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# Aqueous extract of *Zingiber officinale* attenuates carbon tetrachloride induced hepatorenal injury in albino rats

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## ABSTRACT

The rising burden of liver and kidney diseases is taking a global dimension and could threaten public health with devastating consequences. Most patients cannot cope with the cost of conventional treatment particularly in developing nations, hence there is a dire need for a cheaply available but potent alternative in the management of hepatorenal disorders. This study therefore investigates the therapeutic potential of ginger (*Zingiber officinale*) in rat model of hepatorenal toxicity. Twenty-five adult male albino rats were randomly divided equally into five groups. Groups I and II served as positive and negative control respectively and were administered with distilled water and $CCl_4$ respectively. Group III and IV received a single intraperitoneal injection of 3 ml/kg b.w $CCl_4$ and were post-treated with 50 mg/kg b.w. and 100 mg/kg b.w of *Z. officinale* extract respectively. Animals in group V were post-treated with standard drug (silymarin (100 mg/kg b.w.)) after exposure to $CCl_4$. Activities of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), as well as levels of urea, uric acid and bilirubin were determined. Lipid profile as well as reduced glutathione (GSH) were determined in the serum and organs' homogenates. Level of reduced glutathione (GSH) as well as activities of superoxide dismutase (SOD) and catalase (CAT) were also assayed. Exposure to $CCl_4$ caused a marked derangement in lipid profile, inhibition of CAT and SOD, increase in the levels of AST, ALP, ALT, bilirubin, urea and uric acid coupled with depletion in GSH level relative to control animals. Oral intervention of *Z. officinale* extract in $CCl_4$-exposed animals resulted in the restoration of deranged lipid profiles, activity of antioxidant enzymes as well as liver and kidney biomarkers. The study suggests that *Z. officinale* has potentials that can be exploited for hepato-protection and nephroprotection.

**Keywords:** Hepatorenal injury, Nephroprotection, Plant extract, *Zingiber officinale*.

## INTRODUCTION

Carbon tetrachloride ($CCl_4$) is a major industrial and environmental pollutant that spreads quickly due to its ability to evaporate into the environment [1]. Due to the presence of phase and phase II enzymes, the liver is the primary site of xenobiotic metabolism where compounds (drugs and toxins) are biotransformed into water-soluble intermediates for enhanced excretion. Whenever the efficiency of the liver detoxication system is compromised, toxic metabolites may covalently bind to oxygen-rich macromolecules, distorting their structure and function. Free radicals can interact with critical macromolecules causing deleterious effects such as peroxidation of polyunsaturated membrane lipids of critical organs leading to a variety of pathological disorders [2]. Specifically, trichloromethylperoxy radical ($CCl_3O_2$), an intermediate of $CCl_4$ metabolism in the liver can react with lipids and proteins causing oxidative derangement in their native state. In addition, trichloromethyl ($CCl_3$) free radicals can initiate the chain of events that culminate in membrane lipid peroxidation with devastating effects such as compromised mitochondrial permeability, dysfunctional endoplasmic reticulum and loss of membrane integrity as evidenced by histopathological lesions [3-5]. Reports have suggested that $CCl_4$ administration increased cholesterol, triglycerides, and free fatty acids in rat liver and kidney [6,7]. The toxicant ($CCl_4$) has also been identified a culprit in male genotoxicity in mouse bone marrow and germ cells [6,7].

Nowadays, the search for new drugs for the management of diseases has centered on medicinal plants. These drugs play an important role in the renewal of hepatocytes, which may enhance xenobiotic metabolism and management of diseases [8]. Ginger, the rhizome of *Zingiber officinale* Roscoe, is a crop largely distributed across the tropics of Asia, Africa, America, and Australia [9]. It has been used as spice and medicine for over 2000 years and more recently as dietary supplement [10]. *Z. officinale* has a pungent flavor due the presence of volatile oils with potential analgesic, sedative, antipyretic, and antibacterial properties [11]. *Z. officinale* is a rich source of phytochemicals with protective effects against several diseases. In recent times, the global burden of liver, kidney and heart diseases is on the increase. If left unchecked, it can threaten global public health with devastating consequences. Considering the wide usage of *Z. officinale* in traditional medicine, there is a need to investigate its

medicinal potential in the management of liver and kidney diseases This is the rationale behind this study.

## MATERIALS AND METHODS

### Collections of plant materials

Samples of *ginger rhizome* was purchased from the main market (Oja – Oba) in Ado - Ekiti, air dried, pulverized and stored in an airtight container.

### Reagents and Chemicals

Randox Biochemical kits were used for all biochemical parameters determined.

