

Original Research Article

EVALUATION OF THE EFFECTIVENESS OF *TELFAIRIA OCCIDENTALIS* LEAVE EXTRACT IN THE AMELIORATION OF CARBON TETRACHLORIDE INDUCED LIVER INJURIES AND OXIDATIVE DAMAGE IN RATS

ABSTRACT

Aims: The study was aimed to investigate the efficacy of *Telfairia occidentalis* (T.occidentalis) extracts in the attenuation of CCl₄-mediated oxidative stress.

Place and Duration of Study: Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria, between June 2014 and December 2015.

Methodology: Twenty four male wistar albino rats (180-220g) were divided into four groups (1-4) of six rats each. Rats in Group 1 received only water, Group 2 received single dose of equal mixture of carbon tetrachloride (CCl₄) and olive oil (50%, v/v, 1.25 ml/kg i.p.), while Group 3 and 4 received CCl₄ + 50 mg/kg and CCl₄ + 100 mg/kg of *T. occidentalis* respectively. The antioxidant activity of *T. occidentalis* was evaluated *in vitro* using DPPH and ABTS radical scavenging assay.

Results: Our results showed that CCl₄ induction elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and thiobarbituric acid reactive substances (TBARS) levels significantly (P<0.05) while activity of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were significant (P<0.05) reduced. However, administering *T. occidentalis* extract at the doses of 50 and 100 mg/kg caused reversal of the effects significantly. *T. occidentalis* was found to contain high level of total phenolic content (34.65 mg/g in GAE/g dried weight). The implication of these

results is that *T. occidentalis* may be useful in the management of oxidative stress- related organ injuries.

Conclusion: The antioxidant and hepatoprotective activities observed in this study could be due to the ability of phenolics compounds constituents of *T. occidentalis* which are able to absorb, neutralize and quench free radicals.

Key Words: *Telfairia occidentalis*, antioxidants, lipid peroxidation, carbon tetrachloride, hepatoprotective.

1. INTRODUCTION

The liver plays a major role in the digestion, metabolism and storage of nutrients [1]. Today an increasing impact of liver disease and liver injury is being recognized; presently liver diseases such as cirrhosis, steatosis, and hepatitis are a leading health problem after cardiovascular diseases, cancer and AIDS [2]. There are several lines of evidence which implicated oxidative stress and inflammation in the etiology of liver diseases, cardiovascular diseases, and cancer [3] [4]. Due to numerous side effects associated with synthetic drugs used in treating hepatic disorders traditional herbal drugs, spices, fruits, vegetables, and medicinal plants have gained popularity over the past decades owing to their safety and efficacy [5]

One plant species that is popularly consumed for its medicinal values in Nigeria is *Telfairia occidentalis* the herbal preparation of the plant has been employed in the treatment of liver problems, anaemia, chronic fatigue, diabetes, and enhancing sperm quality [6-8]. In previous studies leaf of *T. occidentalis* have been shown to be very rich in iron, antioxidants,

phytochemicals (phenolic compounds) and ascorbic acid. It has also been demonstrated to have antimicrobial, anti-inflammatory and free radical scavenging properties [9] [10].

Carbon tetrachloride (CCl₄), which produces reactive free radicals when metabolized, has been widely used as a solvent for induction of hepatic damage in animal models [11]. CCl₄ increases lipid peroxidation in hepatic cells and induces liver damage and necrosis [12]. It has been suggested that antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl₄ induced liver injury [13]. Based on the reported strong antioxidant potentials of *T. occidentalis*, the possibility of *T. occidentalis* in the prevention of CCl₄-induced toxicity is hereby investigated in this study.

2. MATERIALS AND METHODS

2.1. Reagents

6-Hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sufonic acid) ABTS, Gallic acid, thiobarbituric acid (TBA), nicotinamide adenine dinucleotide reduced (NADH) were obtained from Sigma–Aldrich Chemical Co. Ltd. (England) Nitrobluetetrazolium (NBT), 5,5_-dithiobisnitro benzoic acid (DTNB) was obtained from Fluka (Buchs, Switzerland). All other chemicals used were analytical grade.

2.2. Plant Material

The fresh leaves of *T. occidentalis* were collected from the research farm of Faculty of Agricultural science, Ladoke Akintola University of Technology, Ogbomosho. The identification and authentication of the plant was done by Prof A.J. Ogunkunle at Department of Pure and

Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, where a specimen was deposited in the herbarium. The leaves was dried at room temperature and blended to a coarse powder.

2.3. Preparation of *Telfairia occidentalis* extract.

Powdered leaves of *T. occidentalis* (200 g) were soaked in 600 ml of methanol for 72 hours. The extract was filtered and the solvent was removed from the extract with a vacuum rotary evaporator at 45°C. The concentrated dried methanolic extract was then stored at -20°C before use.

