

Research Article

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

¹Luteino Lorna Hamman, ¹Sani Hyedima Garba, ²Tamunotonye Watson Jacks, ¹Joseph Vandi Zirahei, ^{1*}Nathan Isaac Dibal and ¹Martha Orendu Oche Attah

¹Department of Human Anatomy, University of Maiduguri, Maiduguri, Nigeria

²Department of Human Anatomy, Rivers State University, Port Harcourt, Nigeria

*Corresponding author: nathandibal@unimaid.edu.ng, doi.org/10.55639/607fedc

ARTICLE INFO:

ABSTRACT

Keyword:

Hepatocytes,
Liver,
Kidney,
toxicity,
Zingiber officinale

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 up to 1600 mg/kg 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.

Corresponding author: Nathan Isaac Dibal, Email: nathandibal@unimaid.edu.ng
Department of Human Anatomy, University of Maiduguri, Maiduguri, Nigeria

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 health care (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, grinded, 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

$LD50 = \sqrt{D0 \times D100}$ 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

Phases	Groups	Number of Rats	Treatment
1	1	3	10 mg/kg
	2	3	100 mg/kg
	3	3	1000 mg/kg
2	1	1	1600 mg/kg
	2	1	2900 mg/kg
	3	1	5000 mg/kg

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 μ m 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, $LD50 = \sqrt{2900 \times 5000}$ mg/kg = $\sqrt{14,500,000}$ mg/kg = 3,807 mg/kg.

Table 2: Oral acute toxicity test of ethanol extract of *Zingiber officinale* in rats

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

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).

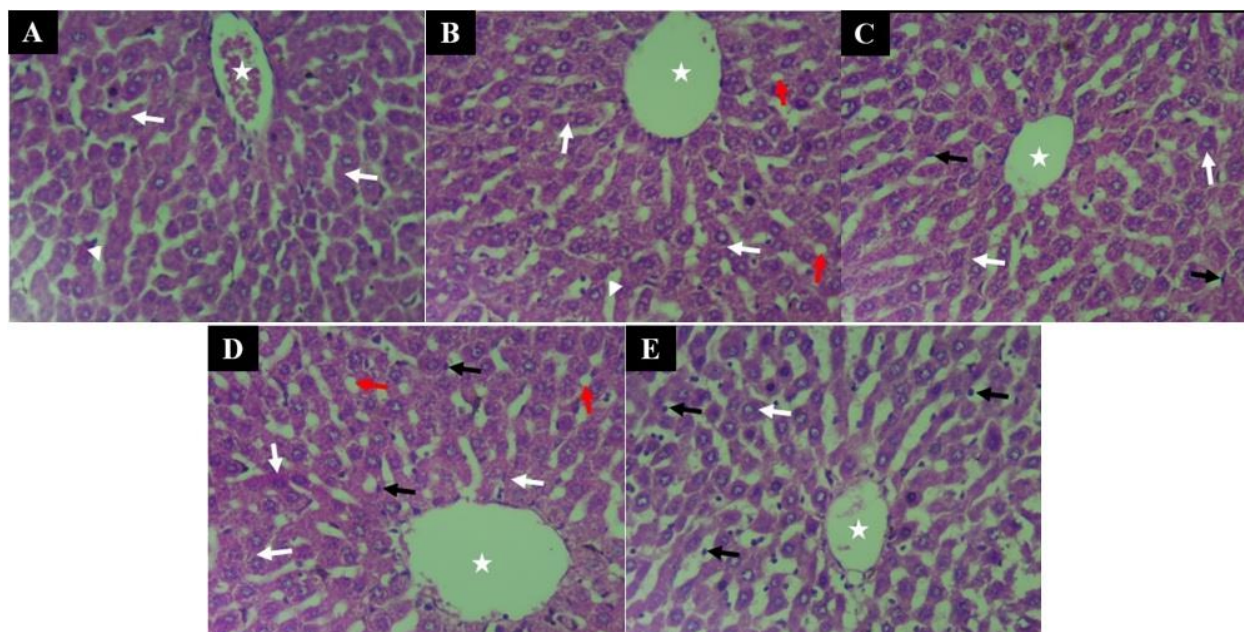


Figure 1: Photomicrograph of the liver of rats showing central vein (white stars), hepatocytes (white arrows), pyknosis (black arrows), and vacuole (red arrows). A= control, B= 25mg/kg *Zingiber officinale*, C= 50mg/kg *Zingiber officinale*, D= 75mg/kg *Zingiber officinale*, E= 150mg/kg *Zingiber officinale*.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

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).