### Extraction of the extract

Rhizomes of *Z. officinale* were chopped to increase its surface area and air-dried for 43 days. The air-dried samples were ground to fine powder using a blender. Five hundred (500) g of the powdered ginger was soaked in 3000 ml of distilled water for 72 hours. It was then filtered using cheese cloth, and the filtrate obtained was freeze-dried to obtain the crude extract, which was kept in a closed container and refrigerated.

### Animals

Twenty-five (25) male Wistar albino rats weighing 120 g – 200 g was obtained from a reputable the animal breeding colony. They were acclimatized for 2 weeks and housed in clean wire meshed cages under standard conditions temperature (24 ± 1 ºC), relative humidity, 12 /12-hour light and dark cycle. They were given unrestricted access to their feed and portable water daily. A good hygiene was ensured by routine changing and replacement of rat beddings every day for the period of experiment. Experimental animals were handled as described by the National Institutes of Health guide for care and use of Laboratory animals (NIH Publications No 8023, revised 1978). The animals, (25 male Wistar albino rats) were randomly divided into five groups (I-V) of five animals each as indicated in Table 1.0:

**Table 1.0:** Experimental Treatment of Animals

| Group | Treatment |
|---|---|
| I | Animals that were not exposed to $CCl_4$ at all |
| II | Animals were exposed to 3 ml/kg bw. $CCl_4$ only without treatment with extract |
| III | Animals were exposed to 3 ml/kg bw. $CCl_4$ and treated with 50 mg/kg bw of *Z. officinale* extract |
| IV | Animals were exposed to 3 ml/kg bw. $CCl_4$ and treated with 100 mg/kg bw of *Z. officinale* extract |
| V | Animals were exposed to 3 ml/kg bw. $CCl_4$ and treated with 100 mg/kg bw of silymarin |

### Preparation of homogenates and serum

Rats were dissected and portion of whole blood was collected in plain sample bottles and allowed to stand for 1 h. Serum was prepared by centrifugation at 3000 rpm for 15 min at 25°C. The clear supernatant was collected by decantation and used for the estimation of serum biochemical parameters. The liver, heart and kidney were excised using surgical scissors and forceps. They were trimmed of fatty tissue, washed in distilled water, blotted with filter paper and weighed. They were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm at 4 ˚C for 30 min. The supernatant obtained was collected and stored under 4 ˚C and then used for biochemical analyses.

### Serum Enzyme Biomarkers

### 1. Assay of Aspartate Aminotransferase (AST) Activity

AST activity was determined according to the principle described by Reitman and Frankel [12]. One hundred microliter each of serum, liver, kidney and heart homogenates was measured in separate test tubes and mixed with 100 mM potassium phosphate buffer (pH 7.4), 100 mM L-aspartate and 2 mM α-oxoglutarate. The reaction mixture was then incubated for 30 min at 37 ºC. Five hundred microliters of 2 mM dinitrophenylhydrazine was added to the reaction mixture and re-incubated for 20 min at 25 ºC. Finally, 5.0 ml of 0.5 M NaOH was added and the absorbance of the resulting solution was read at 546 nm against the reagent blank.

### 2. Assay of Alanine Amino transferase (ALT) Activity

Activity of ALT was determined according to the method of Reitman and Frankel [12]. Five hundred microliter of a reagent A containing 100 mM potassium phosphate buffer (pH 7.4), 0.2 M L-alanine and 2 M α-oxoglutarate (2.0 mol/l) was added to 0.1 ml of sample and the mixture was incubated for 30 min at 37 ºC. Furthermore, 0.5 ml of reagent B containing 2, 4-dinitrophenylhydrazine (2.0 mmol/l) was added and the resulting solution re-incubated at 20 ºC for 20 min. Finally, 5 ml of NaOH was added and the solution was allowed to stand for 5 min at 25 ºC and the absorbance was read at 546 nm against the blank.

### 3. Assay of Alkaline Phosphatase (ALP) Activity

ALP activity in the serum and organs' homogenates was measured as described by Englehardt *et al*. [13] using commercial assay kits (Randox laboratories, UK) according to manufacturer's instructions contained in the kit's manual. Briefly, 1.0 ml of a reagent containing: (1 M diethanolamine buffer (pH 9.8), 0.5 mM $MgCl_2$ and 10 mM p-nitrophenol phosphate as substrate) was added to 20 µl of sample and mixed thoroughly. Absorbance of the final reaction mixture was taken at 405 nm for 3 min at intervals of 1 min.

### Lipid Profile

### 1. Estimation of Total Cholesterol Level

Total cholesterol level was determined as described by method of Trinder [14] using commercially available kits (Randox laboratories, UK). Ten microliters (10 µl) of standard and 10 µl each of samples were measured into separate labeled test tubes. Thereafter, 1 milliliter of working reagent [Pipes buffer (80 mM at pH 6.8), 4-aminoantipyrine (0.25 mM), phenol (6 mM), peroxidase (≥ 0.5 U/ml), cholesterol esterase ion (≥0.15 U/ml) and cholesterol oxidase (0.10 U/ml)] was added, thoroughly mixed and incubated for 10 min at 25 °C. Absorbance of the resulting solution was taken at 500 nm against the reagent blank.