2.4. Determination of Total Phenolic Compounds in *Telfairia occidentalis*

The content of total phenolic compounds in *T. occiidental**is* was determined by Folin–Ciocalteu method as described by Miliauskas *et al.*, [14]. Briefly, 1 ml aliquots of 0.024, 0.075, 0.0105 and 0.3 mg/ml ethanolic gallic acid solutions were mixed with 5ml Folin-ciocalteu reagent (diluted ten-fold) and 4ml (75g/L) sodium carbonate. The absorption was read after 30 min at 20°C at 765 nm and the calibration curve was drawn. One ml of *T. occiidental**is* extract (1mg/ml) were mixed with the same reagents as described above, and after 1 hour the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in *T. occiidental**is* methanol extract in gallic acid equivalents (GAE) was calculated by the following formula:

$$C = c \cdot V/m'$$

Where: C-total content of phenolic compounds, mg/g plant extract, in GAE; c-the concentration of gallic acid established from the calibration curve, mg/ml; V- the volume of extract, ml; m'-the weight of pure plant methanolic extract, g.

2.5. Trolox Equivalent Antioxidant Capacity (TEAC) with Manganese dioxide

The assay was performed as previously described by Schelesier *et al.*, [15]. The ABTS radical cation was prepared by filtering a solution of ABTS (in PBS) through manganese dioxide powder. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2µm syringe filter. This solution was diluted in 5mM PBS pH 7.4, adjusted to an absorbance of 0.700 ± 0.020 at 734nm and preincubated at room temperature prior to use for 2 hours. 1 ml of ABTS^{•+} solution and various concentrations of the extracts (diluted with water) were vortexed for 45 seconds in reaction tubes, and the absorbance (734nm) was taken exactly 2 minutes after initiation of mixing. PBS blanks were run in each assay. The antioxidant activity of the extracts was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\% \text{ antioxidant activity} = ((A_{(\text{ABTS}^{\bullet+})} - A_{(\text{Extracts})}) / (A_{(\text{ABTS}^{\bullet+})}) \times 100.$$

2.6. Trolox Equivalent Antioxidant Capacity with Potassium Persulfate

The assay was performed essentially as described by Re *et al.*, [16]. ABTS radical cation was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–24 h before use. The ABTS^{•+} solution was diluted with water for the hydrophilic assay and with ethanol for the lipophilic assay and adjusted to an absorbance of 0.700 ± 0.020 at 734nm. For the photometric

assay, 1ml of the ABTS^{•+} solution and various concentrations of the extracts were mixed for 45 seconds and measured immediately after 1 minute at 734nm. The antioxidant activity of the extracts was calculated by determining the decrease in absorbance at different concentrations by using the following equation.

$$\% \text{ antioxidant activity} = ((A_{(\text{ABTS}^{\bullet+})} - A_{(\text{Extracts})}) / (A_{(\text{ABTS}^{\bullet+})}) \times 100.$$

2.7. DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging Activity

The assay was performed as previously described by Schelesier *et al.*, [17]. The radical solution is prepared by dissolving 2.4 mg DPPH[•] in 100 ml methanol. For the photometric assay 1.95 ml DPPH[•] solution and 50 µl antioxidant solution were mixed. At first, the absorbance of the disposable cuvette with 1.95 ml DPPH[•] was measured as blank, then the antioxidant solution was added and mixed. The reaction was measured at 5 min interval at 515 nm until $\Delta A = 0.003 \text{ min}^{-1}$. The anti-oxidative activity was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\% \text{Inhibition activity} = ((A_{(\text{DPPH}^{\bullet})} - A_{(\text{Extracts})}) / (A_{(\text{DPPH}^{\bullet})}) \times 100$$

2.8. Animal model and experimental design

Twenty four male wistar albino rats (180-220g) were obtained from the animal house at LAUTECH Agricultural Department, Ogbomoso, Oyo state and they were maintained under standard environmental conditions and had free access to feed and water. Animal studies were approved by the Committee for Ethical Animal Care and Alternatives to Animal Use in Research, Testing, and Education of 1986. Seven days after acclimatization, the rats were

divided into four groups with six rats each. Group 1 served as control, group 2 received single dose of equal mixture of carbon tetrachloride (CCl₄) and olive oil (50%, v/v, 1.25 ml/kg i.p.) on the 7th day. Group 3 and 4 animals were treated with *T. occidentalis* leave extract at dose level of 50 and 100 mg/kg per day p.o., respectively for 7 days and on the 7th day; a single dose of equal mixture of carbon tetrachloride and olive oil (50% v/v 1.25 ml/kg i.p.) was administered.

