the brain, kidneys and lungs (Clifton, 2007).

The liver is the largest visceral organ and the largest gland in the human body. The liver forms as an endodermal lining outgrowth of the foregut called the hepatic diverticulum growing into the surrounding mesoderm of the septum transversum. The developing liver bulges into the abdominal cavity, thereby stretching the septum transversum to form the ventral mesentery consisting of the falciform ligament (containing the left umbilical vein that regresses after birth to form ligamentum teres) and the lesser omentum (divided into the hepatogastric ligament and hepatoduodenal ligament that contains the portal triad of bile duct, portal vein, and hepatic artery) (Dudek, 2010). In the fetus, the liver is important in the manufacture of red blood cells while in the adult, it plays important roles such as the production and secretion of bile, detoxifying the blood, storage of carbohydrate, production of blood coagulants, anticoagulants, and bile pigments and storage of certain vitamins, iron, and copper (Chung and Chung, 2012).

This experimental study therefore is to research on the hepatoprotective and curative effects of ginger (*Zingiber officinale*) on adult female wistar rats which have been induced with mercury.

MATERIALS AND METHOD

Duration of Experiment and Breeding of Animals

Total of 24 adult female wistar rats were used for this study and were all housed in big mesh grid cages at the animal house of the College of Health Sciences, Nnewi at normal room temperature of about 27°C-30°C. The cages were always cleaned daily to maintain good environmental hygiene and prevent infection. The animals were acquired from the animal farm house of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus and allowed to acclimatize for 14 days while the actual administration of extracts to the test animals lasted for 28 days bringing the duration of the entire experiment to 42 days. The care and handling of these animals were in total compliance with the National Regulations for Animal Research.

Materials for the Study

The materials used for this experiment includes the following:

- A. Twenty-four adult female wistar rats which were divided into three groups 1, 2 3 and 4 of 6 animals each, in which Groups 2, 3 and 4 served as the test groups while Group 1 was the control group.
- B. Ginger seeds (*Zingiber officinale*) purchased at Nkwo market in Nnewi, Anambra state, Nigeria.
- C. Mercury solution purchased from the Onitsha main market in Onitsha, Anambra state, Nigeria.
- D. Hand grinder to grind the seeds into fine powder form.
- E. Growers mash purchased from the Nkwo market in Nnewi, were produced by Premier Feed Mills Company Limited, Sapele, Delta State, Nigeria, and were used as feed for the animals throughout the duration of the experiment.
- F. Four big mesh grid cages.
- G. Analytic Weighing Balance manufactured by Yongkang Zhezhong weighing apparatus factory in China.
- H. Syringes and canula for administering the extracts.
- I. Compound light microscope.

- J. Slides and cover slips.
- K. Refrigerator to store the mercury and stock solution.
- L. Distilled water to prepare the stock solution of the ginger.
- M. Rotatory microtome.
- N. Measuring cylinders and sample bottles.

Experimental Protocols

Twenty-four apparently healthy adult female wistar rats were assigned to four different mesh grid cages in a group of six each for acclimatization, for a period of 14 days, prior to the commencement of the experimental administration. Group 1 served as the control group receiving the water and feed only while Groups 2, 3 and 4 served as the experimental test groups each receiving 500mg/kg of ginger, 2ml/kg of mercury and combination of 500mg/kg ginger and 2ml/kg mercury respectively. These solutions were administered for 28 days respectively. Twenty-four hours after the last administration, the animals were anaesthetized using diethyl ether and then each of them was placed in the anatomical position from which the liver was approached through the lower abdomen, by making a transverse incision.

Preparation of Solution

The ginger (*Zingiber officinale*)s seeds after purchase at the Nkwo market in Nnewi, Anambra State had its outer coats removed with knives and its seeds cut into little pieces and sundried before they were further dried using water bath at temperature of about 50⁰C weighing about 200g. The dried seeds were ground to fine powder and macerated in 1000ml of distilled water, it was shook at intervals for 48 hours after which the mixture was sieved using parceling cloth. It was further filtered with no.1 whatman filter paper. The filtrate was then put in 1000ml of distilled water and used as the stock solution. The mercury and the stock solution were stored in a refrigerator pending use.