### 2. Evaluation of Concentration of Triglyceride

Triglyceride level was determined according to the method of Tietz, [15] using commercially available kits (Randox laboratories, UK). Ten microliter of triglyceride standard and 10 µl each of serum and organs' homogenates were measured into separate labeled test tubes. One milliliter of the working reagents containing : R1a (buffer) made

up of: 40 mM Pipes buffer (pH 7.6), 5.5 mM 4-chloro-phenol, 17.5 mM magnesium-ion; R1b (enzyme reagent containing 0.5 mM 4-amino phenazone, 1 mM ATP, lipase ((≥150 U/ml), glycerol-kinase (≥0.4U/ml), glycerol-3-phosphate oxidase (≥1.5 U/ml) and peroxidase (≥0.5 U/ml)) was added, mixed thoroughly and incubated at 25 °C for 10 min. Absorbance of the final reaction solution was read at 546 nm against the blank.

**3. High Density Lipoprotein (HDL-c)-Cholesterol Assay**

The method described by Grove [16] was adopted in the estimation of HDL- cholesterol in the serum and organs' homogenates. Briefly, reaction mixture containing 200 µl of serum, 200 µl of the cholesterol standard, 500 µl of the diluted precipitant R1 (0.55 mM phosphotungstate, 25 mM magnesium chloride) was mixed thoroughly and incubated at 25 °C for 10 min. The mixture was then centrifuged for 10 min at 4000 rpm to obtain a clear supernatant, which was separated off within 2 h. Cholesterol content in the serum and organs' homogenates was then estimated using the CHOD-PAP reaction method. One hundred microliter of the sample supernatant was added to 1 ml of cholesterol reagent and mixed together in a test tube. The standard test tube contained 100 µl of the cholesterol standard supernatant and 1 ml of cholesterol reagent. The reagent mixture was mixed thoroughly and incubated for 10 min at 25 °C. Absorbance of the sample ($A_{sample}$) and standard ($A_{standard}$) was then measured within 60 min at 500 nm against the reagent blank.

**4. Low Density Lipoprotein (LDL) - Cholesterol Determination**

The concentration of low-density lipoprotein in the serum was calculated as described by Friedwald *et al.* [17].

**Antioxidant assay**

**1. Determination of Catalase Activity**

Catalase activity assay was performed as described by Sinha [18]. Two hundred microliter of serum and organs' homogenates was mixed separately with 0.8 ml distilled $H_2O$. The reaction mixture contained 2 ml 800 µM hydrogen peroxide and 2.5 ml of potassium phosphate buffer in a 10 ml flat bottom flask. Appropriately diluted enzyme aliquot was rapidly mixed with the reaction mixture by a mild swirling motion at 25 °C. One milliliter of the reaction mixture was withdrawn and released into 1 ml dichromate/acetate reagent at 60 s interval. The hydrogen peroxide content of the withdrawn sample was determined as reported by Sinha [18].

**2. Determination of Superoxide Dismutase (SOD) Activity**

Enzymatic activity of SOD was assayed by the method of Misra and Fridovich [19]. An aliquot of 10-fold dilution of serum and organs' homogenates was added in separate test tubes to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate in the spectrophotometer. Enzymic reaction was initiated by adding 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture and thorough mixing was achieved by gentle inversion of the reaction contents. The reference cuvette contained all assay components except the enzyme which was replaced with water. Increase in absorbance at 480 nm was monitored for 150 s at 30s interval.

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min of catalysis.

**3. Determination of Reduced Glutathione (GSH) Level**

Level of GSH in the serum and organs' homogenates was determined according to the method of Beutler *et al.* [20]. Appropriate volume of 10-fold dilution of serum and organs' homogenates was added to 3 ml of precipitant, mixed thoroughly and incubated for 5 min at 25 °C and then filtered. One milliliter of the filtrate was added of 4 ml of 0.1 M potassium phosphate buffer and 0.5 ml of Ellman's reagent. A blank, containing all assay components except the sample, was prepared was prepared. Absorbance of the resulting solution was read at 412 nm against reagent blank.

**4. Determination of Total Protein (TP) in Serum**

Total protein content of the serum and organs' homogenates was determined following the Biuret method as described by Weichselbaum [21] using commercially available kits (Randox laboratories, UK).

**Statistical Analysis**

All values are expressed as mean ± SD. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 11.09 for windows (Anthony and Richard, 2006). The significance level was set at $p < 0.05$.