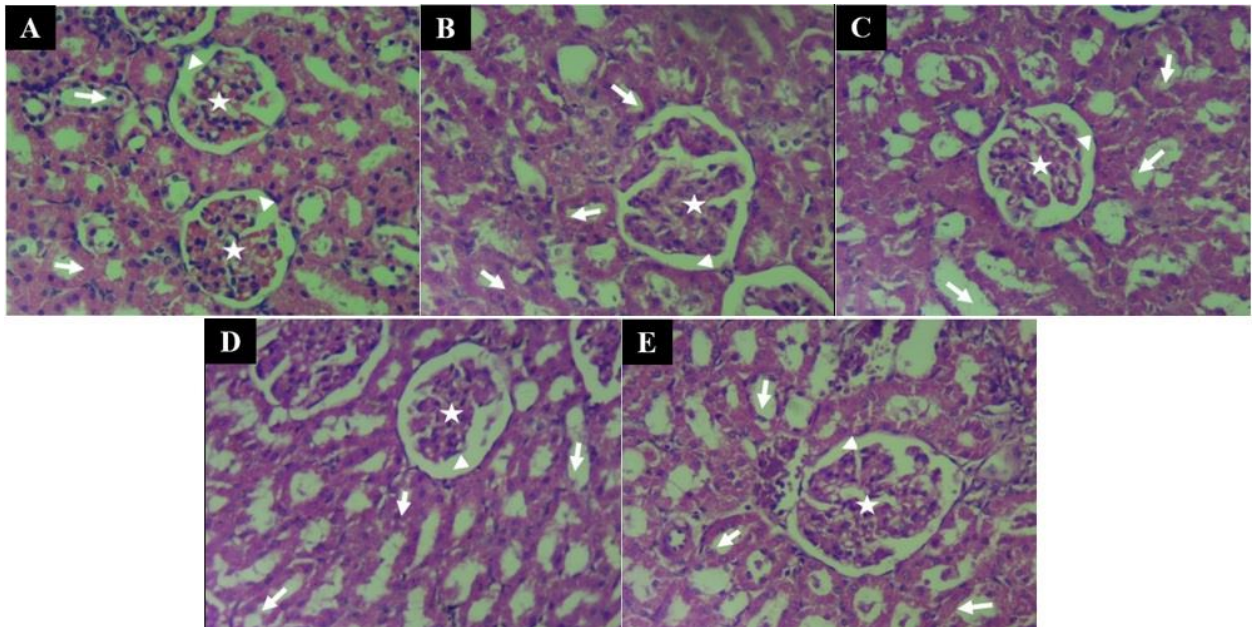


Figure 2: Photomicrograph of kidney of rats showing glomerulus (white stars), renal tubules (white arrows), and Bowman’s capsule (arrowheads). A= control, B= 25mg/kg *Zingiber officinale*, C= 50mg/kg *Zingiber officinale*, D= 75mg/kg *Zingiber officinale*, E= 150mg/kg *Zingiber officinale*. H&E x200 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 LD₅₀ of *Zingiber officinale* ethanol extract in rats was calculated to be 3807 mg/kg. LD₅₀ 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* LD₅₀ to be less than 2000 mg/kg (Anosike *et al.*, 2009; Supu *et al.*, 2018; Benny *et al.*, 2021), others reported a higher LD₅₀ 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

Abdulrazaq, N.B., Cho, M.M., Win, N.N., Zaman, R. and Rahman, M.T. (2012). Beneficial effects of ginger (*Zingiber officinale*) on carbohydrate metabolism in streptozotocin-induced diabetic rats. *British Journal of Nutrition*, 108: 1194–1201.

Ali, S.A., Kadhemi, M.A. and Ali, H.A. (2020). Protective effect of ethanolic extract of *Zingiber officinale* against mercuric chloride-induced renal toxicity in rats. *Indian Journal of Public Health Research and Development*, 11(3): 1118-1123.

Al-Nahain, A., Jahan, R. and Rahmatullah, M. (2014). *Zingiber officinale*: A Potential Plant against Rheumatoid Arthritis. *Arthritis*, 159089.

Anosike, C.A., Obidoa, O., Ezeanyika, L.U.S and Nwuba, M.M. (2009). Anti-inflammatory and anti-ulcerogenic

activity of the ethanol extract of ginger (*Zingiber officinale*). *African Journal of Biochemistry Research*, 3(12): 379-384.

Arablou ,T., Aryaeian, N., Valizadeh, M., Sharifi, F., Hosseini, A. and Djalali, M. (2014).The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. *International Journal of Food Science and Nutrition*, 65:515-20.

Bello, I., Bakkouri, A.S., Tabana, Y.M., Al-Hindi, B., Al-Mansoub, M.A., Mahmud, R. and Asmawi, M.Z. (2016). Acute and Sub-Acute Toxicity Evaluation of the Methanolic Extract of *Alstonia scholaris* Stem Bark. *Medical Sciences*, 4: 4.

