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Studies of ethanolic extract of ginger (*Zingiber officinale*) on the histology of the liver using adult male rats

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Abstract
The use of botanical medicine is ancient and plant chemicals are still the backbone of our pharmacopoeia because more than 50% of drugs used in Western pharmacopoeia are isolated from herbs or derived from modification of chemicals first found in plants. *Zingiber officinale* is one of the most widely used herbs and food flavouring agent and commonly known as ginger. This study was done to evaluate the possible histological effect(s) of ethanolic extract of *Zingiber offinale* on the liver of adult male albino rats. Twenty five (25) adult wistar rats weighing between 125-200g were divided into five groups of five (5) rats each. Group C, D and E served as experimental groups while group A and B served as the control groups. Group C was administered a dose of 100mg/kg of the extract, group D was given oral dose of 250mg/kg of the extract while group E was given oral dose of 500mg/kg of *Zingiber officinale* extract. Administration of extract lasted for fourteen days at the end of which the animals were sacrificed using chloroform-inhalation method. The liver was harvested as tissue samples from sacrificed animals for pathological examination using routine histological procedure and stained with the haematoxylin and eosin stains. Histological examinations of liver showed that after treatment with low and medium doses (100, 250mg/kg) the ginger extract produced little damaging effects on the liver histology, but at higher doses (500mg/kg) the liver shows marked dilatation of the central vein and sinusoidal spaces as well as loss of cytoplasmic material. Therefore, *Zingerber officinale* should be used with caution because it may have deleterious effects on the liver cells at high doses.

1. Introduction

Plants are the basic source of knowledge of modern medicine. The burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care (Ahmed M. and Hussain, 2013)Several plants of diverse origins have been exploited by trial and error over many generations for therapeutic purposes. In Africa and in most of the developing countries, plants' properties are empirically appreciated. The adverse effects of chemical drugs, their increasing costs and greater public access to information on traditional medicine have also led to an increase in interest in

alternative treatments. The reason is that traditional medicine is a medicine of proximity, less constraining and non-expensive (Agunbiade *et al.*, 2012). The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases (Palaksha and Ravishankar 2012) Traditional medicines are used by about 60% of the world population in both developing and developed countries where modern medicines are predominantly used while an estimated 60-80% Africa's population depends solely on herbal remedies for its primary health care needs (Adesuyi, *et al.*, 2011). Ginger is mentioned in ancient Chinese, Indian and middle Eastern writings and has long been prized for its aromatic, culinary and medicinal properties. After theancient Romans imported ginger from China, almost two thousand years ago, its popularity in Europe remained centred in the Mediterranean region until the middle ages when its use spread throughout other countries (Zhang and Durst T., 2003).

Zingiber officinale has been found by researchers to contain essential chemicals responsible for its therapeutic functions. These are the medically active constituents of ginger and are also responsible for ginger's characteristic odour and taste. The aromatic constituents include zingiberene and bisabolene while its pungent constituents which are usually credited with the anti-nausea and anti-vomiting effects of ginger include gingerols and shogaols (Satoh and Murata, 2002).Ginger is widely used in different parts of the world as spice for cooking different kinds of food. In India, ginger is used in all sub-varieties of the Indian cuisines; it is used in the production of a candy called inji-murappa (ginger candy). In western cuisine, ginger is traditionally restricted to sweet foods such as ginger ale, ginger bread, ginger cake and ginger biscuit. In Asia, it is used as flavouring agents for candies, cookies, crackers and cakes. In Nigeria, it is used as a major cooking spice for the preparation of stew, pepper soup, meat seasoning and for making ginger biscuits and sweets.

The liver is our greatest chemical factory. It builds complex molecules from simple substances absorbed from the digestive tracts. It neutralizes toxins and manufactures bile which aids in fat digestion (Buraimoh *et al*, 2011). The liver also functions in converting drugs into forms that can be readily eliminated from the body. In the world of today, there is a burden Imposed on the liver due to numerous drugs in circulation and toxic food components ingested by the public. Antioxidants like chlorophyll, xanthenes and alpha carotene have the ability to change the membrane on cells; this will make them toxins trying to enter and damage the cell; (Aslam *et al*, 2005).

2. Materials and Method

2.1. Preparation of Ethanolic Extract of *Zingiber Officinale* Plant

Ginger (zingiber officinale roscoe) rhizome was purchased from the local market at Calabar south (watt market), Calabar. The roots were identified and authenticated by the botanist in the botany department, university of Calabar, Calabar.

