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Hepatoprotective and nephroprotective property of *Zingiber officinale* leaves in acetaminophen induced liver and kidney damage in Wistar rats

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ABSTRACT

It is necessary to search for safe compounds for management of kidney and liver diseases, due to side effects of currently available drugs. This research studied the hepatoprotective and nephroprotective effects of *Zingiber officiale* leaves in Wistar rats using acetaminophen as toxicant. Thirty rats were classified to five categories comprising of six animals each. The first group was the normal control and was given only vehicle (distilled water). Groups 2 served as acetaminophen control and was given acetaminophen (750mg/kg). Groups 3, 4, 5 received acetaminophen (750mg/kg). Groups 3, 4, 5 also received silymarin (reference drug; 50mg/kg), *Zingiber officiale* (200mg/kg), *Zingiber officiale* (400mg/kg) respectively. All administration was done by oral means for 21 days. Experimental rats were thereafter euthanized by cervical dislocation. Blood and other tissues were harvested. Results showed that rats in normal control, silymarin and *Zingiber officinale* groups had significantly ($p < 0.05$) lower serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, urea and creatinine levels in comparison with rats in acetaminophen control. Rats in acetaminophen control had lower concentration of protein in serum, in comparison with normal control, silymarin or *Zingiber officiale* treated groups. Furthermore, reduced glutathione concentration was lowered and malondialdehyde concentration was raised in tissues (liver and kidney) in acetaminophen control rats in comparison with other groups. There was no significant difference in the ALP, AST, total protein, albumin, bilirubin, urea, creatinine, GSH and MDA levels of treatment groups (3, 4 and 5). These findings that methanol leaf extract of *Zingiber officiale* possess hepatoprotective, nephroprotective and antioxidant effects in rats. The study is therefore relevant in the discovery of new compounds for ameliorating the burden of liver and kidney diseases.

Key words: Acetaminophen, *Zingiber officiale,* silymarin, leaf, malondialdehyde, reduced glutathione

INTRODUCTION

Chronic liver disease and chronic kidney disease are main reasons for deaths worldwide. Treatment is expensive, hence there is need to search for alternative means of treatment (Prinja *et al.*, 2018; Gedney, 2021). Reports have shown that varieties of natural products possess hepatoprotective and nephroprotective effects. *Suaeda vermiculata*, *Descurainia Sophia*, *Tinospora crispa*, *Punica granatum*, *Arbutus pavarii*, *Costus afer*, *Pistacia atlantica* have been suggested to have hepatoprotective and nephroprotective activities (Tienda-Vázquez *et al.*, 2022). Acetaminophen (paracetamol) is a commonly used analgesic. When used wrongly, it becomes injurious to hepatocytes (Jannu *et al.*, 2012). Upon metabolism by hepatocytes, it yields N-acetyl-P-benzoquinoneimine which bonds with thiol group of cytochrome P-450 enzymes. The phenomenon results in lipid peroxidation and ultimately damage of the liver cells. Furthermore, many of the hepatotoxic agents including acetaminophen

subsequently impair functioning of the kidneys mainly through lipid peroxidation or other oxidative forms (Haque *et al*., 2014).

Zingiber officinale is commonly called 'ginger'. Traditionally, the root is utilized as a remedy for stomach upset, allergy, catarrh, and sinus problems (Kumar *et al*., 2011). The leaves have been employed as flavor for foods in Asian traditional medicine. They have been used to reduce toothache, promote digestion and reduce constipation (Shahrajabian, *et al*., 2019). They also possess antioxidant activities and phytochemicals such as flavonoids, tannins, saponins and glycosides (Ibukun and Oluwadare, 20211). With all the medicinal attributes of *Zingiber officinale* leaves, there is dearth of scientific information on its possible hepatoprotective and nephroprotective effect. Consequently, this research was embarked upon to ascertain hepatoprotective and nephroprotective activities of leaves of *Zingiber officinale*, in Wistar rats. This research is significant in the discovery of new agents for the management of liver and kidney diseases.

METHODS

Chemicals, Drugs and Kits

Ethylenediamine tetra acetic acid (EDTA), sodium citrate, 5',5'–dithiois–2-nitrobenzoicacid (DTNB), thiobabituric acid (TBA), trichloroacetic acid (TCA), hydrochloric acid, reduced glutathione (GSH) and methanol were acquired from Sigma-Aldrich , U.SA.

Silymarin was obtained from Micro Labs Limited (INDIA). Acetaminophen was acquired from Emzor Pharmaceuticals Industries Limited (NIGERIA).

