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Antioxidant potency of n-butanol fraction of *Ficus glumosa* leaves against oxidative stress induced by carbon tetrachloride in the kidneys of rats

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Abstract. *Abu MS, Yakubu OE, Onuche JI, Tatah SV. 2022. Antioxidant potency of n-butanol fraction on* Ficus glumosa *leaves against oxidative stress induced by carbon tetrachloride in the kidneys of rats. Nusantara Bioscience 14: 40-46.* The kidneys play several essential roles in the body, including regulating the water and ions reabsorb from glomerular filtrate in kidney tubules. It is controlled by several hormones such as antidiuretic hormone (ADH), aldosterone, and angiotensin II. This report presented the repairing effect of the n-butanol fraction of *Ficus glumosa* Delile on nephrotoxicity induced by CCl_4 in rats. Rats were divided into 7 groups with 5 animals each. Groups 1 and 2 were used as normal and vehicle controls, respectively. Group 3 was induced but treated with neither extract nor standard drug. However, Groups 3, 4, and 5 were induced by CCl_4 and administered with varying doses of the n-butanol fraction. Group 6 was induced by CCl_4 and treated with a standard antioxidant drug. The results showed that treatments with an n-butanol fraction of *F. glumosa* leave and silymarin significantly ($p<0.05$) restored the activities of SOD, GPx, and CAT comparable to normal values, i.e., 2.10±0.07 U/L, 43.8±2.49 U/L, and 34.2±2.59 U/L, respectively. In addition, the treatments reduce the MDA level in the kidney of rats treated with an n-butanol fraction comparable to 0.34±0.05 mmol/L in the normal rats. Similarly, there was a significant ($p<0.05$) reduction of urea from 5.24 mg/dL to 3.5 mg/dL (standard value) in the treated groups. Creatinine significantly ($p<0.05$) reduced from 71.2 mg/dL in the treated groups to 43.3 mg/dL in the normal group. Electrolytes, Na^+, K^+, and Cl^- levels in the CCl_4-induced rats after administration of n-butanol fraction of *F. glumosa* leaves significantly ($p<0.05$) decreased to 137.8±2.59 mmol/L, 3.98±0.54 mmol/L, and 92.8±1.92 mmol/L respectively. The significant reversal of the rats' biochemical parameters can be ascribed to the ameliorative potency of the n-butanol fraction on methanol extract of *F. glumosa* leaves on the glomerular and tubular cells, which may have improved renal function in the injured kidney.

Keywords: Antioxidant, carbon tetrachloride, *Ficus glumosa*, kidney, n-butanol fraction extract, oxidative stress

INTRODUCTION

The kidneys are organs located in the retroperitoneal space at the back of the abdominal cavity with a bean-shaped. These organs are essential to the urinary system and serve homeostatic functions such as electrolyte regulation, acid-base balance maintenance, and blood pressure regulation via salt and water balance (Vasudevan et al. 2011; Biff 2015). The kidneys also function as a natural blood filter, removing water-soluble wastes such as urea and ammonium, which are then excreted in the urine (David et al. 2015). In addition, it reabsorbs water, glucose, and amino acids into circulation and produces hormones (such as calcitriol and erythropoietin) and a key enzyme (renin) that acts in negative feedback (Le 2013). Renal failure interferes with these essential functions resulting in inefficient removal of waste products and an imbalance in osmotic pressure. It can occur quickly (acute renal failure) or gradually (acute kidney injury), often as the result of ischemia, toxins, or mechanical trauma (Setyaningsih et al. 2006; David et al. 2015).

Ficus glumosa Delile is a small to medium-sized tree with a height of 5-10 m and 2 m in diameter (Umar et al. 2013). However, it can grow into a huge tree at 2 m and 24 m in height. The branches are widely spreading, more or less horizontal, and are frequently supported by stilt roots (Alfred 2014). The bark is yellow, grey, or green-grey, smooth to somewhat rough with a few flaking pieces; the slash is reddish with white streaks; the branchlets are twiggy, finely hairy, with substantial leaf scars (Orwa et al. 2009). Bioactivities of leaf, stem bark, and gum extracts of *F. glumosa* have been previously investigated for antioxidant, anti-diabetic, anti-diarrheal, diuretic, hematological, hypolipidaemic, and toxicological effects (Mutungi et al. 2021). This study investigated the ameliorative effects of leaf extract on nephrotoxicity and oxidative stress induced by CCl_4 in albino rats.