2.5kg of fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried for two weeks and crushed into powdered form using an electric blender

2000g (2kg) of this powdered ginger was macerated completely in 5000ml of 99.9% ethanol and shaken vigorously. It was allowed to stand for 48 hours at room temperature and was stirred at intervals.

After 48 hours, the dissolved ginger in ethanol was filtered using at first a material with small pores after which it was filtered again using No1 whatmann paper (filter paper) and funnel. The filtrate was collected in a tray and was air dried for 5 days. This was to ensure the complete evaporation of the ethanol used.

The ginger paste obtained was collected from the tray with the aid of a spatula into a container and was measured using an electric weighing balance. 50g of ginger paste was extracted and was then dissolved in 100ml of extra virgin olive oil (which served as the vehicle). This extract was kept in a dry place at room temperature.

2.2. Breeding/Grouping of Animals

Twenty-five adult albino male wistar rats weighing between 90g to 130g were purchased from the department of pharmacology animal farm, university of Calabar, Calabar. These animals were housed in well ventilated animal cages and were kept in the animal house of the department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar. The animal house was properly fitted with bright light and environmental temperature always kept at a range of 28 to 32 degrees Celsius. The house was constantly kept clean and disinfected.

The animals were fed with growers mesh obtained from vital feed located in Calabar and Distilled water daily with the aid of water bottles and were allowed to acclimatize for a period of 14 days.

After the fourteenth day of acclimatization, the rats weighed between 125-200g. They were then randomly selected into five groups with each group containing five rats in well labelled cages.

2.3. Plant Extract Administration

The animals were divided into five groups with five rats each.

GROUP A: was the normal control group. The animals

were administered normal laboratory diet (growers mesh) and distilled water only.

GROUP B: was the vehicle control group (virgin olive oil control group). They were administered 2ml of virgin olive oil.

GROUP C: was the low dose group. They were administered 100mg/kg body weight of the ethanolic extract of Zingiber officinale.

GROUP D: was the medium dose group. The animals were administered 250mg/kg body weight of the ethanolic extract of Zingiber officinale.

GROUP E: was the high dose group. The animals were administered 500mg/kg body weight of the ethanolic extract of Zingiber officinale.

Each animal in the experimental groups was administered the plant extract based on its body weight and administration was done using the oral route throughout the period of the experiment (which lasted for 14 days) after which the animals were sacrificed, liver harvested and processed for histological observation.

3. Result

The following results were obtained from the histological process using Haematoxylin and Eosin staining method.

CONTROL (GROUP A): The control group A (Plate. 1) received no extract of zingiber officinale but was given feed and distilled water. A section of the liver tissue shows a preserved cytoarchitecture with a well outlined central vein (CV) with hepatocytes (HC) radiating outward from it. The hepatocytes have an abundant eosinophilic cytoplasm with round to oval shaped basophilic nuclei.

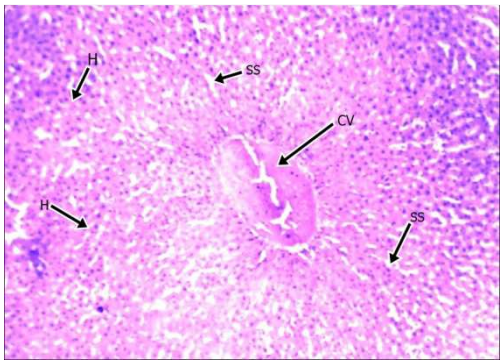
OLIVE CONTROL (GROUP B): The vehicle control group B (Plate. 2) received feed and distilled water as well as 2ml of the vehicle (olive oil) daily. Section of the liver shows a preserved architecture with a well outlined central vein (CV) and plates of hepatocytes (HC) radiating outwards like spokes of a wheel. The hepatocytes have abundant eosinophilic cytoplasm and round to oval basophilic nuclei. The sinusoidal spaces (SS) well demonstrated. The interlobular space contains the portal triad (PT) consisting of bile duct (BD), hepatic artery (HA) and the portal vein (PV). Limiting plates are intact.

LOW DOSE (GROUP C): The low dose group (Plate. 3) received 100mg/kg body weight of ginger extract for a period of 14 days. Section of the liver shows well outlined central vein (CV) with hepatocytes (H) radiating from it. Portal triad (PT) is intact. Sinusoidal spaces (SS) are dilated.