AST, ALT, ALP, protein, albumin, urea and creatinine kits were from Fortress Diagnostics Limited (UK), with catalogue number BXC0202, BXC0212, BXC0183, BXC0171, BXC0221, BXC0121 and BXC0111 respectively. Total bilirubin kit was product of Randox Laboratories Ltd. (UK) with catalogue number BR411.

Experimental animals

Rats (Wistar strain and males, 120g-140g) were acquired from an animal house of University of Medical Sciences (UNIMED) and housed in cages (12 hours light / dark cycles). Prior to experimentation, acclimatization of rats was for done fourteen days. The animals consumed food and water *ad libitum*. Ethical approval for utilization of animals was granted by UNIMED Animal Research Ethics Committee with the number NHREC/TR/UNIMED-HREC-Ondo St/ 15/06/21.

Plant Materials

Zingiber officinale leaves were obtained from a garden in Ondo, Nigeria and verified by a plant biologist in Department of Plant Biology, University of Benin. Herbarium specimen with voucher number UBHz 368 was deposited at the herbarium.

Plant Extraction

Clean leaves (500g) were dried in air, pulverized, immersed in absolute methanol (4.5mL) for three days. Filtration was thereafter carried out using chiffon filter. A rotary evaporator was utilized in concentrating the filtrate (temperature, 40 °C and pressure of 150 mbar), the resulting paste was further dried in an incubator at 40°C and stored in air-tight sterile bottle. The percentage yield of the extract was 5.1%).

Experimental design

Thirty male rats (120g-140g) were randomly distributed into five classes having six animals each. Hepatotoxicity and nephrotoxicity were induced in the rats using acetaminophen and rats were concomitantly treated with plant extract or silymarin. The animals received the following by oral means for 14 days:

Group 1: Normal control (distilled water; 1mL)

Group 2: Negative control (acetaminophen 750mg/kg)

Group 3: Acetaminophen 750mg/kg + Silymarin 50mg/kg (reference drug)

Group 4: Acetaminophen (750mg/kg) + *Zingiber officinale* (200mg/kg)

Group 5: Acetaminophen (750mg/kg) + *Zingiber officinale* (400mg/kg)

Collection of Blood Samples

Following experimentation, the rats were euthanized by cervical dislocation. Blood was collected through cardiac puncture into plain bottles tubes and kept for thirty minutes at room temperature. The serum was centrifuged at 2500 g for 15minutes.

Preparation of Tissue Homogenates

Liver and kidney tissues were dissected from the animals and kept in bottles containing sodium phosphate buffer (0.4M, pH 7). One gram of liver tissue was crushed using a homogenizer in 9mL of buffer, while 0.5g of kidney was homogenized in 4.5mL of buffer. The homogenized tissues were centrifuged at 1000g for 15 minutes and the supernatant was used for subsequent analyses.

Biochemical Analysis

Liver Function Tests

AST, ALT, ALP, protein and albumin were measured in serum using standard kits

Kidney function tests

Urea and creatinine concentrations in serum were measured using kits

Measurement of oxidative stress indices

MDA and GSH levels in liver and kidney homogenates were measured to ascertain the level of oxidative damage to the organs

Malondialdehyde (MDA) Concentration

Buege and Aust (1978) protocol was employed.

Procedure

To 1mL of the homogenate of the sample was added 2mL of Trichloroacetic acid-Thiobabituric acid-HCL reagent followed by thorough mixing by swirling. The resulting solution was heated for 15minutes in boiling water bath. The flocculent precipitate, after cooling, was removed via centrifugation at 1000 revolution for 10 minutes. Absorbance of the clear supernatant was read at 535nm against a reference blank.

Calculation

The MDA concentration of the sample is calculated using the extinction coefficient of 1.56 x$10^5 M^{-1} cm^{-1}$. So that mathematically:

MDA = A x V x 1000 / Molar Extinction Coefficient of MDA x v x l x Y

Where:

A= Absorbance at 530nm

V= Total volume of the reaction mixture = 3mL

l = Light path = 1cm

v = Volume of sample = 1mL

Y= Weight of tissue in reaction mixture (g)

GSH Concentration

The protocol of Ellman (1959) was utilized in carrying out this assay.

Procedure

Liver or heart homogenate (0.5ml) was added to 0.5ml of 20% trichloroacetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 2000 rpm. The supernatant (200 µL) was then transferred to a new set of test tubes and added with 1.8 mL of the Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Absorbance was measured at 412 nm against reagent blank. Absorbance values were compared with a standard curve generated from known GSH standards.