## RESULTS

**General observation**

Administration of $CCl_4$ caused a significant increase in the serum lipid profile and in the profile of lipids (cholesterol, triglycerides, and LDL) in the various organs examined while it significantly reduced the HDL activity in both the serum and the various organs (Table 2A-D). Treatment with *Z. officinale* extract at 50 and 100 mg/kg bw restored deranged lipid profile in a manner similar to silymarin 100 mg/kg bw – treated animals (Table 2A-D). Exposure to $CCl_4$ also caused a marked increase in AST, ALT, ALP, urea, uric acid, creatine kinase and bilirubin (Table 3A-D). However, treatment with *Z. officinale* extract reversed the toxic effect of $CCl_4$ in a dose-dependent manner comparable to silymarin. Antioxidant enzymes (SOD and CAT) as well as GSH was depleted by exposure to $CCl_4$ (Table 4A-C). Treatment with *Z. officinale* extract restored the antioxidant activity of the enzymes in a dose dependent fashion similar to silymarin treated animals.

**Table 2A:** Effect of *Z. officinale* extract on serum lipid profile of $CCl_4$-exposed rats

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w) | $CCl_4$ + Ginger (100 mg/kg b.w ) | $CCl_4$ + Silymarin (100 mg/kg b. w.) |
|---|---|---|---|---|---|
| CHOL | 73.28±1.84 | 140.21±0.00** | 41.34±0.83 | 34.94±0.25 | 77.76±0.96 |
| TRIG | 23.05±1.30 | 46.22±1.18** | 32.01±1.08 | 28.79±1.16 | 25.35±0.81 |
| HDL | 15.91±0.681 | 10.10±0.04* | 10.53±0.27* | 13.40±0.04 | 13.35±0.28 |
| LDL | 54.10±10.53 | 120.87±0.46** | 24.45±1.00 | 15.78±0.38 | 59.34±1.22 |

**Table 2B:** Effect of *Z. officinale* extract on kidney lipid profile of $CCl_4$-exposed rats

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| **CHOL** | 23.96±1.80 | 100.89±0.00*** | 92.11±40.79** | 38.49±0.65 | 24.16±5.89 |
| **TRIG** | 7.75±0.65 | 11.6±0.50** | 9.65±0.20 | 9.89±0.08 | 9.51±3.74 |
| **HDL** | 6.40±0.07 | 3.57±0.11*** | 4.71±0.29* | 4.19±0.02* | 5.61±0.25 |
| **LDL** | 16.01±1.71 | 32.6±0.68*** | 24.25±0.28* | 17.95±0.01 | 16.64±5.16 |

**Table 2C:** Effect of *Z. officinale* extract on hepatic lipid profile of $CCl_4$-exposed rats

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| CHOL | 72.96±1.01 | 119.75±1.52*** | 95.48±1.77* | 83.67±1.26* | 65.36±1.19 |
| TRIG | 1.40±0.16 | 7.57±1.63*** | 7.07±1.12*** | 2.58±0.27 | 1.74±0.25 |
| HDL | 53.62±0.11 | 34.19±0.10*** | 40.13±0.08** | 49.42±0.30 | 54.15±0.19 |
| LDL | 6.53±1.06 | 67.44±3.41*** | 31.84±1.69 | 27.82±1.26 | 6.13±1.77 |

**Table 2D:** Effect of *Z. officinale* extract on cardiac lipid profile of $CCl_4$-exposed rats

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| **CHOL** | 30.58±0.50 | 57.46±0.76*** | 42.55±1.13 | 42.11±1.58 | 32.57±9.13 |
| **TRIG** | 17.34±0.09 | 36.17±0.20** | 32.01±0.38* | 33.79±15.00* | 24.61±12.17 |
| **HDL** | 11.27±0.19 | 8.31±0.01*** | 9.61±0.24* | 10.13±0.09 | 10.64±0.13 |
| **LDL** | 16.11±0.57 | 41.93±0.74*** | 28.01±1.03 | 25.22±4.18 | 10.46±4.38 |

*Data represent mean ± SEM of an experiment performed in triplicate.*

*** indicates mild difference from the negative control*

**** indicates significantly different from the negative control*

**Table 3A:** Effect of *Z. officinale* extract on selected organs' function enzymes and biomarkers in the serum

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| ALP | 85.37±0.00 | 145.96±0.00*** | 122.32±0.01* | 107.16±1.23 | 96.39±0.00 |
| ALT | 66.09±0.88 | 106.69±1.72*** | 82.89±0.96 | 73.17±0.49 | 66.92±0.46 |
| AST | 75.19±1.06 | 113.01±1.08*** | 78.20±0.76 | 74.68±1.06 | 73.36±0.49 |
| T. BIL | 63.73±0.43 | 102.48±0.36*** | 92.51±0.44* | 87.70±0.39 | 68.11±0.67 |
| UREA | 42.80±0.68 | 99.61±0.66*** | 67.50±0.67 | 49.23±0.67 | 50.77±0.57 |
| URIC | 28.40±0.10 | 50.80±0.23*** | 38.82±0.66 | 31.12±0.78 | 31.11±0.53 |