MATERIALS AND METHODS

Fractionation of crude methanol extract of *F. glumosa* leaves

In this study were used crude methanol extract (20 g) was re-dissolved in 300 mL of distilled water. It is partitioned in a separating funnel with 400 mL of n-hexane

repeatedly with vigorous shaking. Then the mixture was allowed to stand for 30 minutes to separate into distinct hexane and aqueous layers. Next, the n-hexane fraction was collected and concentrated using a water bath. Next, to obtain the ethyl acetate fraction, the aqueous layer was repeatedly partitioned on 400 mL of ethyl acetate. Then, the aqueous layer was saturated with distilled water and repeatedly partitioned on 400 mL of n-butanol, after which the n-butanol fraction and aqueous residue were obtained. Finally, the fractions were concentrated using a water bath maintained at 45°C to obtain concentrated fractions. The concentrated fractions were kept in sealed containers and refrigerated for further use at 2- 4 °C.

Experimental design to assess the ameliorative effect of n-butanol fraction of *F. glumosa* leaves extract on CCl_4-induced kidney toxicity and oxidative stress

Animal grouping

In this study, a total of 35 albino rats were used, which were divided into 7 groups of 5 animals each:

Group 1: Normal control rats received feed and tap water only. It served as the normal control group.

Group 2: Rats were treated with 1 mL olive oil /kg body weight and served as the vehicle control group.

Group 3: In olive oil, rats were treated with 1 mL/kg body weight 50% Carbon tetrachloride (CCl4). It served as the CCl_4 control group without extract or standard drug treatment.

Group 4: Rats were treated with 1 mL/kg body weight 50% CCl_4 in olive oil + 100 mg/kg body weight/day n-butanol fraction.

Group 5: Rats were treated with 1 mL/kg body weight 50% CCl_4 in olive oil + 300 mg/kg body weight/day of the n-butanol fraction.

Group 6: Rats were treated with 1 mL/kg body weight 50% CCl_4 in olive oil + 500 mg/kg body weight/day of the n-butanol fraction.

Group 7: Rats were treated with 1 m/kg body weight 50% CCl_4 in olive oil + 100 mg/kg body weight/day of silymarin as a standard drug reference.

Ethical clearance and animal testing regulations

All animal ethical protocols were obtained and followed the animal testing regulations at Ahmadu Bello University Zaria, Zaria, Nigeria.

Induction of nephrotoxicity and oxidative stress using CCl_4 and treatment of the n-butanol fraction of F. glumosa *leaves*

On the 1st day, the experimental animals were pre-treated with 1 mL/kg body weight of 50% solution of CCl_4 in olive oil (IP). Then followed by oral administration of the CCl_4 extract intoxication after 24 hours. Then, the administration of the n-butanol fraction was continued for 21 days and challenged with 1 mL/kg body weight of 50% solution of CCl_4 once weekly. Finally, the animals were fasted for 24 hours after the last administration of the extract to be sacrificed at the end of the experiment for sample collection and analyzed subsequently (Akram et al. 2010).

Collection and preparation of animal samples

Collection and preparation of sera samples

The animals were anesthetized using chloroform and sacrificed by decapitation at the end of 21 of treatment. Then, for biochemical analysis, blood samples were collected from the throat in plain and EDTA bottles for hematological analysis. First, blood samples in plain tubes were allowed to clot; after that, then the sera were separated using Labofuge 300 centrifuge (Heraeus) at 3,000 rpm for 10 minutes. Then, the collected sera were subjected to biochemical analysis.

Collection of kidneys for homogenization

Immediately after the blood was collected, kidneys were quickly excised, then trimmed of connective tissues and rinsed with physiological saline to eliminate blood contamination, dried with filter paper by blotting, and then weighed (to calculate the relative weight) were kept on ice. Next, one gram of each kidney was randomly homogenized in 10 mL of buffer (50 mM potassium phosphate buffer, pH 7.4) using a pestle and mortar. Next, the rest of the organs for histopathological studies were placed in freshly prepared 10% formalin. Finally, the homogenate was centrifuged at 4,000 rpm (2,700 x g) for 15 minutes, and the supernatant was collected using a Pasteur pipette.