Tissue Processing

The tissues were processed according to standard procedures for easy study and understanding under the compound light microscope. Fixation was done in 10% formal saline for 2 hours to preserve the cellular components of the liver tissues, these tissues were then dehydrated in ascending grades of alcohol of 50%, 70%, 90% and 100% for 2 hours each before then being cleared in three changes of xylene for a period of 1 hour 30 minutes to remove alcohol from these tissues. The xylene was then removed and substituted with molten paraffin wax maintained at a temperature of 3⁰C-5⁰C above the melting point of the paraffin wax, the tissues were then placed in proper orientation in embedding cassettes containing molten paraffin wax and allowed to cool and solidify before being transferred into a bath of ice block for easy detaching of the mould made from the embedded cassettes. The moulds containing these livers were then mounted and trimmed to remove excess wax and sectioned to 5 microns with the aid of a rotatory microtome. The tissue sections were deparaffinised in 40% alcohol and hydrated in a water bath preheated to about 50⁰C-55⁰C before being allowed to dry on clean slides, the slides were dried in an incubator at about 37⁰C overnight for adherence of the tissue. The sectioned tissue slide was stained using haematoxylin and eosin staining technique for the tissues structures to give their characteristic colours before being mounted using the Dibutylphthalate Polystyrene Xylene mountant and finally covered with cover slip to avoid air bubbles before viewing under the compound light microscope.

RESULT

The following results were obtained during this experiment:

Behavioural and Physical Observations

During the period of administration, the animals of groups 3 and 4 were observed to be less active than those of groups 1 and 2. A reduction in the food intake and water were observed also in groups 3 and 4 and a visible reduction in body sizes.

Histopathological Analysis

The following histopathological findings were gotten after the tissues were processed by haematoxylin and eosin staining techniques:

GROUP 1:

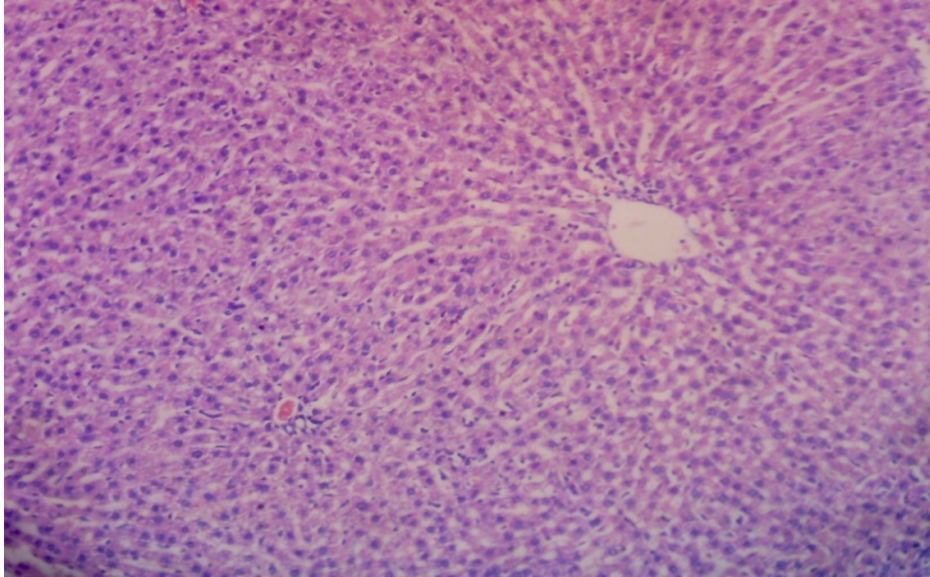


Fig 1: Micrograph of group 1 $\times 100$ showing normal Liver showing central vein.