**Table 3B:** Effect of *Z. officinale* extract on selected kidney function enzymes and markers

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| **ALP** | 85.37±0.00 | 145.96±0.00*** | 120.11±8.20 | 80.78±1.59 | 96.39±0.00 |
| **ALT** | 56.14±0.94 | 158.71±7.56*** | 76.12±1.04 | 69.35±0.4 | 64.06±0.97 |
| **AST** | 69.58±1.28 | 99.74±1.08*** | 89.97±1.06 | 72.98±1.24 | 75.27±1.47 |
| **UREA** | 52.69±0.67 | 93.08±0.00*** | 57.98±0.00 | 54.23±0.00 | 58.85±0.00 |
| **URIC** | 24.36±0.29 | 50.48±0.33*** | 42.62±0.44 | 33.53±0.29 | 25.55±0.55 |

**Table 3C:** Effect of *Z. officinale* extract on selected liver function enzymes and markers

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| ALP | 55.08±0.00 | 112.91±0.00*** | 93.64±1.49 | 67.93±14.29 | 65.41±1.38 |
| ALT | 44.99±3.23 | 116.55±3.18*** | 90.82±1.19 | 75.86±1.88 | 66.03±0.96 |
| AST | 69.07±1.55 | 104.59±4.32*** | 84.45±0.97 | 74.85±1.28 | 68.64±1.74 |
| T. BIL | 24.85±1.28 | 46.48±0.18*** | 41.05±0.595 | 32.26±0.138 | 26.65±1.15 |

**Table 3D:** Effect of *Z. officinale* extract on selected cardiac function enzymes

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| **ALP** | 23.87±1.59 | 38.56±0.00*** | 30.29±0.00 | 24.79±8.82 | 27.54±0.00 |
| **ALT** | 2.18±0.49 | 15.05±0.39*** | 9.79±0.93 | 5.56±0.67 | 4.56±0.47 |
| **AST** | 11.97±0.77 | 27.09±1.44*** | 17.79±1.57 | 12.14±1.06 | 9.88±1.05 |

*Data represent mean ± SEM of an experiment performed in triplicate.*

** *indicates mild difference from the negative control*

*** *indicates significantly different from the negative control*

**Table 4A:** Effect of *Z. officinale* extract on selected antioxidant enzymes and toxicity markers in the serum

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| SOD | 8.47 ± 0.49 | 5.02 ± 0.35*** | 5.83 ± 0.29* | 6.97 ± 1.93 | 7.80 ± 1.04 |
| CAT | 4.36 ± 0.18 | 1.84 ± 0.05*** | 3.12 ± 0.72 | 3.93 ± 0.43 | 3.77 ± 0.60 |
| GSH | 6.81 ± 1.10 | 4.53 ± 1.22** | 5.06 ± 0.41* | 6.21± 0.91 | 6.04 ± 0.87 |
| TP | 3.75 ± 0.20 | 1.67 ± 0.60*** | 2.62 ± 1.26 | 2.76 ± 0.83 | 2.59 ± 0.18 |

**Table 4B:** Effect of *Z. officinale* extract on selected antioxidant enzymes and toxicity markers in the kidney

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| **SOD** | 2.04±0.10 | 1.42 ± 0.42* | 1.63 ± 0.37 | 2.17 ± 0.19 | 2.11 ± 0.32 |
| **CAT** | 1.14 ± 0.13 | 0.26 ± 0.21 | 0.47 ± 0.05 | 1.05 ± 1.83 | 0.97 ± 0.22 |
| **GSH** | 1.93± 0.03 | 0.39 ± 0.01*** | 1.29 ± 0.06 | 1.73 ± 0.17 | 1.79 ± 0.03 |
| **TP** | 1.88 ± 0.13 | 1.07 ± 0.09*** | 1.30 ± 0.07 | 1.66 ± 0.12 | 1.58 ± 0.43 |

**Table 4C:** Effect of *Z. officinale* extract on selected antioxidant enzymes and toxicity markers in the liver

| Parameters | Negative Control | Positive Control $CCl_4$ (0.3 ml/kg b.w.) | $CCl_4$ + Ginger (50 mg/kg b.w.) | $CCl_4$ + Ginger (100 mg/kg b.w) | $CCl_4$ + Silymarin (100 mg/kg b.w) |
|---|---|---|---|---|---|
| **SOD** | 4.31± 1.47 | 2.08 ± 0.75*** | 2.40 ± 0.31* | 3.72 ± 0.43 | 4.22 ± 0.43 |
| **CAT** | 3.16 ± 0.10 | 1.90 ± 0.15*** | 2.76 ± 0.43 | 3.35 ± 0.25 | 3.02 ± 0.23 |
| **GSH** | 3.89± 0.03 | 1.05 ±0.05* | 2.46 ± 2.10 | 3.28 ± 2.32 | 2.65 ±0.02 |
| **TP** | 6.47 ± 1.21 | 2.43 ± 0.68*** | 3.13 ± 0.18 | 4.62 ± 0.43 | 5.43 ± 1.84 |