Analysis of Data

Results were presented as Mean±SEM. Testing of difference in mean between the groups was done using one way analysis of variance (ANOVA). Tukey-Kramer test was utilized in checking the degree of significance at p values less than 0.05 ($p < 0.05$). Statistical analysis was performed and graphs were plotted using graph pad prism version 9.

RESULTS

Serum liver function tests

Table 1 depicts the liver function enzymes of rats. Results showed that ALT, AST and ALP activities of rats in acetaminophen control were significantly greater ($p < 0.05$) that those of normal control, silymarin and extract treated groups. ALT activity of rats in *Zingiber officinale* (400mg/kg) was significantly lower than that of Silymarin and *Zingiber officinale* (200mg/kg), suggesting a higher liver protection property. There was no significant difference in AST and ALP activities of Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. The normal control had significantly lower ALT activity than Silymarin and *Zingiber officinale* (200mg/kg) groups. Also, the normal control had significantly lower AST and ALP activities than Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. The serum bilirubin, total protein and albumin concentrations of rats are shown in table 2. Albumin and total protein concentration showed significant increase ($p < 0.05$) in normal control, silymarin and extract classes in contrast to acetaminophen control. Bilirubin concentration in the acetaminophen control was significantly higher ($p < 0.05$) than all other groups. There was no significant difference in bilirubin, albumin and total protein of Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. The normal control had significantly lower ALT activity than Silymarin and *Zingiber officinale* (200mg/kg) groups.

Table 1: Liver Function Enzymes of Rats

	ALT(U/L)	AST(U/L)	ALP(U/L)
Normal control	35.55 ± 2.05[a]	5.45 ± 1.35[a]	22.05 ± 1.22[a]
Acetaminophen control	97.77 ± 3.52[b]	52.47 ± 2.25[b]	60.26 ± 0.21[b]
Silymarin (50mg/kg)	49.18 ± 1.52[c]	17.96 ± 2.32[c]	45.20± 1.25[c]
***Zingiber officinale* (200mg/kg)**	44.39 ± 1.32[c]	11.19 ± 1.33[c]	42.75 ± 2.23[c]
***Zingiber officinale* (400mg/kg)**	31.30 ± 2.55[a]	14.39 ± 5.20[ac]	40.22 ± 3.07[c]

Results are shown as Mean±SEM (n = 6 per group). Different alphabets in same column indicates significant difference between means ($p < 0.05$)

Table 2: Serum Bilirubin, Total Protein and Albumin Concentration of Rats

	Bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Normal control	0.68 ± 0.24[a]	10.32 ± 0.64[a]	5.56± 0.52[a]
Acetaminophen control	1.91 ± 0.06[b]	3.85± 0.17[b]	2.32 ± 0.23[b]
Silymarin (50mg/kg)	0.71 ± 0.44[a]	4.92 ± 0.25[c]	4.65 ± 0.34[ac]
***Zingiber officinale* (200mg/kg)**	0.57 ± 0.23[a]	4.88 ± 0.20[c]	4.81 ± 0.73[ac]
***Zingiber officinale* (400mg/kg)**	0.67 ± 0.22[a]	5.23 ± 0.15[c]	4.25 ± 0.43[ac]

Results are shown as Mean±SEM (n = 6 per group). Different alphabets in same column indicates significant difference between means ($p < 0.05$)

Kidney Function Markers of Rats

Urea and creatinine concentrations in acetaminophen control were significantly greater ($p < 0.05$) that those of normal control, silymarin and extract treated groups (figure 1). There was no significant difference in urea and creatinine concentrations of Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. The normal control had significantly lower urea creatinine concentration than Silymarin and *Zingiber officinale* (200mg/kg) groups.

