Research Article

Acute Toxicity and Effect of Prolonged Oral administration of Zingiber officinale ethanol extract on Liver and Kidney Histology in Rats

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ABSTRACT
The study aimed to evaluate the effect of 28-day repeated administration of Zingiber officinale on the histology of the liver and kidney in Wistar rats. An acute toxicity test was carried out using 12 rats. In phase one, nine rats were allotted into three groups and received Zingiber officinale ethanol extract at 10mg/kg, 100mg/kg, and 1000mg/kg respectively. In phase two, three rats received Zingiber officinale ethanol extract at 1600mg/kg, 2900mg/kg, and 5000mg/kg respectively. Twenty-five rats were distributed into five groups. The groups received distilled water and ethanol extract of Zingiber officinale at 25 mg/kg, 50 mg/kg, 75 mg/kg, and 150 mg/kg respectively for 28 days. On the 29th day, all the rats were euthanized. The oral LD50 of Zingiber officinale ethanol extract was calculated to be 3800 mg/kg in rats. The rats that received Zingiber officinale ethanol extract at 1600mg/kg, 2900mg/kg, and 5000mg/kg respectively showed no signs of toxicity and mortality. The liver of rats that received Zingiber officinale at 150 mg/kg showed distorted hepatocytes and pyknosis. The kidney of 75 mg/kg and 150 mg/kg Zingiber officinale treated rats showed normal glomerulus and normal Bowman’s capsule with severe renal tubular distortion. In conclusion, Zingiber officinale is less toxic. However, long-term administration might lead to liver and kidney damage and eventual death.

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INTRODUCTION

Zingiber officinale, also known as ginger, is a rhizome belonging to the family Zingiberaceae. It is used as a spice for flavoring food in many African and Asian countries (Imtiyaz et al., 2013). The ginger plant has a long history of cultivation in Africa and Asia; it was reported to originate in China and then spread to Africa, the Caribbean, and South-East Asia (Ensminger et al., 1983; McGee, 2004). Both local claims and scientific evidence have proven that Zingiber officinale poses antidiarrheal, antihyperglycaemic, antidiabetic, and antihypertensive and many other pharmacological properties (Cheng et al., 2007; Arablou et al., 2014; Daily et al., 2015). It was estimated that about 80% of most African and Asian countries use herbal medicine for some aspects of primary healthcare (Al-Nahain et al., 2014). The use of herbal medicine has gained recognition globally because pharmaceutical products are expensive for most of the World’s least developed and developing nations, where many people live below 2 US dollars per day (Braun et al., 2010; Ekor, 2013). Many pharmaceuticals currently available to physicians have a long history of use in herbal medicine (Katiyar et al., 2012; Yuan et al., 2016). The World Health Organization reported that almost 25% of modern drugs originated from plants (Veeresham, 2012; Yuan et al., 2016). People seek alternatives to modern medical interventions by looking into traditional and eastern herbs. With the increased use of traditional medicine in developing countries, one needs to take precautions for their efficacy and safety.

A toxicity test is the extent of the potential hazard posed by a substance and measures the degree of its accumulation within tissue and organs following daily administration. The potential hazard and level of accumulation of toxic substances in tissues might lead to serious health problems (Parasuraman, 2011). The clearance rate of the test substance is not usually considered during toxicity testing, but they are important indications of the rate of accumulation of substances within tissues (Patrice et al., 2016). Toxicity tests are performed to determine the minimum dose causing lethality and the degree of damage to some tissues and organs, most especially the liver and kidney (Bello et al., 2016). A toxicity test can be classified as either acute (single dose), sub-acute (daily dose between 2-4 weeks), sub-chronic (daily dose for up to 3 months), or chronic (daily dose for up to 12 months) (Erhirhie et al., 2018; Dibal et al., 2020). The present study aimed to evaluate the effect of 28-day repeated administration of Zingiber officinale on liver and kidney histology in Wistar rats.

MATERIALS AND METHODS

Plant collection and extraction

Zingiber officinale were oven-dried, ground, and soaked in absolute ethanol in a ratio of 100 g to 2.5 litres for 24 hours. The solution was filtered and concentrated to dryness in an oven at 40 °C.