*Data represent mean ± SEM of an experiment performed in triplicate.*

** *indicates mild difference from the negative control*

*** *indicates significantly different from the negative control*

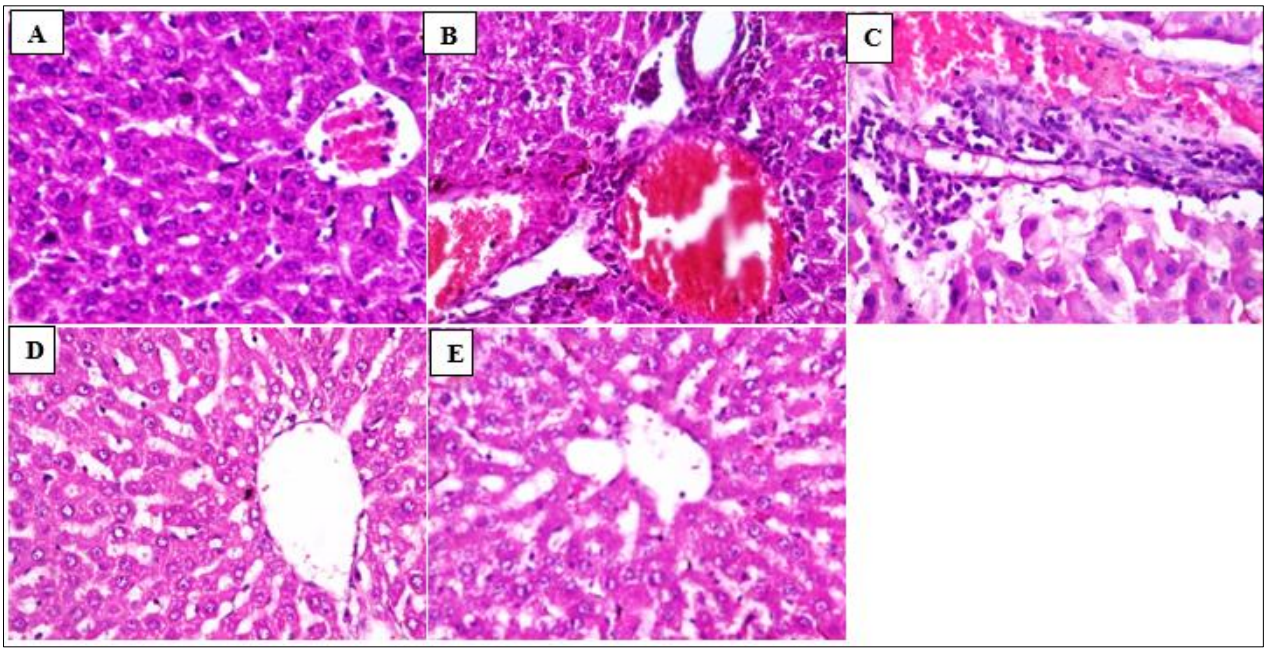


**Figure 1:** Microhistograph of liver slices of animals exposed to $CCl_4$ and treated with *Z. officinale* extract

**A: Normal Control- animals that were not exposed to $CCl_4$ at all**

Normal central venules without congestion, the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated, no noticeable pathological lesion.

**B: Animals exposed to $CCl_4$ but not treated with extract or drug**

Poor histoarchitecture, severe congestion of the portal vein as well as severe peri portal infiltration of inflammatory cells, the liver parenchyma also show area with destroyed liver plates with severe hemorrhage and necrosis, presence of degenerated liver cells, moderate aggregate of inflammatory cells in the liver parenchyma.

**C: Animals exposed to $CCl_4$ and post treated with *Z. officinale* at 50 mg/kg b.w.**

Distorted architecture, there is moderate congestion of the portal vein, notable presence of peri portal inflammatory cells, the morphology of the hepatocytes shows some degenerated hepatocytes and other nomal liver cells.

**D: Animals exposed to $CCl_4$ and treated with *Z. officinale* extract at 100 mg/kg b.w.** Normal central venules and portal tract without congestion, normal morphology of the hepatocytes and sinusoids, no pathological lesion seen.

**E: Animals exposed to $CCl_4$ and treated with silymarin at 100 mg/kg b.w.**

Central venules appeared normal with no congestion, the liver cells appeared normal in their morphology, the sinusoids are normal and not infiltrated, no noticeable pathological lesion.