Benny, M., Shylaja, M.R., Antony, B., Gupta, N.K., Mary, R., Anto, A. and Jacob, S. (2021). Acute and sub-acute toxicity studies with ginger extract in rats. *International Journal of Pharmaceutical Sciences and Research*, 12(5): 2799-2809.

Bhargava, R., Chasen, M., Elten, M. and MacDonald, N. (2020). The effect of ginger (*Zingiber officinale* Roscoe) in patients with advanced cancer. Supportive care in cancer: official *Journal of the Multinational Association of Supportive Care in Cancer*, 28(7): 3279-3286.

Braun, L.A., Tiralongo, E., Wilkinson, J.M., Spitzer, O., Bailey, M., Poole, S., et al. (2010). Perceptions, use and attitudes of pharmacy customers on complementary medicines and pharmacy practice. *BMC Complementary and Alternative Medicine*, 10:38.

Chen, J. C., Li-jian, H., Tin-yum, H., Shih-Lu, W. and Sheng-Chu, K. (2007). Ginger and its Bioactive Components inhibit E-Coli Heat Labile Enterotoxin-induced Diarrhoea in Mice. *Journal of Agricultural and Food Chemistry*, 55(21): 8390-8398.

Daily, J.W., Yang, M., Kim, D.S. and Park, S. (2015). Efficacy of ginger for treating

- Type 2 diabetes: A systematic review and meta-analysis of randomized clinical trials. *Journal of Ethnic Foods*, 2: 36-43.
- Dibal, N.I, Garba, S.H. and Jacks, T.W. (2020). Repeated 28-day oral dose toxicity of onion skin quercetin in mice. *Comparative Clinical Pathology*, 29: 1219-1227.
- Ekor, M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4(177): 1-10.
- El-Aziz, G.S.A., Mustafa, H.N., Saleh, H.A., Alshali, R.A., Alsaggaf, S. and El-Fark, M.M.M. (2018). *Zingiber officinale* alleviates maternal and fetal hepatorenal toxicity induced by prenatal cadmium. *Biomedical and Pharmacology Journal*, 11(3): 1369-1380.
- Ensminger, A.H., Ensminger, M.E., Kondale, J.E. and Robson, J.R.K. (1983). *Foods & nutrition encyclopedia*. Clovis; California, USA: Pegus Press.
- Erhirhie, E.O., Ihekwereme, C.P. and Ilodigwe, E.E. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology*, 11(1): 5-12.
- Imtiyaz, S., Rahman, K., Sultana, A., Tariq, M. and Chaudhary, S.S. (2013). *Zingiber officinale* Rosc.: A traditional herb with medicinal properties. *Association of Humanitas Traditional Medicine*, 3(4): e26.
- Katiyar, C., Gupta, A., Kanjilal, S. and Katiyar, S. (2012). Drug discovery from plant sources: An integrated approach. *AYU*, 33(1): 10-19.
- Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. and Altman, D.G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*, 8(6): e1000412.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4): 275-87.
- McGee, H. (2004). *On food and cooking. The science and lore of the kitchen 2nd Edition*. Harold McGee (Ed). New York, 435-436.
- Motawi, T.K., Hamed, M.A., Shabana, M.H., Hashem, R.M. and Naser, A.F.A. (2011). *Zingiber officinale* acts as a nutraceutical agent against liver fibrosis. *Nutrition and Metabolism*, 8: 40.
- OECD (2002). *OECD Guideline for Testing of Chemicals: Acute Toxic Class Method (No.423)*. 21 OECD, Paris.
- Parasuraman, S. (2011). Toxicological screening. *Journal of Pharmacology and Pharmacotherapeutics*, 2(2): 74-79.
- Patrice, M.B.A., Timothée, O.A., Félix, Y.H., Nicaise, B.F., Gildas, G. and Jean, A.Y. (2016). Chemical component and acute toxicity study of *Erythrococca anomala* (Euphorbiaceae). *Asian Journal of Biomedical and Pharmaceutical Sciences*, 6(57): 4-8.
- Supu, R.D., Diantini, A., Levita, J., (2018). Red ginger (*Zingiber officinale* var. *Rubrum*): its chemical constituents, pharmacological activities and safety. *Fitofarmaka Jurnal Ilmiah Farmasi*, 8(1): 25-31.
- Unnikrishnan, M.C. and Kuttan, R. (1988). Cytotoxicity of extracts of spices to cultured cells. *Nutrition and Cancer*, 11: 251-57.
- Veeresham, C. (2012). Natural products derived from plants as a source of drugs. *Journal of Advance Pharmaceutical Technology and Research*, 3: 200-201.
- Yuan, H., Ma, Q., Ye, L. and Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*, 21: 559.