GROUP 2:

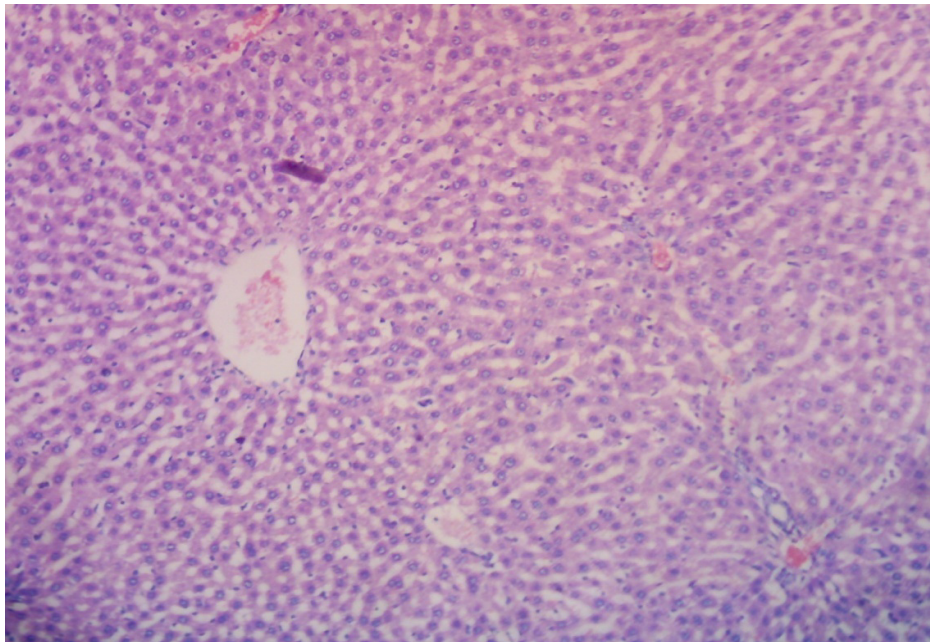


Fig. 2: Micrograph of Group 2 $\times 100$ showing no significant changes.

GROUP 3:

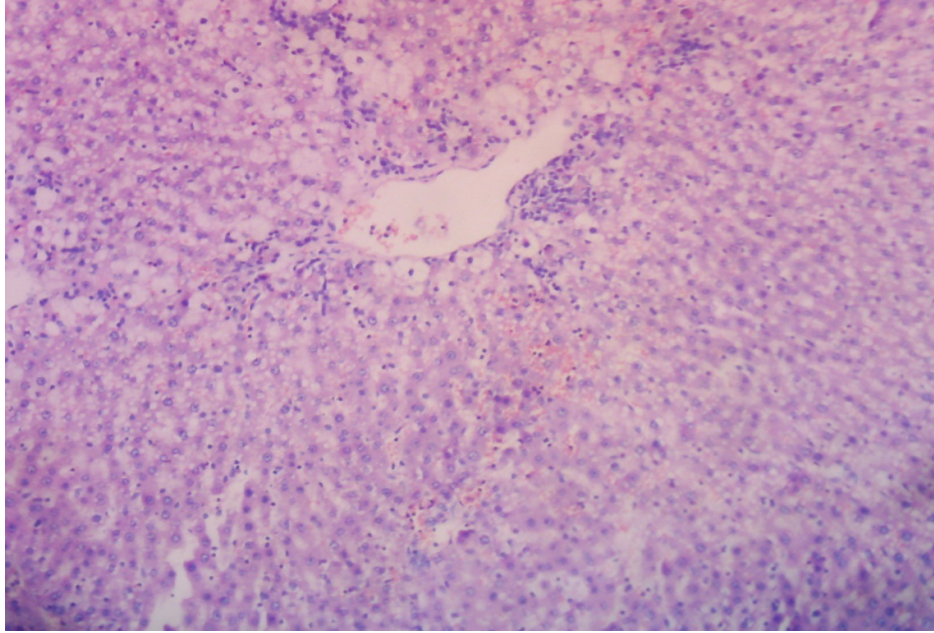


Fig. 3: Micrograph of Group 3 $\times 100$ showing mild disorientation of hepatocytes with small lipid vacuoles and few nuclei displaced peripherally.