## DISCUSSION

In recent times, there is a global concern about the ever-increasing burden of liver and kidney diseases which portends a major impediment to the attainment of sustainable development goal on health [22]. Hence, there is a dire need for concerted efforts at stemming the tide viz-a viz the use of medicinal plants which are locally available and potent. In the present study, *Z. officinale* extract proved effective as therapeutic agent when administered to experimental animals initially exposed to hepatotoxic and nephrotoxic assault of $CCl_4$.

Numerous experimental models of hepatorenal diseases involving rats have been employed to test the medicinal potentials of selected plants traditionally used for managing liver and kidney diseases. For healthy animals, there is a standard profile of lipids expected in the membrane of their cells. Hence, hepato-and nephro-toxicants such as $CCl_4$ are commonly used to cause derangement in the normal lipid profile as a mark of their toxicity [23, 24]. In the present study, exposure to $CCl_4$ resulted in severe derangement in both renal and hepatic lipid profile of experimental animals. Cholesterol level in the serum, and organs' homogenates surged after exposure to $CCl_4$ when compared to animals that were not exposed at all (Table 2A-D). The $CCl_4$-tiggered surge in cholesterol level implies that the toxicant or its toxic metabolites must have attacked critical enzymes of cholesterol biosynthetic pathway such that the sensing mechanisms for regulation of cholesterol biosynthesis have been repressed leading to an unregulated synthesis of cholesterol. Furthermore, since cholesterol is an integral component of the membrane, it is possible for hepatic and renal membrane structure to be altered leading to a compromise in their functions. Treatment with *Z. officinale* extract mitigated the derangement in cholesterol synthesis, thereby restoring its level back to that similar to animals exposed but treated with silymarin. This observation is in agreement with [25] and can be linked to the phytochemicals present in the extract as earlier profiled [26].

A single intraperitoneal exposure of experimental animals to $CCl_4$ caused a marked increase in triglyceride level in the serum and organs' homogenates. This further suggest that the derangement in lipid profile caused by the toxicant is not limited to cholesterol alone. This observation is in agreement with earlier reports of [22]. However, treatment of exposed rats with *Z. officinale* extract showed a significant restoration of triglyceride level in a manner comparable with animals treated with silymarin. This attests to the efficacy of the plant as potent therapeutic alternative to conventional drugs in the management of liver and kidney related ailments. The restorative potentials can perhaps be linked to the myriads of antioxidant phytochemicals present in the extract as earlier reported [25].

Toxicity of an agent is often identified by its ability to diminish the level of high-density lipoprotein (HDL) in the serum and organs homogenates (Table 2A-D). Specifically, HDL level is reliable predictor of the health status of experimental animals [27]. In the present study, exposure to $CCl_4$ triggered a significant depletion in HDL in the serum and hepatorenal tissues of experimental rats. This observation suggests a marked depletion in the antioxidant strength of experimental animals. Oral administration of Z. *officinale* relieved the

oxidative stress on the organs as the level of HDL was restored to normal. This is in agreement with the report of [27] and can be attributed to the high level of polyphenols and flavonoids in the extract as earlier reported [25]. Low level lipoprotein level surged following exposure of experimental rats to $CCl_4$. This indicates that the toxicant triggered the synthesis of 'bad cholesterol' further worsening the deranged lipid profile. Oral treatment with *Z. officinale* extract cause a reversal of the trend restoring the hepatic and renal LDL to basal comparable with animals that were not exposed to the toxicant at all. This implies that *Z. officinale* contains bioactive agents that attenuate free-radical imposed oxidative stress on the organs.

Functionality and integrity of the hepatorenal system is routinely measured using biomarkers such as AST, ALT and ALP [24]. An increase in the serum level of these biomarkers can imply an hepatorenal injury [28]. A marked increase in serum ALP level of intoxicated animals suggests a possible biliary obstruction, dehydration as well as reduced flow of blood to the kidneys as earlier reported [29]. Fundamentally, the deranged lipid profile earlier observed must have allowed for the leakage of these enzymes that should ordinarily be kept within the hepatocytes and renal cytoplasm, hence, the increased level in the blood serum. When treated with graded doses of *Z. officinale* extract, serum level of these biomarkers were restored in a manner that is comparable to intoxicated animals that treated with silymarin (Table 3A-D). This strongly suggest the potential of *Z. officinale* extract as a therapeutic candidate that can be exploited in the management of liver and kidney diseases. The observation is in line with the reports of [27]. The observed therapeutic effect can be attributed to the avalanche of phytochemicals with potent antioxidant and medicinal properties present in the extract as suggested by [26].