MEDIUM DOSE (GROUP D): The medium dose group (Plate. 4) received 250mg/kg body weight of the extract for a period of 14 days. Section shows a mild constriction of the central vein (CV) with hepatocytes radiating from it. Limiting plate (endothelial lining of the CV) is intact. Hepatocytes (HC) made up of eosinophilic cytoplasm and oval shaped basophilic nuclei. The sinusoidal spaces (SS) are mildly constricted. Portal triad (PT) is distinct containing the bile duct (BD), hepatic artery (HA) and the portal vein (PV).

HIGH DOSE (GROUP E): The high dose group (Plate. 5) received 500mg/kg body weight of the extract for a period of 14 days. Section shows constriction of the central vein (CV) and its detachment from the hepatocytes (HC) due to the distortion of the endothelial cells of the sinusoids and of the hepatocytes in the perivascular area. Portal triad (PT) is prominent with dilated portal vein (PV). Sinusoids (SS) are constricted. Pyknosis of some nuclei is also observed as they lose their colour. There is cytoplasmic disintegration with loss of cytoplasmic material.

Group A (Normal Control)



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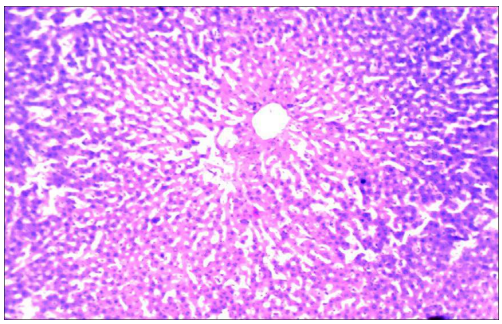

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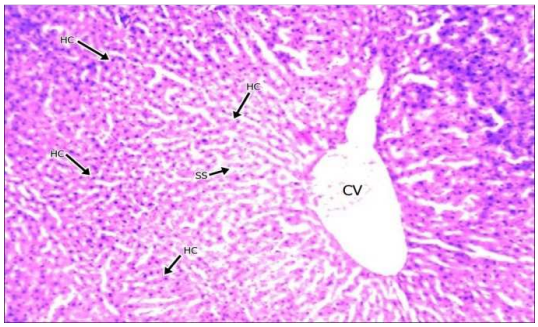
Plate 1. *A section of the liver tissue shows a preserved cytoarchitecture with a well outlined central vein (CV) with hepatocytes (HC) radiating outward from it. The hepatocytes have an abundant eosinophilic cytoplasm with round to oval basophilic nuclei.*

Group B (Olive Control)



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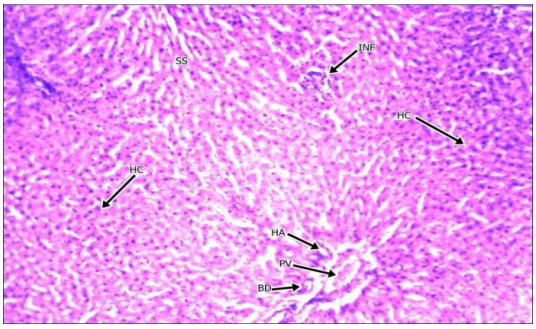

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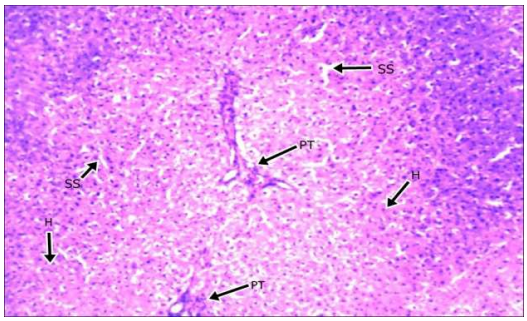
Plate 2. *Section of the liver shows a preserved architecture with a well outlined central vein (CV) and plates of hepatocytes (HC) radiating outwards like spokes of a wheel. The hepatocytes have abundant eosinophilic cytoplasm and round to oval basophilic nuclei. The sinusoidal spaces (SS) are well demonstrated. The interlobular space contains the portal triad (PT) consisting of bile duct (BD), hepatic artery (HA) and the portal vein (PV). Limiting plates are intact.*

Group C (Low dose)



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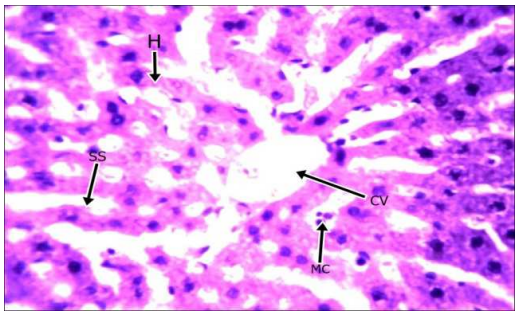