Toxicological studies on renal function of experimental animals

Determination of serum urea concentration

Serum urea concentration was assessed using the method described by (Fawcett and Scout 1960).

Principle**:** Urease breaks down urea into carbon dioxide and ammonia. Ammonia reacts with salicylate and hypochlorite to form a colored compound, dicarboxy indophenol, in an alkaline medium. The reaction is catalyzed by sodium nitroprusside. The intensity of color produced is measured spectrophotometrically at 578 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{salicylate} \longrightarrow \text{dicarboxyindophenols (blue compound)}$$

Procedure: Reagent (1 mL) containing sodium nitroprusside and urease was added into three clean test tubes that have been contained 0.01 mL sample, 0.01 mL standard reagent, and 0.01 mL distilled water and labeled as a test sample, standard, and reagent blank, respectively and mixed. Test tubes were incubated at room temperature (25-30°C) for 10 minutes. The absorbance of the test sample and standard were read against the reagent blank at 578 nm.

The serum urea concentration was calculated using the formula below:

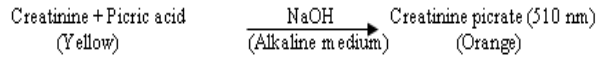
$$\text{Urea Conc.} \left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

Blood Urea Nitrogen Concentration (mg/dL) = 0.467 x Urea Concentration (mg/dL)

Determination of serum creatinine concentration

The colorimetric method was used to determine serum creatinine concentration, according to Bertels and Bohmer (1973).

Principle: Creatinine in the serum reacts with alkaline picrate to form a colored complex. The rate formation of the colored complex is directly proportional to creatinine concentration. This reaction rate reaction (intensity of orange color produced) is measured colorimetrically at 510 nm and is compared to the standard.



Procedure: A working reagent (1 mL) containing picric acid and sodium hydroxide was added into two clean test tubes. The test tubes contained 0.1 mL of the test sample and 0.1 mL of standard solution and were labeled as test samples and standard and mixed thoroughly. After 20 seconds, the standard (ST1) and test sample (TS1) absorbance were read at 510 nm. Precisely 80 seconds later, absorbance for (ST2) and (TS2) of the standard and sample were read at 510 nm against distilled water (blank).

The concentration of creatinine in serum (mg/dL) was calculated using the formula below:

$$\text{Creatinine Conc.}\,(\text{mg/dl}) = \frac{TS2 - TS1}{ST2 - ST1}\ x\ \text{Concentration of Standard}$$

(ST= Standard, TS= Test Sample)

Estimation of serum sodium, potassium, and chloride ions

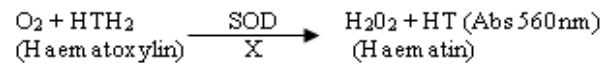
A flame photometer Model 143, equipped with an automatic diluter Model 144 (dilution ratio of 200:1) (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A.), was used. The flame photometer was calibrated using distilled water twice and a standard with a Na^+ concentration of 140 mequiv/L and a K^+ concentration of 5 mequiv/L (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A). In addition, the instrument's stability was checked with the standard solution after each sample measurement.

Determination of in vivo antioxidant status in the kidney of experimental animals

Estimation of Superoxide Dismutase (SOD) activity

Superoxide dismutase activity was measured using the method described by (Martin et al. 1987).

Principle: Auto-oxidation of hematoxylin (by increasing absorbance at 560 nm) is inhibited by SOD activity at pH 7.8. The percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample was determined by measuring the amount of haematin formed at 560 nm.



Procedure: Phosphate buffer 0.05 M, pH 7.8) (920 µL) was added to a clean test tube, followed by the addition of 40 µL of the sample (tissue homogenate), which was labeled as the test sample. A reagent test (blank/ without sample) was also prepared by adding 40 µL of assay buffer (phosphate buffer 0.05 M, pH 7.8) to another clean test tube. The mixtures were shaken and incubated for 2 minutes at room temperature. Also, 40 µL of hematoxylin was added to both sample and reagent test tubes (blank) and mixed quickly to start the auto-oxidation reaction. Following the addition of 40 µL of hematoxylin, the absorbance of the sample and reagent test (blank) was read at 560 nm every 30 seconds for 5 minutes against distilled water.