2.9. Preparation of serum and liver homogenate

Twenty-four hours after the animals were administered with a single dose of carbon tetrachloride (CCl₄) they were sacrificed by chloroform anesthesia. Blood samples of each animal were collected by heart puncture and were allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 4000×g for 5 min and analyzed for various biochemical parameters including serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), and serum lactate dehydrogenase (LDH). The liver samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for assay of superoxide dismutase, catalase, reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

2.10 Biochemical analysis

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in serum were determined using enzymatic kits (Labkit, Spain) according to the manufacturer's instructions

2.11 Measurement of hepatic lipid peroxidation

MDA levels were measured by the double heating method [18]. The method is based on spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 0.5mL of liver homogenate was mixed with 2.5mL of trichloroacetic acid (TCA, 10%, w/v) solution followed by boiling in a water bath for 15 min. After cooling to room temperature, the samples were centrifuged at 3000 rpm for 10 min and 2mL of each sample supernatant was transferred to a test tube containing 1mL of TBA solution (0.67%, w/v). Each tube was then placed in a boiling water bath for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was calculated based on the absorbance coefficient of the TBA–MDA complex ($\epsilon = 1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$) and it was expressed as nmol/mg protein.

2.12 Superoxide dismutase activity assay

SOD activity was measured according to method of Kakkar, et al. [19]. Assay mixture contained 0.1mL of supernatant, 1.2mL of sodium pyrophosphate buffer (pH 8.3; 0.052 M), 0.1mL of phenazine methosulphate (186 μM), 0.3mL of nitrobluetetrazolium (300 μM) and 0.2mL of NADH (750 μM). Reaction was started by addition of NADH. After incubation at 30°C for 90 s, the reaction was stopped by addition of 0.1mL of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0mL of n-butanol. Colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of NBT reduction/mg protein.

2.13 Determination of Reduced Glutathione

GSH was assayed by the method of Jollow et al. [20], with slight modification. An aliquot of 0.5mL of each tissue homogenate was precipitated with 0.5 mL of trichloroacetic acid (10% w/v). The precipitate was removed by centrifugation. 0.8 mL of the filtered sample was mixed with 0.3 DTNB (4 mg/mL) and 0.9mL phosphate buffer (0.1 M, pH 7.4). The yellow colour developed was read at 412 nm. Reduced glutathione was expressed as $\mu\text{g}/\text{mg}$ of protein.

2.14 Catalase assay activity

Catalase activity was measured by the method of Aebi [21]. An aliquot (10 μl) of each tissue supernatant was added to cuvette containing 1.99 μl of 50mM phosphate buffer (pH 7.0). Reaction was started by addition of 1000 μl of freshly prepared 30mM H_2O_2 . The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm. Activity of catalase was expressed as U/mg of protein.

2.15 Statistical Analysis

Results are expressed as means $\pm$ SEM. Statistical analyses were performed using one way analysis of variance followed by Tukey's test. All analyses were done using Graph Pad Prism software Version 5.00 and $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Trolox Equivalent Antioxidant Capacity (TEAC) assays [mmol^{-1} of three antioxidants; Trolox, Gallic acid and *Telfairia occidentalis* leave extract

In the three versions of the TEAC assay; the TEAC value of Trolox is 1.00. Gallic acid responded in all the assays as the strongest antioxidant. The TEAC value analyzes in TEAC III (hydrophilic version) was higher in Gallic and lower in *T. occidentalis* when compared with the value obtained under TEAC II and TEAC III (lipophilic version). In addition, both TEAC II and TEAC III (lipophilic version) showed comparable antioxidant activity both in Gallic and *Telfairia occidentalis* (Table 1).

Table 1. Trolox Equivalent Antioxidant Capacity (TEAC) assay of Trolox, Gallic acid and *Telfairia occidentalis*

Assay/Antioxidant	Trolox	Gallic	<i>Telfairia occidentalis</i>
TEAC II	1.00	4.40	0.99
TEAC III(hydrophilic)	1.00	5.78	0.81
TEAC III (lipophilic)	1.00	4.25	0.91

3.2. Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of *Telfairia occidentalis*

The *T. occidentalis* leave extract demonstrated a concentration dependent scavenging activity by quenching DPPH radicals (data not shown) and was compared with gallic acid, as a positive control. The IC₅₀ values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by *Telfairia occiadenalis* and gallic acid were 259.52 µg/dL and 16.33µg/dL respectively (Table 2).