Animal treatment

Thirty-seven female Wistar rats were purchased at National Veterinary Research Institute Vom, Nigeria. They were kept in the Animal House of the Department of Human Anatomy, University of Maiduguri, Borno State, and given food and water ad libitum. The research was conducted following the ARRIVE guidelines and the OECD guidelines for testing chemicals (OECD, 2002).

Experimental design

The acute toxicity test was carried out in two phases according to the method described by Lorke (1983), using a total of 12 rats. In phase one, nine rats were allotted into three groups of three rats each. The rats received Zingiber officinale ethanol extract at 10 mg/kg, 100 mg/kg, and 1000 mg/kg. In phase two, three rats received Zingiber officinale ethanol extract...
at 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg respectively (Table 1). The rats were observed for 24 hours and fourteen days for toxicity signs and/or mortality. The LD50 was calculated as

\[ \text{LD50} = \sqrt{D_0 \times D_{100}} \]

Where; D0=Highest dose with no mortality and D100=lowest dose that caused mortality.

Table 1: Experimental protocol for acute toxicity testing of ethanol extract of Zingiber officinale in rats

<table>
<thead>
<tr>
<th>Phases</th>
<th>Groups</th>
<th>Number of Rats</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>1000 mg/kg</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1600 mg/kg</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>2900 mg/kg</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>5000 mg/kg</td>
</tr>
</tbody>
</table>

Subacute toxicity test involved the use of twenty-five rats which were distributed into five groups of five rats each. Group 1 served as the control and received distilled. In contrast, groups 2-4 served as the experimental groups and were administered ethanol extract of *Zingiber officinale* at 25 mg/kg, 50 mg/kg, 75 mg/kg, and 150 mg/kg respectively for 28 days. On the 29th day, all the rats were euthanized. The liver and kidney were dissected out and fixed in 10% Neutral buffered formalin, dehydrated in graded alcohol, and embedded in paraffin wax. It was sectioned at 5 µm using a rotary microtome, cleared in xylene, and stained with Hematoxylin and Eosin stain.

RESULTS

Adverse effect of Zingiber officinale ethanol extract in rats

The rats that received *Zingiber officinale* ethanol extract at 10 mg/kg, 100 mg/kg, 1000 mg/kg, and 1600 mg/kg showed no signs of toxicity and mortality. The rat that received 2900 mg/kg *Zingiber officinale* ethanol extract exhibited body weakness and lost appetite for a few days after administration while the rat that received *Zingiber officinale* ethanol extract at 5000 mg/kg died 48 hours post-administration with signs such as; body weakness, loss of appetite, erected hair and bulging of the eyeball (Table 2).

Mortality and Oral Lethal dose (LD50) of *Zingiber officinale* ethanol extract in rats

The rat that received *Zingiber officinale* ethanol extract at 5000 mg/kg died 48 hours after administration. The LD50 was evaluated at 3,807 mg/kg. D0=2900 mg/kg and D100=5000 mg/kg. Therefore, \[ \text{LD50} = \sqrt{2900 \times 5000} \text{ mg/kg} = \sqrt{14,500,000} \text{ mg/kg} = 3,807 \text{ mg/kg}. \]
Table 2: Oral acute toxicity test of ethanol extract of *Zingiber officinale* in rats

<table>
<thead>
<tr>
<th>Phases</th>
<th>Groups</th>
<th>Number of Rats</th>
<th>Dose</th>
<th>Toxic Signs</th>
<th>Number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>10 mg/kg</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>100 mg/kg</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>1000 mg/kg</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1600 mg/kg</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>2900 mg/kg</td>
<td>Body weakness and loss of appetite</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>5000 mg/kg</td>
<td>Erected hair and bulging eyes</td>
<td>1</td>
</tr>
</tbody>
</table>

**Effect of *Zingiber officinale* prolonged administration on the liver of rats**

Photomicrograph of a liver in the control group showed normal central veins, normal hepatocytes, and sinusoids (Figure 1A). The liver of rats that received *Zingiber officinale* at 25 mg/kg also showed normal central vein and normal hepatocytes with few vacuoles (Figure 1B). The liver of rats that received *Zingiber officinale* at 50 mg/kg showed normal hepatocytes and pyknosis (Figures 1C). The liver of rats that received *Zingiber officinale* root at 75 mg/kg showed pyknosis and hepatic vacuoles (Figure 1D). In comparison, the liver of rats that received *Zingiber officinale* at 150 mg/kg showed distorted hepatocytes and pyknosis (Figure 1E).