SOD activity was determined by measuring the ratios of auto-oxidation rates in the presence and absence of the sample. SOD activity in the sample was calculated as follows:

$$\text{Absorbance}_{\text{Reagent test}}\,(A_R) = \text{Absorbance}_{\text{Reagent test 2}} - \text{Absorbance}_{\text{Reagent test 1}}$$

$$\text{Absorbance}_{\text{sample test}}\,(A_S) = \text{Absorbance}_{\text{sample test 2}} - \text{Absorbance}_{\text{sample test 1}}$$

$$\%\ \text{SOD inhibition} = (1 - A_S / A_R) \times 100$$

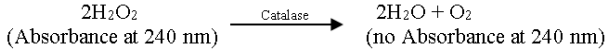
$$\text{SOD activity (U/ml)} = (1 - A_S / A_R) \times 100 \times 1.25$$

One unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the auto-oxidation of hematoxylin to haematin in 1 minute.

Estimation of Catalase (CAT) activity

Catalase activity determination follows the method described by (Aebi 1983).

Principle: Catalase scavenges hydrogen peroxide (H_2O_2) and converts it to water and molecular oxygen.



The catalase activity in the sample was determined by observing the rate of decrease in absorbance at 240 nm.

Procedure**:** Working buffer (50 mM potassium phosphate buffer, pH 7.0, 1000 µL) was added to a cuvette and used to standardize the spectrophotometer at a wavelength of 240 nm. Also, 950 µL of the mixture of working buffer (490 µL of 50 mM potassium phosphate buffer, pH 7.0) and 460 µL of 30 mM hydrogen peroxide (H_2O_2), and 50 µL of a sample (tissue homogenate) were pipetted to another clean cuvette, mixed quickly. A catalase standard was prepared by adding 50 µL of diluted catalase standard to 950 µL of working assay buffer. The decomposition rate of H_2O_2 was measured at 240 nm every 1 minute for 5 minutes. Catalase activity was determined and expressed as (U/mL) of the sample's decomposition rate given as (ΔA240nm/min).

ΔA240nm/min = Change in absorbance per minute.

$$\text{Catalase (U/ml)} = \frac{(\Delta A240\text{nm/min})}{\text{The volume of the reaction mixture}}$$

Estimation of Glutathione Peroxidase (GPx) activity

Glutathione Peroxidase Assay is a modified method of Paglia and Valentine (1967). Principle**:** Glutathione Peroxidase catalyzes the reduction of hydrogen peroxide

(H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH), forming $NADP^+$. As a result, it decreases absorbance (340 nm) and recycles the GSH. Since Glutathione Peroxidase is the reaction rate-limiting enzyme, the decrease in absorbance at 340 nm is directly proportional to the Glutathione Peroxidase activity.

Procedure: All reagents were prepared and used at room temperature, and samples (tissue homogenate) were placed on ice. The NADPH reagent (β-nicotinamide adenine dinucleotide phosphate and GSH reduced) was reconstituted with NADPH diluent (glutathione reductase in buffer with stabilizer and 4mM NaN_3) and was labeled as working NADPH. Next, 50 µL of the sample was added to a clean test tube, followed by 50 µL working NADPH. Also, 50 µL of working H_2O_2 (0.3 mL of 3% H_2O_2 diluted to 10 mL with assay buffer) was added to the sample test tube and equilibrated for 1 minute. Next, the blank tube was prepared by replacing the sample with 50 µL of distilled water. The mixtures in both tubes were transferred to cuvettes, and absorbance was read at 340 nm for 5 minutes with 30 seconds recording intervals against the blank sample.

Glutathione Peroxidase activity was calculated from the net rate and expressed as (U/mL).

$$GPx = \frac{2\,(mRates - mRateb)\,150}{6.22 \times 50}$$

Where;

$mRate_s$ = 1000 x ΔA_{340}/min of sample

$mRate_b$= 1000 x ΔA_{340}/min of blank

6.22 = NADPH 340 nm millimolar absorption coefficient at 1 cm path length.

150 µL = volume of the reaction mixture

50 µL = volume of sample

2= Correction factor for 2 moles GSH oxidized to 1 mole GSSG per mole NADPH oxidized.

Estimation of Thiobarbituric Acid Reactive Substance (TBARS)

A thiobarbituric acid reactive substance (TBARS) in the tissues was estimated using the method described by (Fraga et al. 1988).