3.3. The Total Phenolic Content of *Telfairia occidentalis* leaves extract

The phenolic content of *T. occidentalis* extract was determined using Folin-Ciocalteu assay and by constructing a standard curve using gallic acid. The total amount of phenolic compounds present in *T. occidentalis* was found to be 34.65 mg/g in Gallic acid equivalent (Table 2)

Table 2. Total phenolic content and DPPH radical scavenging value of *Telfairia occidentalis*

Sample	Total Phenol ^a	DPPH Scavenging activity (IC 50) ^b
<i>Telfairia occidentalis</i>	34.7	259.5 ± 7.8
Gallic	ND	16.3 ± 1.5

Each value represents the mean ± SEM. ($n=3$).

^a Total phenolic content was expressed as mg gallic acid equivalents/g dried extract.

^b Expressed as µg/mL

ND, not detected

3.4. Effect of treatment with Carbon Tetrachloride on the levels of AST, ALT and ALP activities

CCl₄ treatment resulted in significant ($p < 0.05$) rise in the activities of AST, ALT and ALP in the serum when compared to the control group (Figs. 1). Administrations of *Telfairia occidentalis* at 50mg/kg and 100mg/kg doses lower the activities of these marker enzymes significantly ($p < 0.05$).

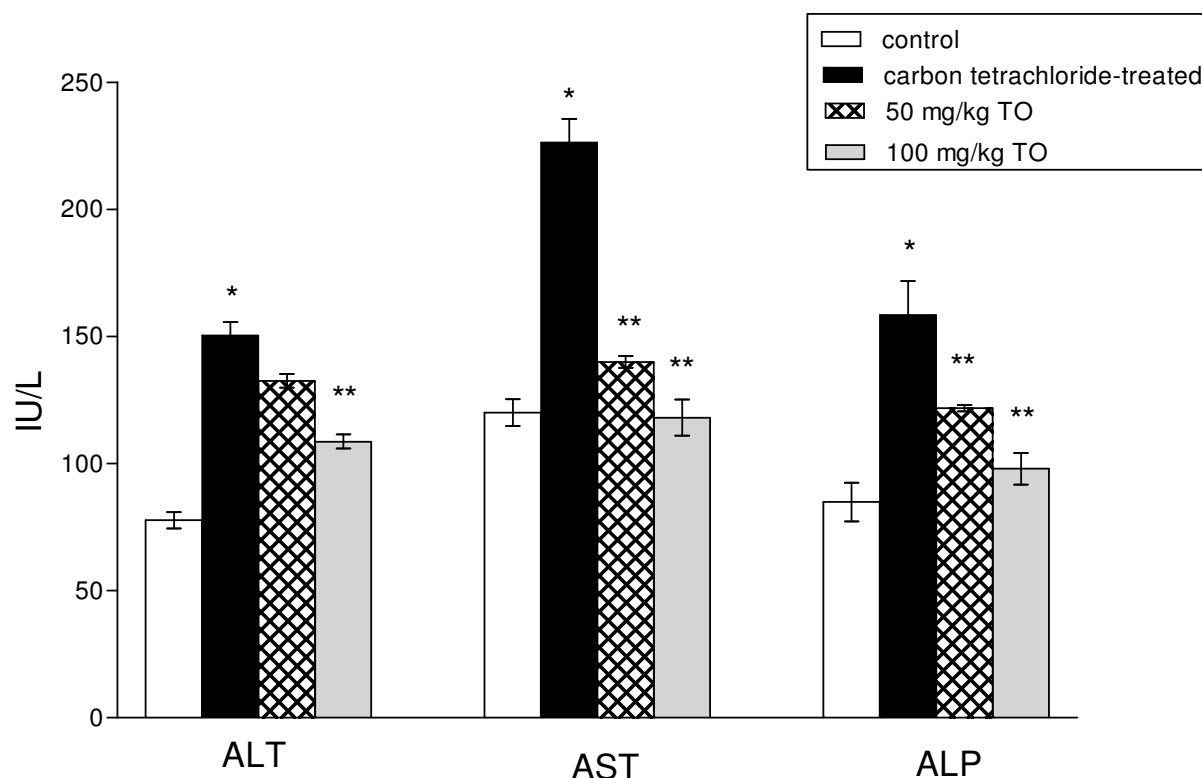
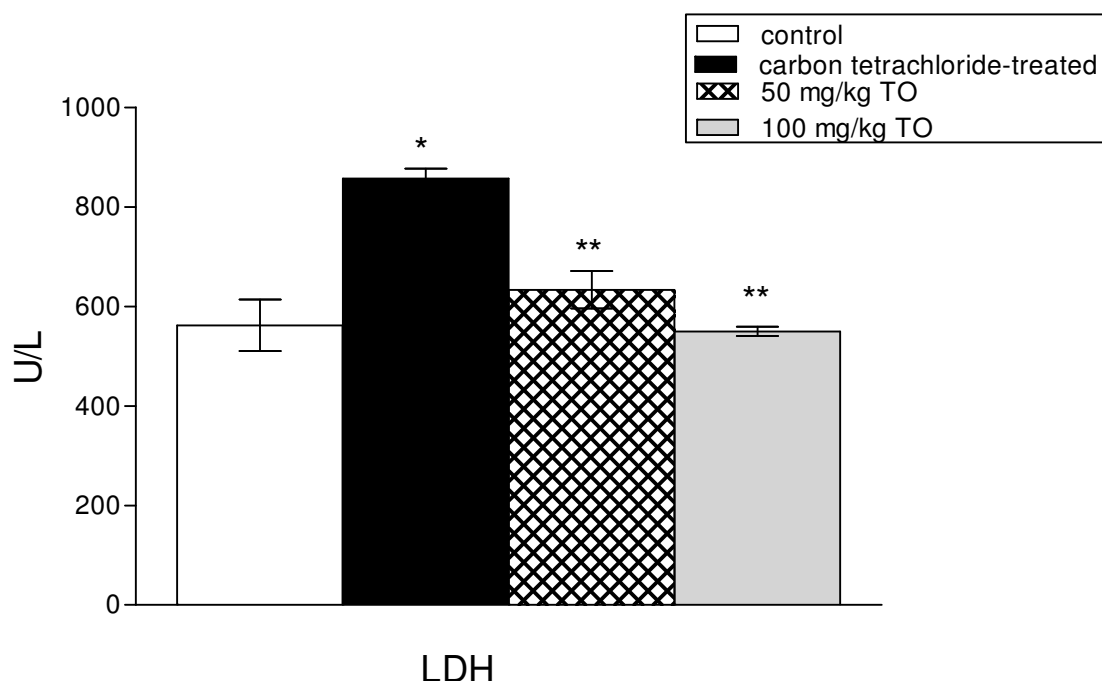