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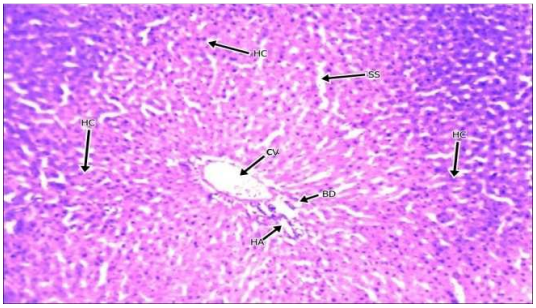
Plate 3. *Section of the liver shows well outlined central vein (CV) with hepatocytes (H) radiating from it. Portal triad (PT) is intact. Sinusoidal spaces (SS) are dilated.*

Group D (Medium dose)



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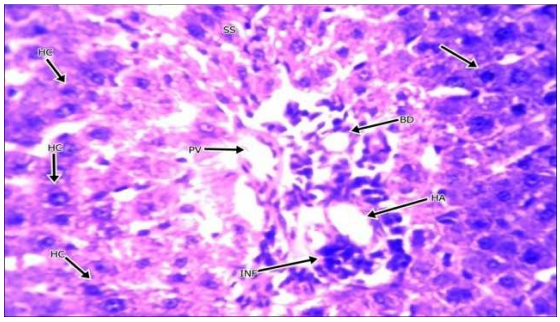

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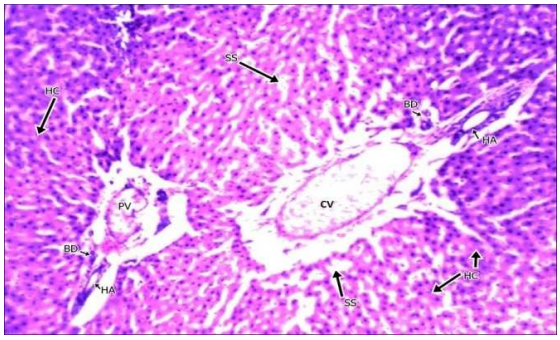
Plate 4. *Section shows a mild constriction of the central vein (CV) with hepatocytes radiating from it. Limiting plate (endothelial lining of the CV) is intact. Hepatocytes (HC) have abundant eosinophilic cytoplasm and oval basophilic nuclei. The sinusoidal spaces (SS) are mildly constricted. Portal triad (PT) is distinct containing the bile duct (BD), hepatic artery (HA) and the portal vein (PV).*

Group E (High dose)



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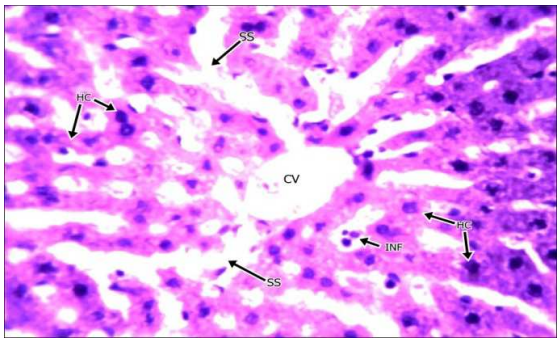

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Plate 5. *Section shows constriction of the central vein (CV) and its detachment from the hepatocytes (HC) due to the distortion of the hepatocytes in the perivascular area. Portal triad (PT) is prominent with dilated portal vein (PV). Sinusoids (SS) are constricted. Pyknosis of some nuclei as they lose their colour. There is cytoplasmic disintegration with loss of cytoplasmic material.*

4. Discussion

Zingiber officinale commonly known as ‘ginger’ has its origin traced to Asia. It has a lot of medicinal uses as far as herbal medicine is concerned. It has been proven to have anticovulsant, antidiuretic, anti-inflammatory, diuretic, antifungal, antihypertensive, antispasmodic, antitumor, and anti cancer. It has other medicinal values which are too

numerous to mention (Farooq *et al*, 2006).

Ginger is widely used in different parts of the world as a spice for cooking different kinds of food and also in the baking industries as flavour and spice for making biscuits, bread and cakes (Futwell and Rietshel, 1993). It can be eaten raw and as food additive. Certain people also used zingiber officinale in ginger ale, ginger breads, ginger snaps, ginger cake and ginger biscuits (Hashimoto *et al* 2002).