GROUP 4:

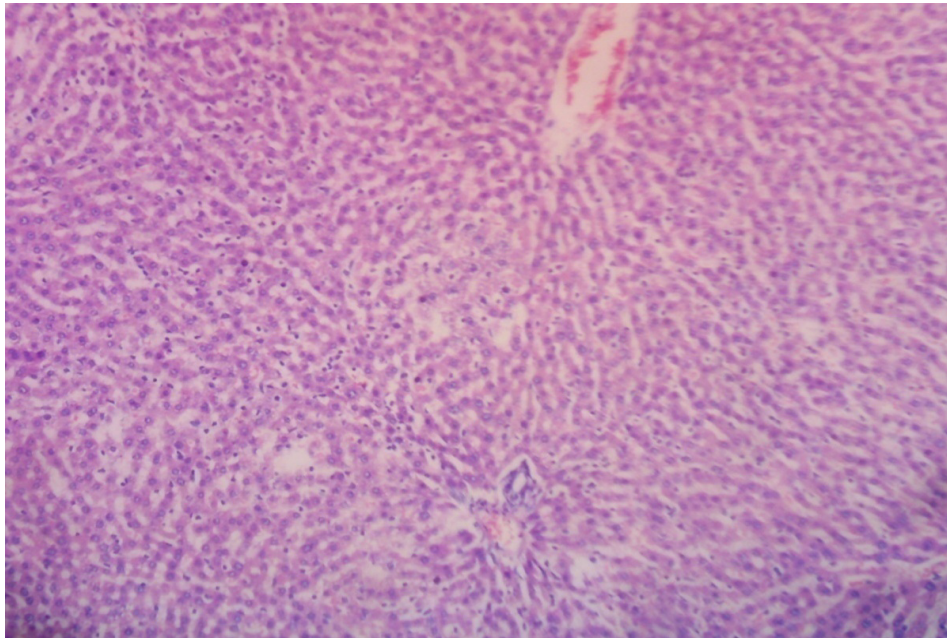


Fig. 4: Micrograph of Group 4 $\times 100$ showing most of the hepatocytes are distended with large lipid vacuoles and peripherally displaced nuclei.

DISCUSSION

The results of this study agree with previous researchers that mercury has toxicological effect on the liver of wistar rats. The liver showed necrosis, infiltration by inflammatory cells and congestion of the central vein and distortion of the general liver cell architectures.

It was observed during the studies that generally, the group in which the rats were treated with ginger was able to tolerate the mercury induced in their system much longer. Most of them did not show any sign of histological differences in their liver at all suggesting that the anti-oxidant properties of ginger could protect to a large extent against the toxicity effects of xenobiotic agents such as the mercury used in this study although there was mild distension of hepatocytes with small liver vacuoles and a few peripherally displaced nuclei.

CONCLUSION

The solution of ginger used in this study was found to have some hepatoprotective effects as seen from the cellular architecture of the liver tissues of the animals. Therefore demonstrating the potential ability of ginger to protect against mercury induced toxicity in the liver of wistar rats. Rats' tissues are very similar in many aspects to those of human and the findings of this study suggests that ginger should be recommended to individuals exposed to mercury poisoning as it could provide some protection against mercury toxicity and perhaps ameliorate the effects of the mercury toxicity on the liver which is a very vital organ. This study also suggests that however considerable works have been done to exploit the protective effects of ginger yet countless possibilities for further investigations still remain as there are hardly any reports on the toxic effects of ginger, studies should be conducted to assess for the possible adverse effects of ginger, especially at higher concentrations and when consumed over longer periods.

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