Principle: Malondialdehyde formation is the basis for the TBA method used for the extent of lipid peroxidation evaluation. At low pH of 2-3 and high temperature (60^0C), Malondialdehyde (MDA) binds thiobarbituric acid (TBA) to form a pink complex (MDA-TBA) adduct, which absorbs maximally at 532 nm.

Procedure: Tissue homogenate (sample) (250 µL), 10 µL of BHT reagent (butylated hydroxytoluene in ethanol), 250 µL acid reagent (1 M phosphoric acid), and 250 µL of TBA reagent (2-thiobarbituric acid reconstituted with 10.5 ml distilled water) were added to a clean sample centrifuge tubes, mixed vigorously. A blank test was prepared by replacing the sample with 250 µL of distilled water. Then, both tubes were incubated for 60 minutes in a water bath at 60^0C. It was then cooled and centrifuged at 10,000 x g for 3 minutes. Finally, the reaction mixture in both tubes was transferred to cuvettes, and the absorbance was read at 532 nm for 5 minutes against a blank sample.

The concentration of TBARS is expressed in Malondialdehyde (MDA) equivalent (µM).

Molar extinction of MDA = 1.56 x 10^5 $M^{-1}cm^{-1}$

MDA concentration = Absorbance / 1.56 x 10^5 $M^{-1}cm^{-1}$

RESULTS AND DISCUSSIONS

Effect of n-butanol fraction of *F. glumosa* on oxidative stress and lipid peroxidation in the kidney

The effect of daily oral administration of the n-butanol fraction of *F. glumosa* for 21 days on the levels of some endogenous antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) and Malondialdehyde (MDA) in the kidney of CCl_4-induced rats are represented in Table 1 showing that the Malondialdehyde (MDA) level was significantly ($p<0.05$) increased. The level of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) of the CCl_4-induced control group were significantly decreased ($p<0.05$) compared to the induced rat but treated with n-butanol fraction groups. However, compared with the normal control group, there was no significant ($p>0.05$) difference in the levels of MDA and endogenous antioxidant enzymes of the induced but treated groups.

Effect of n-butanol fraction of methanolic leaf extract of *F. glumosa* on kidney biomarkers

Figure 2 depicts the urea concentrations in the serum of normal and CCl4-induced rats after the oral administration of the n-butanol fraction of *F. glumosa* for 21 days. The result showed that urea concentration in the serum of CCl_4-induced rats but not treated with the n-butanol fraction of *F. glumosa* leaves was significantly ($p<0.05$) higher than the induced but treated with n-butanol fraction groups.

Creatinine concentrations in the serum of normal and CCl_4-induced rats after oral administration of n-butanol fraction of *F. glumosa* for 21 days are represented in Figure 3. The result showed that the creatinine concentration in the serum of CCl4-induced rats but not treated with n- butanol fraction was significantly ($p<0.05$) higher than the induced but treated with n-butanol fraction and the normal control groups.

Table 2 shows the concentrations of electrolytes (sodium ion Na^+, potassium ion K^+, and chlorine ion CL^-) in the serum of the experimental albino rats after oral administration of the n-butanol fraction of *F. glumosa* for 21 days. The result showed a significant ($p<0.05$) increase in the concentrations of these ions in the serum of the CCl_4-induced but not treated with n-butanol fraction group compared to the normal and induced but treated with n-butanol fraction groups.

Table 1. MDA, SOD, CAT, and GPx concentrations of CCl_4-induced albino rats treated orally with an n-butanol fraction of *F. glumosa* leaves

Group	MDA (mmol/L)	SOD (U/L)	CAT (U/L)	GPx (U/L)
Normal control	0.34 ± 0.05^{b}	2.10 ± 0.07^{b}	43.80 ± 2.49^{b}	34.20 ± 2.59^{b}
Olive oil only	0.36 ± 0.05^{b}	2.04 ± 0.15^{b}	42.00 ± 1.58^{b}	28.60 ± 6.22^{b}
CCl_4 only	0.84 ± 0.05^{ac}	1.68 ± 0.04^{ac}	17.00 ± 2.00^{ac}	15.20 ± 0.04^{ac}
CCl_4 + 100mg Extract	0.78 ± 0.05^{ac}	1.82 ± 0.08^{bc}	32.40 ± 2.88^{ab}	26.60 ± 4.93^{b}
CCl_4 + 300mg Extract	0.44 ± 0.08^{b}	2.02 ± 0.12^{b}	33.00 ± 0.71^{b}	27.40 ± 3.21^{b}
CCl_4 + 500mg Extract	0.36 ± 0.05^{b}	2.00 ± 0.10^{b}	40.60 ± 4.39^{b}	30.40 ± 3.21^{b}
CCl_4 + 100mg sylimarin	0.36 ± 0.06^{b}	2.10 ± 0.07^{b}	42.40 ± 3.78^{b}	27.05 ± 6.40^{b}