Ginger contains volatile oils (~1% to 3%) and non-volatile pungent components oleoresin Zick *et al.,* (2008). A variety of active components were identified in the oleoresin of ginger including gingerols and shogaols. Gingerols are a series of homologues with varied unbranched alkyl chain length, whereas shogaols are a series of homologues derived from gingerols with dehydration at the C-5 and C-4 during long-term storage or thermal processing. Other active compounds from the oleoresin portion of ginger were also reported, such as 6-paradol; 6- and 10-dehydrogingerdione; 6-and 10-gingerdione; 4, 6, 8, and 10-gingerdiol; 6-methylgingerdiol; zingerone; 6-hydroxyshogaol; 6-, 8-, and 10-dehydroshogaol; and diarylheptanoids Sang *et al.,* (2009). Among these compounds, gingerols and shogaols are the major constituents of oleoresin, while the other compounds are present in a limited amount, accounting for 1-10% of the overall amount of gingerols and shogaols Sang *et al.,* (2009). Gingerols (especially 6-gingerol) are the major components in the fresh ginger rhizome. The amount of shogaols is increased in the dried ginger, as evidenced by the reduction of the ratio of 6-gingerol to 6-shogaol from 10:1 in fresh ginger to 1:1 in dried ginger Wu *et al.,* (2010).

Since ginger extracts contain various components, it would be important to identify which compounds are responsible for their pharmacological effects. It was demonstrated that 6-, 8-, and 10-gingerols and 6-shogaol showed efficacy in anti-inflammatory, antibacterial, antipyretic, antilipidemic, antitumorigenic, and antiangiogenic effects Park *et al.,* (2009). In addition, 6-gingerol was shown to inhibit leukotriene A4 hydrolase (LTA4H) and suppress anchorage-independent cancer cell growth in colorectal cancer cells (HCT116 and HT29) with IC_{5o}'s of 50 and 35 uM, respectively Jeong *et al.,* (2009). Sang *et al.,* (2009) demonstrated that 6-, 8-, and 10-shogaols exhibited much higher antiproliferative potency than 6-, 8-, and 10-gingerols against human lung cancer cells (H-1299) with IC_5o's of 8 µM for 6-shogaol and 150 µM for 6-gingerol. In addition, 10-gingerol was the most potent among the gingerol Sang *et al.,* (2009) Furthermore, Dugasani *et al.,* (2010) found that 6-shogaol showed the most potent efficacy of antioxidative activity with an IC_{50} of about 8 µM, while 6, 8, and 10-gingerols had IC_5o's of 28, 20, and 12 µM, respectively.

The liver is involved in about 8% of reported adverse effect of drugs reactions. This report is indicative of its central role in metabolism and excretion of many drugs. The liver is involved in dealing and taking up of many metabolites (both toxic and non-toxic) and drugs (Agostini, 1984). The vast and ever increasing number of chemicals used industrially and pharmacologically provide an ever increasing hazard to the liver particularly as certain chemicals which are harmless to most individuals with a special susceptibility is yet unpredictable.

The main function of the liver is digestion and removal of waste products and worn-out cells from the blood. The liver is our greatest chemical factory. It builds complex molecules from simple substances absorbed from the digestive tract. It neutralizes toxins and it manufactures bile which aids in fat digestion (Buraimoh *et al*, 2011). The liver also functions in converting drugs into forms that can be readily eliminated from the body. In the world of today, a complex burden has been imposed on the liver due to the variety of drugs in circulation. But the ability of the liver to perform its function is often compromised by numerous substance humans are exposed to on daily bases, these substances include certain medicinal agents which when taken in overdose or within therapeutic range injure the liver (Gagliano *et al*, 2007). Although liver diseases or damage is stereotypically linked to drugs or alcohol, the truth is that there are over 100 known forms of liver diseases caused by a variety of factors and affecting people ranging from infant to older adult. Liver damage includes hepatitis; inflammation caused by viruses (viral hepatitis) and by some liver toxins e.g. alcohol (alcoholic hepatitis) or drugs with harmful metabolites (Anderson *et al*, 1997).

The observed effect is largely due to the presence of 6-8-10 gingerols and 6-10-shogaols which are the affective components responsible for its pharmacology effect. Park *et al.,* (2007).

From the results, it can be noted that at high dose, ginger extract may be deleterious to the liver cells due to the fact that there is distortion of the hepatocytes in the perivascular area, pyknosis of some nuclei and cellular disintegration.

5. Conclusion

From the above results, it can be said that the administration of the ethanolic extract of Zingiber officinale can cause hepatocellular damage. This effect is dose dependent as greater effects were seen in animals that were administered high doses. This effect may be due to some phytochemicals contained in the plant.

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