Fig.1 Effect of *Telfairia occidentalis* extract on serum levels of AST (IU/L), ALT (IU/L) and ALP (IU/L) during CCl₄ treated oxidative stress in rats. Values are mean± SEM.* Group 2 (CCl₄ treated rats) compared with Group 1 (control rats). ** Groups 3 and 4 (*Telfairia occidentalis* treated rats) compared with Group 2 (CCl₄ treated rats).

3.5. Effect of treatment with carbon tetrachloride on the level of Lactate dehydrogenase activity

CCl₄ treatment resulted in significant ($p < 0.05$) rise in the level LDH when compared to the control group (Figs. 2). Oral administrations of *Telfairia occidentalis* at two different doses (50mg/kg and 100mg/kg) lower the level of this marker enzyme, LDH significantly ($p < 0.05$)



247
 248 Fig.2 Effect of *Telfairia occidentalis* extract on serum levels of LDH (IU/L), during CCl₄induced
 249 oxidative stress in rats. Values are mean± SEM.* Group 2 (CCl₄ treated rats) compared with
 250 Group 1 (control rats). ** Groups 3 and 4 (*Telfairia occiadentalis* treated rats) compared with
 251 Group 2 (CCl₄ treated rats).

252
 253
 254 **3.6. Effect of treatment with CCl₄ on the levels of superoxide dismutase and reduced**
 255 **glutathione**

256 Administration of CCl₄ caused a significant ($p < 0.05$) decrease in SOD and GSH levels in rats
 257 when compared with normal animal. The *Telfairia occiadentalis* at 50 and 100 mg/kg showed
 258 significant ($p < 0.05$) increase in SOD and GSH levels when compared to CCl₄ treated rats. (Fig.
 259 3)