Note: n= 5; the values are in mean±standard deviation; the values with different superscripts down the columns are significantly different at $p<0.05$; a= significantly different from the normal control group ($p<0.05$); b= significantly different from the group treated with CCl_4 without extract or the standard drug treatment ($p<0.05$); c= significantly different from the group treated with CCl_4 and the standard drug treatment ($p<0.05$); MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

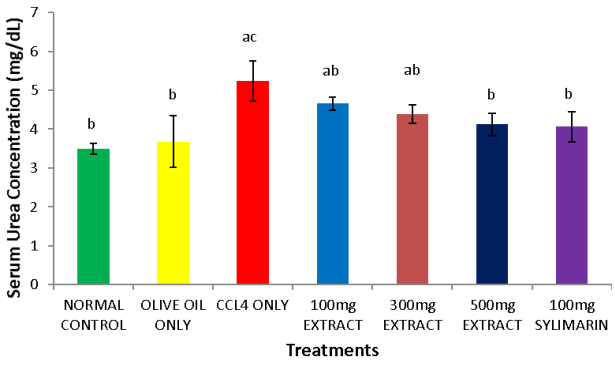


Figure 2. Serum urea levels of CCl_4-induced albino rats treated orally with an n-butanol fraction of *F. glumosa* leaves. Note: n= 5; the values are in mean±standard deviation; values with different superscripts across the bars are significantly different at $p<0.05$; a= significantly different from the normal control group ($p<0.05$); b= significantly different from the group treated with CCl_4 without extract or the standard drug treatment ($p<0.05$); c= significantly different from the group treated with CCl_4 and the standard drug treatment ($p<0.05$)

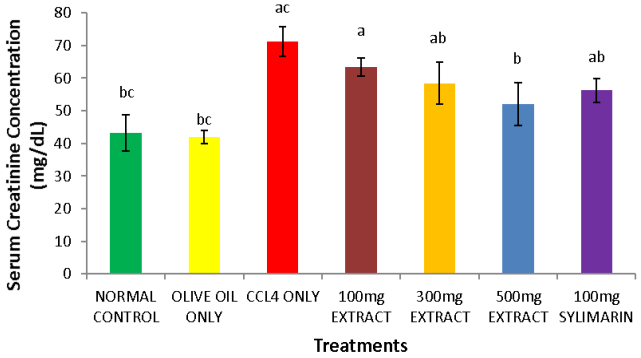


Figure 3. Serum creatinine concentration of CCl_4-induced albino rats treated orally with an n-butanol fraction of *F. glumosa* leaves. Note: n=5; the values are in mean±standard deviation; the values with different superscripts across the bars are significantly different at $p<0.05$; a= significantly different from the normal control group ($p<0.05$); b= significantly different from the group treated with CCl_4 without extract or the standard drug treatment ($p<0.05$); c= significantly different from the group treated with CCl_4 and the standard drug treatment ($p<0.05$)

Table 2. Sodium ion (Na^+), potassium ion (K^+), and chlorine (Cl^-) concentrations in the serum of CCl_4-induced albino rats treated orally with an n-butanol fraction of *F. glumosa*

Group	Na+ (mmol/L)	K+ (mmol/L)	Cl- (mmol/L)
Normal control	137.80 ± 2.59^{b}	3.98 ± 0.54^{b}	92.80 ± 1.92^{b}
Olive oil only	132.20 ± 2.88^{b}	4.24 ± 0.36^{b}	97.40 ± 2.07^{b}
CCL4 only	156.60 ± 4.51^{ac}	6.32 ± 0.41^{ac}	114.00 ± 7.91^{ac}
CCL4 + 100mg Extract	139.80 ± 2.28^{b}	4.26 ± 0.18^{b}	100.00 ± 2.92^{b}
CCL4 + 300mg Extract	132.00 ± 2.12^{b}	4.28 ± 0.18^{b}	98.60 ± 3.05^{b}
CCL4 + 500mg Extract	138.80 ± 2.77^{b}	4.30 ± 0.29^{b}	99.40 ± 2.97^{b}
CCL4 + 100mg sylimarin	137.00 ± 9.12^{b}	4.46 ± 0.23^{b}	97.80 ± 1.48^{b}