![Figure 1: Photomicrograph of the liver of rats showing central vein (white stars), hepatocytes (white arrows), pyknosis (black arrows), and vacuole (red arrows). A= control, B= 25mg/kg *Zingiber officinale*, C= 50mg/kg *Zingiber officinale*, D= 75mg/kg *Zingiber officinale*, E= 150mg/kg *Zingiber officinale*. H&E x200 Magnification.](Image)
Effect of *Zingiber officinale* prolonged administration on the kidney of rats

Micrographs of the control kidney showed normal glomerulus, normal Bowman’s capsule, and renal tubules (Figure 2A). The kidney of 25 mg/kg and 50 mg/kg *Zingiber officinale* treated rats showed normal glomerulus and normal Bowman’s capsule with mild distortion of renal tubules (Figure 2B & 2C). The kidney of 75 mg/kg and 150 mg/kg *Zingiber officinale* treated rats showed normal glomerulus and normal Bowman’s capsule with severe renal tubular distortion (Figure 2D & 2E).

![Micrographs of the control kidney](image)

**Figure 2:** Photomicrograph of kidney of rats showing glomerulus (white stars), renal tubules (white arrows), and Bowman’s capsule (arrowheads). A= control, B= 25mg/kg *Zingiber officinale*, C= 50mg/kg *Zingiber officinale*, D= 75mg/kg *Zingiber officinale*, E= 150mg/kg *Zingiber officinale*. H&E x200 Magnification.

**DISCUSSION**

The current study suggests that ethanol extract of *Zingiber officinale* might exert a toxic effect at 2900 mg/kg and 5000 mg/kg. In the present study, the oral LD\(_{50}\) of *Zingiber officinale* ethanol extract in rats was calculated to be 3807 mg/kg. LD\(_{50}\) is defined as the dose of a substance that will result in at least 50% mortality when administered to a given number of animals. While some studies reported *Zingiber officinale* LD\(_{50}\) to be less than 2000 mg/kg (Anosike et al., 2009; Supu et al., 2018; Benny et al., 2021), others reported a higher LD\(_{50}\) of 4525 mg/kg (Abdulrazaq et al., 2012). *In-vitro* studies on human lymphocytes and ovarian cells of Hamster showed an ethanolic extract of *Zingiber officinale* to be more toxic than the aqueous extract (Unnikrishnan and Kuttan, 1988). Therefore, care must be taken with the continuous use of ethanol extract of ginger to prevent and treat diseases (Bhargava et al., 2020). The body weakness, loss of appetite, erected hair, and bulging of the eyeball observed in rats that received 5000 mg/kg could lead to death due to liver and kidney failure.

Also, 28-day repeated administration of *Zingiber officinale* at 25 mg/kg and 50 mg/kg had no effect on both liver and kidney tissues. Earlier studies reported that *Zingiber officinale* ameliorates maternal and fetal hepatorenal toxicity in rats (El-Aziz et al., 2018; Ali et al.,
2020). *Zingiber officinale* was reported to prevent liver fibrosis by reducing collagen deposit, downregulating free radical elevation, and normalizing hepatic architecture (Motawi et al., 2011).

**CONCLUSION**

*Zingiber officinale* ethanol extract is relatively less toxic, with an LD50 of 3800 mg/kg. Continuous administration of *Zingiber officinale* at doses up to 2000 mg/kg over a long period might lead to liver and kidney damage and eventual death. Therefore, care must be taken when consuming repeated doses of *Zingiber officinale* over a prolonged period despite its numerous benefits.

**ETHICS APPROVAL**

The research was approved by the Department of Human Anatomy ethical committee (UM/HA/PGR13.14-08800).

**COMPETING INTERESTS**

None

**REFERENCES**