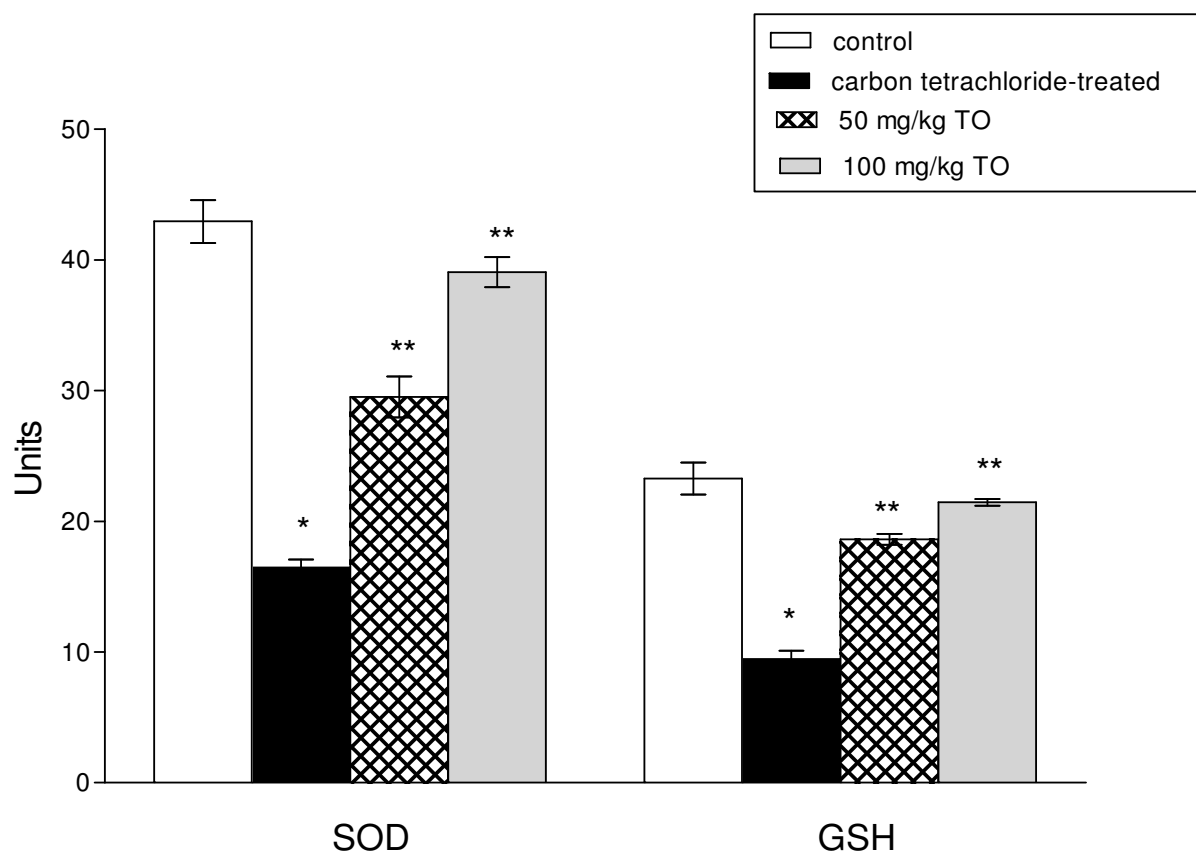
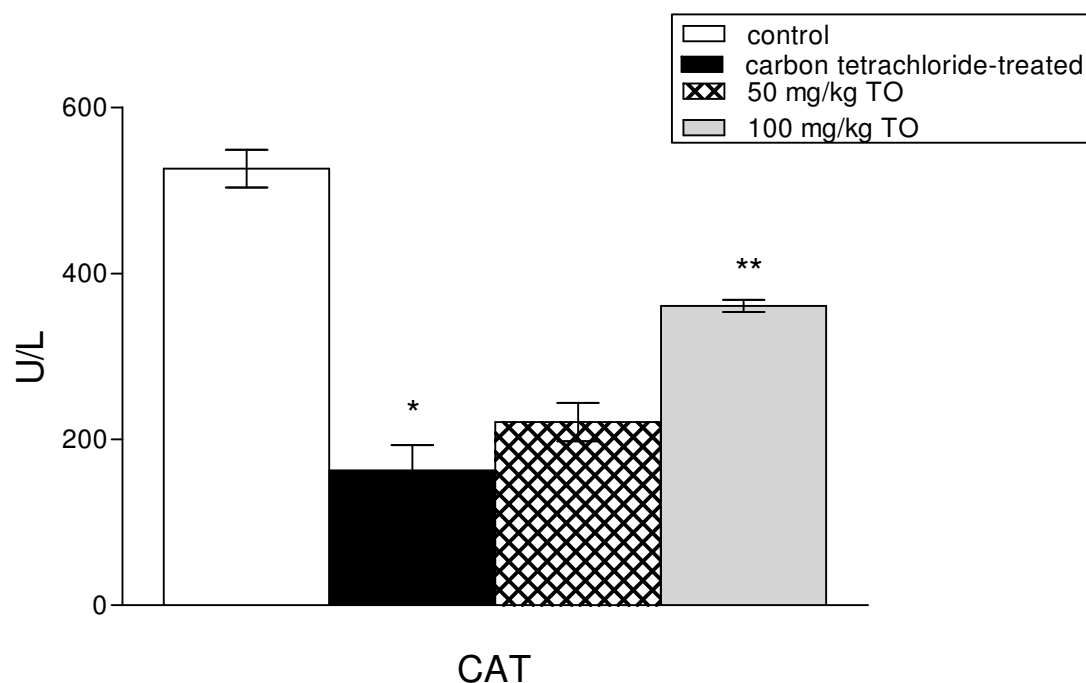