Following the catabolic degradation of heme in the liver, spleen and bone marrow, bilirubin is produced which is a biomarker of liver integrity. When produced, bilirubin is rapidly conjugated to glucuronic acid forming a soluble excretable product. Whenever the bilirubin level in the blood rises beyond a certain threshold, liver injury can be inferred. In the present study, liver and serum bilirubin was markedly increased after rats were exposed to $CCl_4$ relative to animals that were not exposed to toxicant. Perhaps, this observation could suggest an upregulation of heme catabolism and attack on hepatocytic membrane triggered by toxic metabolites of $CCl_4$ biotransformation. Oral administration of *Z. officinale* extract ameliorated the toxicity caused by the toxicant by restoring the bilirubin level to that similar to intoxicated animals that were treated with standard drug. This is in agreement with [26] and further buttresses *Z. officinale* in the treatment of hepatorenal diseases.

The integrity of kidney structure and function is often measured by the level of urea in the serum [30]. Animals exposed to $CCl_4$ toxicity showed significantly higher serum urea than control animals suggesting that $CCl_4$ is both a hepatotoxicant and nephro-toxicant. Administration of graded doses of *Z. officinale* extract via oral gavage showed marked restoration of urea level relative to animals that were expose to the toxicant but not treated at all with the extract. Specifically, the restoration was comparable to animals treated with silymarin after exposure, suggesting the potential of the plant in the management of renal com plications.

Owing to its correlation with the onset of certain diseases such as renal insufficiency, obesity, diabetes and hypertension, uric acid has been routinely used as a marker for monitoring renal function [31]. In the present study, a single intraperitoneal exposure of experimental animals to $CCl_4$ caused a marked increase in serum uric acid when compared to animals that were not exposed. Treatment with graded doses of *Z. officinale* extract reversed the toxic effect of $CCl_4$ restoring the uric acid level in the serum and kidney homogenates to amount comparable to intoxicated animals that were post treated with silymarin. This suggests the potential of *Z. officinale* in the management of free radical induced renal diseases.

Endogenous antioxidant enzymes such as superoxide dismutase and catalase (CAT) have been pivotal in offering protection against reactive oxygen species in the physiological system [32]. These two enzymes work in synergy in the conversion of superoxide radicals to hydrogen peroxide and its subsequent scavenging and deradicalization [33]. Animals exposed to $CCl_4$ in the present study showed a significant depletion in SOD and CAT activities in the serum and organs' homogenates relative to unexposed animals (Table 4A-C). This suggests an unabated surge in superoxide radicals in the serum and organs of exposed rats. Post treatment with *Z. officinale* extract relieved the inhibition of CAT and SOD in the serum and organs homogenates, suggesting that specific phytochemicals present in the extract played a role. Specifically, agents such as polyphenols and flavonoids present in the extract could be responsible for such restorative effect as earlier reported.

It has been suggested that reduced glutathione (GSH) is a reliable predictor of the health status of animals [34]. Agents that deplete GSH level in an organism can be said to be toxic. Administration of $CCl_4$ in the present study caused a marked depletion in GSH in the serum and organs' homogenates of rats, implying that $CCl_4$ is both toxic to the liver and kidney. Treatment of exposed animals with the extract of *Z. officinale* triggered a significant restoration of GSH level in intoxicated animals, although un a dose -dependent fashion. This observation implies that Z. officinale has vast medicinal potential in the management of liver and kidney diseases.

Liver histoarchitecture (Figure 1(A-E)) that was distorted by $CCl_4$ exposure was restored to normal after post treatment with extract of *Z. officinale*. This implies that the free radical induced hepatic damage was cured by the extract. This observation can be linked to the presence of polyphenols and flavonoids present in the extract, further attesting to the therapeutic potentials of the plant in the management of liver diseases.

## CONCLUSION

*Z. officinale* extract ameliorated deranged biochemical parameters such as lipid profile, liver and kidney biomarkers as well as antioxidant enzymes such as catalase and superoxide dismutase. Hepatic and renal histoarchitecture that was distorted by the toxicant was also restored by treatment with *Z. officinale* extract. From the foregoing, *Z. officinale* is a potent medicinal candidate that can be employed in the treatment of liver and kidney related diseases.

### Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by Committee on Care and Use of Experimental Animals, Ekiti State University, Ado Ekiti. The study was carried out in compliance with the ARRIVE guidelines. Ethical approval with certificate number ORD/AD/EAC/22/113 was obtained from the Office of Research and Development, Ekiti State University, Ado Ekiti.

### Consent for publication

Both authors read and agreed that the manuscript be published in its present form.

### Availability of data and material

Data and materials related to the present work will be made available on

request by the corresponding author

### Competing interests

Authors declare that there is no conflict of interest of any kind.

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**Authors' contributions**

O.T. designed the work, collected data, wrote the methods and discussion and edited the final manuscript,

O.J.A. wrote part of the manuscript and analysed the data.

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