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Evaluation of the Effectiveness of *Telfairia occidentalis* Leave Extracts in the Amelioration of Carbon Tetrachloride Induced Liver Injuries and Oxidative Damage in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AOO designed the study and wrote the protocol. Author ABA managed the animals and collected all data. Author AA performed the statistical analysis and wrote the first draft of the manuscript. Author OSO did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was aimed to investigate the efficacy of *Telfairia occidentalis* (*T. occidentalis*) extracts in the attenuation of CCl_4-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 (180-220 g) were divided into four groups (1-4) of six rats each. In group 1 the rats received only water, group 2 received single dose of equal mixture of carbon tetrachloride (CCl_4) and olive oil (50%, v/v, 1.25 mL/kg i.p.), while animals in group 3 and 4 received CCl_4 + 50 mg/kg of *T. occidentalis* and CCl_4 + 100 mg/kg of *T. occidentalis* respectively. The antioxidant activity of *T. occidentalis* was evaluated *in vitro* using DPPH and ABTS radical scavenging assay.

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Results: Our results showed that CCl_4 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 phenolic compounds constituents of *T. occidentalis* which are able to absorb, neutralize and quench free radicals.

Keywords: 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 fruits, vegetables, and medicinal plants have gained popularity over the past decades in the management of several diseases 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 leaves 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 (CCl_4), which produces reactive free radicals when metabolized, has been widely used as a solvent for induction of hepatic damage in animal models [11]. CCl_4 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 CCl_4 induced liver injury [13]. Based on the reported strong antioxidant potentials of *T. occidentalis*, the possibility of *T. occidentalis* in the prevention of CCl_4-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 were 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℃. The concentrated dried methanolic extract was then stored at -20℃ before use.

2.4 Determination of Total Phenolic Compounds in *Telfairia occidentalis*

The content of total phenolic compounds in *T. occiadentalis* 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 5 mL Folin-ciocalteu reagent (diluted ten-fold) and 4 mL (75 g/L) sodium carbonate. The absorption was read after 30 min at 20℃ at 765 nm and the calibration curve was drawn. One mL of *T. occiadentalis* extract (1 mg/mL) were mixed with the same reagents as described above, and after l hour the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in *T. occiadentalis* methanol extract in gallic acid equivalents (GAE) was calculated by the following formula:

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

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 Phosphate buffer saline (PBS) through manganese dioxide powder. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 µm syringe filter. This solution was diluted in 5 mM PBS pH 7.4, adjusted to an absorbance of 0.700 ± 0.020 at 734 mm and preincubated at room temperature prior to use for 2 hours. One mL of $ABTS^{\bullet+}$ solution and various concentrations of the extracts (diluted with water) were vortexed for 45 seconds in reaction tubes, and the absorbance (734 nm) 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:

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

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 hrs before use. The $ABTS^{\bullet+}$ 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 734 nm. For the photometric assay, 1 mL of the $ABTS^{\bullet+}$ solution and various concentrations of the extracts were mixed for 45 seconds and measured immediately after 1 minute at 734 nm. The antioxidant activity of the extracts was calculated by determining the decrease in absorbance at different concentrations by using the following equation.

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

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 $DPPH^{\bullet}$ in 100 mL methanol. For the photometric assay 1.95 mL $DPPH^{\bullet}$ solution and 50 µL antioxidant solution were mixed. At first, the absorbance of the disposable cuvette with 1.95 mL $DPPH^{\bullet}$ was measured as blank, then the antioxidant solution was added and mixed. The reaction was measured at 5 min interval at 515 nm until $\Delta A = 0.003\ min^{-1}$. The anti-oxidative activity was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

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

2.8 Animal Model and Experimental Design

Twenty-four male wistar albino rats (180-220 g) 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. The rats of group 1 served as control animals, those of group 2 received single dose of equal mixture of carbon tetrachloride (CCl_4) 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 extracts 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 (CCl_4) they were sacrificed by chloroform anesthesia. Blood samples of each animal were collected by heart puncture and were allowed to clot for 45 min 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 aminotransefrase (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

Malondialdehyde (MDA) levels were measured by the double heating method as reported by Draper and Hadley [18]. The method is based on spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 0.5 mL of liver homogenate was mixed with 2.5 mL of tricholoroacetic 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 2 mL of each sample supernatant was transferred to a test tube containing 1 mL 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 ($\varepsilon = 1.56 \times 10^5$ $cm^{-1}M^{-1}$) 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 0.1 mL of supernatant,1.2 mL of sodium pyrophosphate buffer (pH 8.3; 0.052 M), 0.1 mL of phenazine methosulphate (186 µM), 0.3 mL of nitrobluetetrazolium (300 µM) and 0.2 mL of NADH (750 µM). Reaction was started by addition of NADH. After incubation at 30℃ for 90 s, the reaction was stopped by addition of 0.1 mL of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 mL 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 0.5 mL 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 mL DTNB (4 mg/mL) and 0.9 mL phosphate buffer (0.1 M, pH 7.4). The yellow colour developed was read at 412 nm. Reduced glutathione was expressed as µg/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 50 mM phosphate buffer (pH 7.0). Reaction was started by addition of 1000 µL of freshly prepared 30 mM 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 ± 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 of Trolox, Gallic Acid and *Telfairia occidentalis* Leave Extracts

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 values analyzed in TEAC II, TEAC III (hydrophilic version) and TEAC III (lipophilic version) were higher in gallic acid and lower in *T. occidentalis*. Based on these facts, the descending order of antioxidant activity of the three substances analyzed is: gallic acid > Trolox > *Telfairia occidentalis*. In addition, both TEAC II and TEAC III (lipophilic version) showed comparable antioxidant activity both in gallic acid and *Telfairia occidentalis* (Table 1).