Fig.3 Effect of *Telfairia occiidentalensis* extract on hepatic level of SOD (U/mg protein) and GSH ($\mu\text{g}/\text{mg}$ protein) during CCl_4 induced oxidative stress in rats. Values are mean $\pm$ SEM.* Group 2 (CCl_4 treated rats) compared with Group 1 (control rats). ** Groups 3 and 4 (*Telfairia occiidentalensis* treated rats) compared with Group 2 (CCl_4 treated rats).

3.7. Effect of treatment with CCl_4 on the level of catalase activity

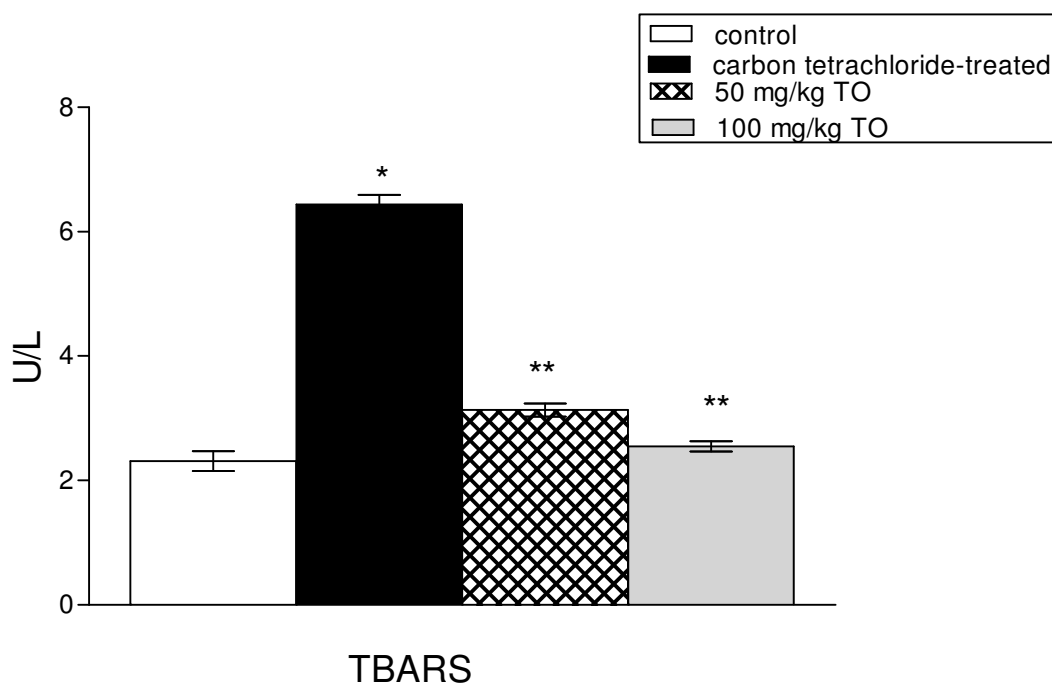
The CCl_4 -treatment caused significant ($p < 0.05$) decrease in the level of CAT in liver homogenate tissue, when compared with control group (Fig. 4). Pretreatment of rats with *Telfairia occiidentalensis* extract at the dose of 50 and 100 mg/kg resulted in significant ($p < 0.05$) increase of CAT level when compared to CCl_4 treated rats.



271
 272 Fig.4 Effect of *Telfairia occiidentalis* extract on hepatic level of catalase (U/mg protein) during
 273 CCl₄ induced oxidative stress in rats. Values are mean± SEM.* Group 2 (CCl₄ treated rats)
 274 compared with Group 1 (control rats). ** Groups 3 and 4 (*Telfairia occiidentalis* treated rats)
 275 compared with Group 2 (CCl₄ treated rats).

276 3.8. Effect of treatment with CCl₄ on the levels of thiobarbituric acid reactive substance

277 Rats treated with CCl₄ showed significant increase in Lipid peroxidation level (LPO) of liver
 278 homogenates when compared to rats in control group as shown in Fig. 5. Pretreatment with
 279 *Telfairia occiidentalis* at two different doses (50mg/kg and 100mg/kg) significantly reduced the
 280 LPO level when compared with CCl₄ treated rats.