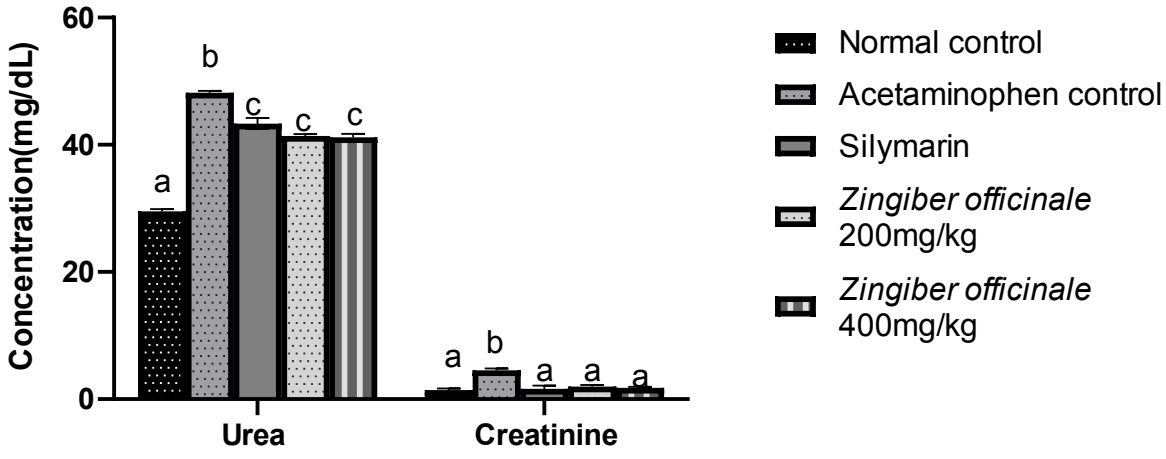

Figure 1: Kidney Function Markers of Rats

Results are shown as Mean±SEM (n = 6 per group). Different alphabets on bars for same parameter represent significant difference between means ($p < 0.05$)

Levels of GSH and MDA in Rats

Results showed that GSH levels in tissues of acetaminophen control were significantly lower ($p < 0.05$) than other groups. Furthermore, MDA levels in tissues of acetaminophen control were greater ($p < 0.05$) than those of normal control, silymarin and extract treated groups (figure 2). There was no significant difference in GSH concentration (liver and kidney) of Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. The normal control had significantly higher GSH concentration (liver and kidney) than Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. There was no significant difference in MDA concentration (liver and kidney) of Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. MDA concentration in liver of normal control was significantly lower than Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups. However, there was no significant difference in MDA concentration in kidney of normal control, Silymarin, *Zingiber officinale* (200mg/kg) and *Zingiber officinale* (400mg/kg) groups.