Note: n= 5; values are in mean±standard deviation; values with different superscripts down the columns are significantly different at $p<0.05$; a= significantly different from the normal control group ($p<0.05$); b= significantly different from the group treated with CCl_4 without extract or standard drug treatment ($p<0.05$); c= significantly different from the group treated with CCl_4 w and standard drug treatment ($p<0.05$)

Discussion

The levels of thiobarbituric acid reactive substances such as malondialdehyde and endogenous antioxidant enzymes are sensitive indices in free radical-induced hepatocellular damage (Meghri et al. 2019). The endogenous antioxidant enzymes are superoxide dismutase, glutathione peroxidase, and catalase. Malondialdehyde (MDA) increased significantly ($p<0.05$) in the kidney tissues of CCl_4-induced but not treated rats may be a result of increasing membrane lipid peroxidation caused by free radicals generated by CCl_4 (Shah et al. 2017). It might also be caused by the failure of antioxidant defense mechanisms to reduce free radicals' excessive formation or detrimental effects (Shaban et al. 2021). SOD, GPx, and CAT decreased significantly ($p<0.05$) in the kidney tissues of CCl_4-induced but not treated rats. It may be due to a high concentration of free radicals generated by CCl_4.

On the other hand, it may lead to inactivation (adaptive response) or inhibition of the synthetic pathways of these endogenous antioxidant enzymes. Thereby it results in low turnover (Fahima et al. 2016). However, treatments with an n-butanol fraction of *F. glumosa* leaves and silymarin could restore SOD, GPx, and CAT activity levels to almost normal and reduce the MDA level. It follows the results of Momoh et al. (2015) that the administration of *Vernonia amygdalina* Delile aqueous leaf extracts exhibited similar actions against liver damage induced by acetaminophen, equally.

The effects of the n-butanol fraction were comparable to the standard drug (Silymarin), which equally increased the activity of the endogenous antioxidant enzymes. Therefore, the result could be attributed to the free radical scavenging activity of the n-butanol fraction of *F. glumosa* leaves due to antioxidant compounds such as phenolic acids, flavonoids, and ascorbic acid (Abu et al. 2020). Therefore, it may have exerted beneficial action against pathophysiological alterations caused by superoxide, hydroxide free radicals, and hydrogen peroxide and restored the antioxidant status in the cells (Saeed et al. 2012; Letiele et al. 2020).

Furthermore, administration of CCl_4 caused nephrotoxicity as indicated by significantly elevated ($p<0.05$) urea and creatinine levels and electrolytes (Na^+ K^+ and Cl^-) concentrations in the serum of the experimental animals. These results agree with previous findings by Venkatanarayana et al. (2012) and Yacout et al. (2012). Kidney toxicity caused a rapid reduction in renal performance due to a decrease in glomerular filtration rate (GFR) and a lack of kidney ability to excrete toxic metabolites produced in the body. In addition, it results in abnormal retention of renal biomarkers like blood urea nitrogen and creatinine (Kumar et al. 2013). This study shows that significant elevation in serum urea, creatinine, and electrolyte levels can be attributed to damaged nephron structural integrity (Khan and Siddique 2012). However, the significant reduction ($p<0.05$) in urea, creatinine, and electrolytes levels in the CCl_4-induced but treated groups may be due to the repairing ability of the n-butanol fraction of *F. glumosa* leaves in the glomerular and tubular cells. Therefore, applying the n-butanol fraction of *F. glumosa* leaves may have improved renal function in kidney disorders.

In conclusion, the significant reversal of the rats' biochemical parameters can be ascribed to the ameliorative potency of the n-butanol fraction of methanol extract of F. glumosa leaves on the glomerular and tubular cells improved renal function in the injured kidney. The outcome of this research implies that the n-butanol fraction of *F. glumosa* leaves extract can be purified to manage kidney disorders.

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