281
 282 Fig.5 Effect of *Telfairia occiidentalis* extract on hepatic level of thiobarbituric acid reactive
 283 substance (TBARS) (nM/mg protein) during CCl₄ induced oxidative stress in rats. Values are
 284 mean±SEM.* Group 2 (CCl₄ treated rats) compared with Group 1 (control rats). ** Groups 3 and
 285 4 (*Telfairia occiidentalis* treated rats) compared with Group 2 (CCl₄ treated rats).

286 4. DISCUSSION AND CONCLUSSION

287 The change in dietary habits and chemoprevention show considerable effective strategy against
 288 oxidative stress and are the main focus of area of research these days [22]. Oxidative stress form
 289 when there is imbalance between free radical generating and scavenging systems has been
 290 implicated in the pathogenesis of wide range of disorders, including neurodegenerative disorders,
 291 cardiovascular diseases, cancer, and ageing [23]. Several epidemiological studies suggest the
 292 importance of a high consumption of secondary plant products widely distributed in fruit and
 293 vegetables in ameliorating the effects of oxidative stress [14] [15]. The current investigation was

undertaken to evaluate the possible protective effect of *T. occiidentalis* against carbon tetrachloride induced hepatotoxicity and oxidative stress in rats.

Among the various methods used to evaluate the total antioxidant activity of vegetables or other plants, the ABTS^{•+} and DPPH radical scavenging assay are among the common applied methods. In the present study, *T. occiidentalis* showed DPPH radical scavenging activity which is attributed to its hydrogen donating ability. In still another approach, ABTS method was used to measure the radical scavenging activity of *T. occiidentalis*. Based on our results, *T. occiidentalis* showed significant radical scavenging activity in a concentration dependent manner.

Antioxidant potential of *T. occiidentalis* extract against CCl₄ induced liver damaged was also evaluated in this study. In many studies, carbon tetrachloride (CCl₄) was used as a toxic substance to induce liver damage in rats [24] [25], this is due to biotransformation of CCl₄ by Cytochrome P₄₅₀ in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical [25] [26]. This formed radical has been reported to damage a number of tissues particularly the liver and kidney of many species [27]. Thus free radicals scavenging ability or the inhibition of free radicals generation are important in the protection against CCl₄ induced liver injury [28].

One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes, such as LDH, ALP, ALT and AST in the circulation after CCl₄ administration. The results obtained in the present study regarding the effect of CCl₄ intoxication

on the concentrations of LDH, ALP, ALT and AST are in agreement with those reported [29] [30]. The elevated activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of the cell membranes in liver [31]. The stabilization of these enzyme levels by the crude extract of *T. occidentalis* and its active principle is a clear indication of the improvement of the functional status of the liver.

Enhanced lipid peroxidation expressed in terms of MDA contents and reduction in liver GSH level in CCl₄ treated rats as observed in our study indicates the damage to the hepatic cells which is confirmed by the earlier reports also [32] [33]. The cleavage of CCl₄ leads to the formation of highly unstable free radicals ($\bullet\text{CCl}_3$ or $\bullet\text{CCl}_3\text{O}_2$) which initiates peroxidation [34]. Extract of *T. occidentalis* showed ability to prevent CCl₄ induced increment of MDA content, suggesting that extract inhibit lipid peroxidation and its propagation in the liver. This result is in agreement with the previous findings [35] [36]. Hence, it may be possible that the mechanism of hepatoprotection by *T. occidentalis* extract is due to its antioxidant potentials.

Carbon tetrachloride induced depletion of hepatic glutathione, superoxide dismutase (SOD) and catalase (CAT). This finding supported the hypothesis that reactive oxygen intermediates generated by CCl₄ leads to the decreased level of glutathione and exhaustion of superoxide dismutase (SOD) and catalase (CAT) [37]. The level of liver GSH as well as SOD and Catalase activities were brought to near normal in rats pretreated with the *T. occidentalis* extract at 50mg/ml and 100mg/ml prior to CCl₄-treatment. The stabilization of these enzymes by the extract is an indication of the improvement of the functional status of the liver. This can probably indicates that the *T. occidentalis* extract either increase the biosynthesis of SOD, catalase and

GSH or reduce the extent of oxidative stress leading to less degradation, or it may have both effects.

In conclusion, it may be mentioned that the altered biochemical profiles due to CCl₄ exposure is reversed towards normalization by *T. occidentalis* extract. The contents of the extract not only protect the integrity of plasma membrane but, at the same time increase the regenerative and reparative capacity of the liver. Beneficial effect of the *T. occidentalis* extract may be due to presence of some phenolic components that have membrane stabilizing effects. This phenolic compounds may directly combine with free radicals and inactivate them which may suppress the intracellular concentration of free radicals.

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