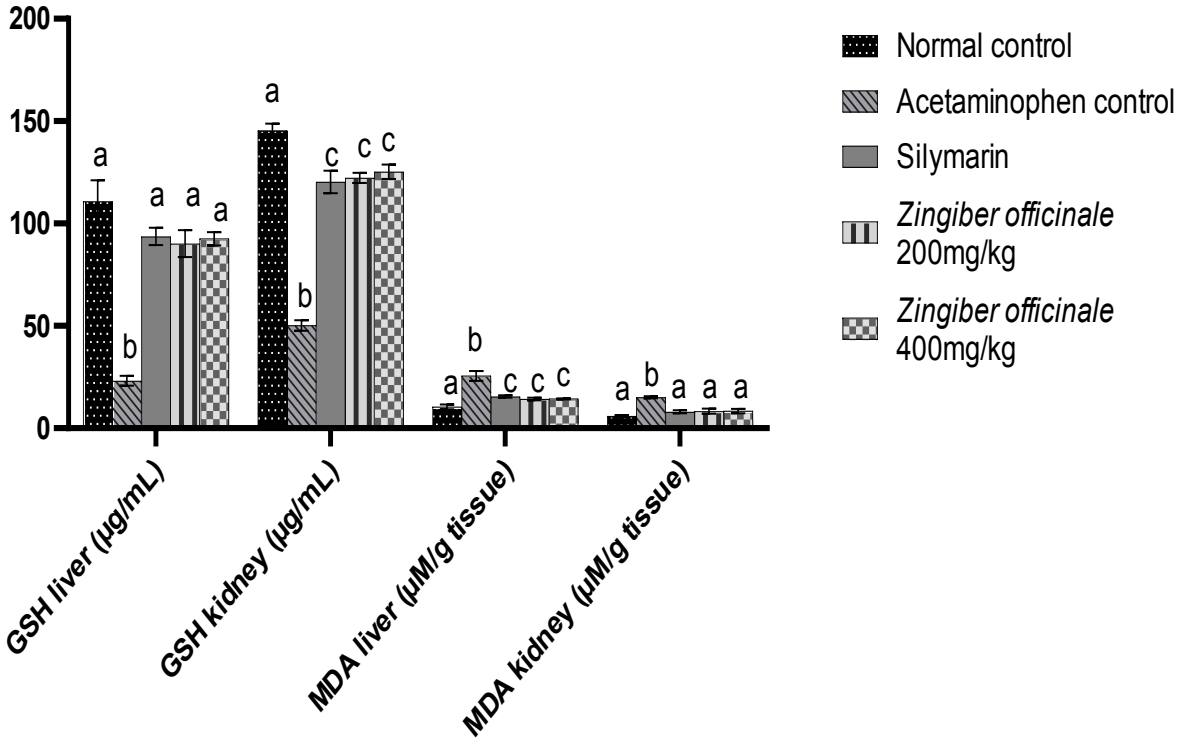

Figure 2: GSH and MDA concentrations in liver and kidney of rats

Results are shown as Mean±SEM (n = 6 per group). Different alphabets in same column represents significant difference between means ($p < 0.05$)

DISCUSSION

This research focused on assessing the hepatoprotective and nephroprotective functions of *Zingiber officinale* leaves in Wistar rats. Acetaminophen was utilized in inducing liver and kidney toxicity in rats. Studies have demonstrated the induction of liver and kidney damages with overdose of acetaminophen in animals under experimentation, therefore, for screening of hepatoprotective and nephroprotevtive agents, acetaminophen has been used as a reliable method of inducing toxicity (Gulnaz *et al.*, 2010; Parmer *et al.*, 2010)

Bilirubin, ALT, ALP and AST are mainly utilized as tracers to assess liver damage (Girish *et al.*, 2009). Administration of acetaminophen resulted in a rise in these parameters. The co administrations of plant extract or silymarin to their different groups prevented an elevation in serum AST, ALT, ALP and bilirubin level. This supports the accepted fact that AST, ALT and ALP in serum reverses to lower levels when hepatocytes are restored (Parmer *et al.*, 2010).

Nearly all serum proteins are made in liver. (Vasudevan *et al.*, 2013). The production of proteins shows the functionality of hepatocytes. Also, the extent of reduction in serum proteins is proportional to the level of liver injury (Daniel and Marshall, 1999). The rats in the acetaminophen control had significantly reduced level of total protein and albumin in comparison with, normal

control, group given the extract and silymarin group. Similar results for serum ALT, AST, Bilirubin, protein in rats that received acetaminophen and plant extract were obtained by Parmar *et al.* (2010).

Urea is obtained from protein metabolism, the amino acids from protein breakdown are deaminated to ammonia which is then converted to urea. The adequate elimination of urea from body is dependent on the renal system. Creatinine is produced as a result of transamination of amino acids such as arginine, glycine and methionine and is excreted primarily by kidneys. Serum or plasma urea and creatinine concentrations are suitable indicators of renal function, since they show glomerular filtration rate (Traynor *et al.*, 2006). In this study, treatment of rats with acetaminophen significantly elevated concentration of urea and creatinine, indicating impairment of kidney function. However, urea and creatinine levels in rats treated with *Zingiber officinale* or silymarin were significantly reduced and comparable to that of rats in normal control. Reports of Gulnaz *et al.* (2010) in which treatment of acetaminophen induced rats with garlic extract restored urea and creatinine values to normal levels corroborates with these results.

Raised MDA concentration in rats tissues intoxicated by acetaminophen is a suggestion of increased lipid peroxidation. However, a lowered lipid peroxidation in tissues of *Zingiber officinale* treated rats indicates that the plant possess antioxidant properties. GSH, a tripeptide is a non-enzymatic biological antioxidant. It participates in removal of free radicals and maintains thiol groups of proteins in bio-membrane (Prakash *et al.*, 2007). GSH level was depleted in tissues of acetaminophen control, indicating reduced antioxidant capacity in the tissues. Extract treated groups had restored levels of GSH. These findings are in agreement with that of (Verma *et al*, 2013) in which treatment of acetaminophen induced rats with extract of *Ageratum conyzoides* led to an increase in amount of GSH and reduction in MDA.
Several phytochemicals including flavonoids, tannins, saponins and glycosides have been previously identified in the methanol leaf extract of *Zingiber officinale* (Ibukun and Oluwadare, 2021). In this study, the observed antioxidant, hepatoprotective and nephroprotective effects of the methanol leaf extract of *Zingiber officinale* may be mediated by these phytochemicals in the extract.

CONCLUSION

This study demonstrated that *Zingiber officinale* leaf extract has significant antioxidant, hepatoprotective and nephroprotective activities and may be a source of agents in ameliorating liver and kidney diseases.

AUTHOR CONTRIBUTIONS

FUNDING

None

COMPETING INTERESTS

None

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