3.2 Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity of *Telfairia occidentalis*

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

3.3 The Total Phenolic Content of *Telfairia occidentalis* Leave Extracts

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).

3.4 Effect of Treatment with Carbon Tetrachloride on the Levels of AST, ALT and ALP Activities

CCl_4 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 (Fig. 1). Administrations of *Telfairia occidentalis* at 50 mg/kg and 100 mg/kg doses lower the activities of these marker enzymes significantly ($p < 0.05$).

3.5 Effect of Treatment with Carbon Tetrachloride on the Level of Lactate Dehyrogenase Activity

CCl_4 treatment resulted in significant ($p < 0.05$) rise in the level LDH when compared to the control group (Fig. 2). Oral administrations of *Telfairia occidentalis* at two different doses (50 mg/kg and 100 mg/kg) lower the level of this marker enzyme, LDH significantly ($p < 0.05$).

3.6 Effect of Treatment with CCl_4 on the Levels of Superoxide Dismutase and Reduced Glutathione

Administration of CCl_4 caused a significant ($p < 0.05$) decrease in SOD and GSH levels in rats when compared with normal animals. The *Telfairia occiadentalis* at 50 mg/kg and 100 mg/kg showed significant ($p < 0.05$) increase in SOD and GSH levels when compared to CCl_4 treated rats (Fig. 3).

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 occidentalis* extract at the dose of 50 mg/kg and 100 mg/kg resulted in significant ($p < 0.05$) increase of CAT level when compared to CCl_4 treated rats.

Table 1. Trolox equivalent antioxidant capacity (TEAC) assay of Trolox, gallic acid and *Telfairia occidentalis*

Assay/Antioxidant	Trolox	Gallic acid	*Telfairia occidentalis*
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

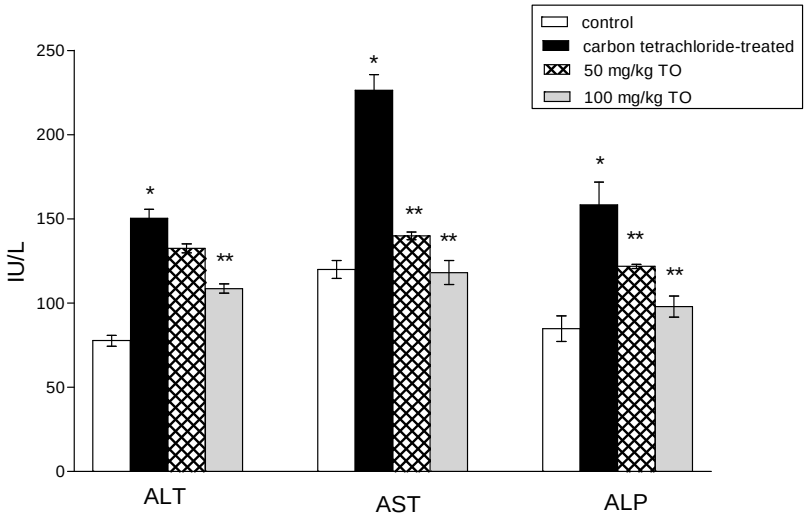

Fig. 1. Effect of *Telfairia occidentalis* extract on serum levels of AST (IU/L), ALT (IU/L) and ALP (IU/L) during CCl_4 treated oxidative stress in rats

*Values are mean±SEM. * Group 2 (CCl_4 treated rats) compared with group 1 (control rats). ** Groups 3 and 4 (Telfairia occidentalis (TO) treated rats) compared with group 2 (CCl_4 treated rats)*

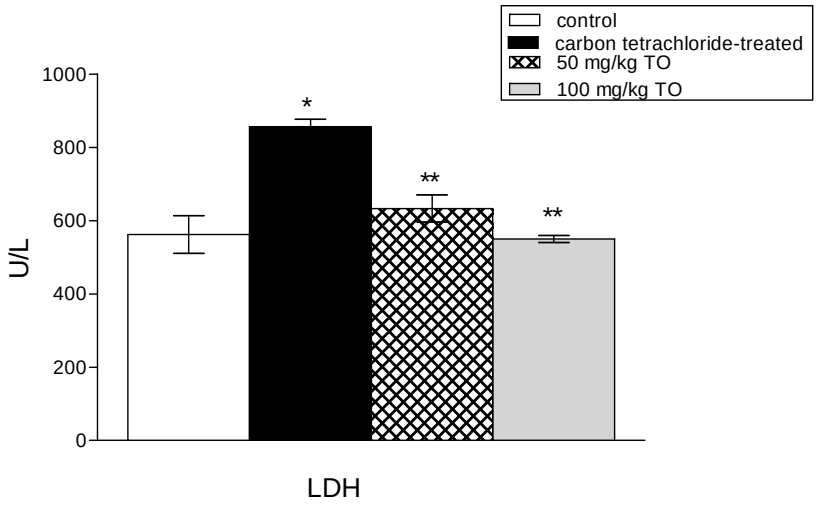

Fig. 2. Effect of *Telfairia occidentalis* extract on serum levels of LDH (IU/L), during CCl_4 induced oxidative stress in rats

*Values are mean±SEM. * Group 2 (CCl_4 treated rats) compared with group 1 (control rats). ** Groups 3 and 4 (Telfairia occidentalis (TO) treated rats) compared with group 2 (CCl_4 treated rats)*

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

Sample	Total phenol[a]	DPPH scavenging activity (IC 50)[b]
Telfairia occidentalis	34.7	259.5 ± 7.8
Gallic acid	ND	16.3 ± 1.5

Each value represents the mean± SEM. (n = 3)
aTotal phenolic content was expressed as mg gallic acid equivalents/g dried extract.
bExpressed as µg/mL
ND, not detected

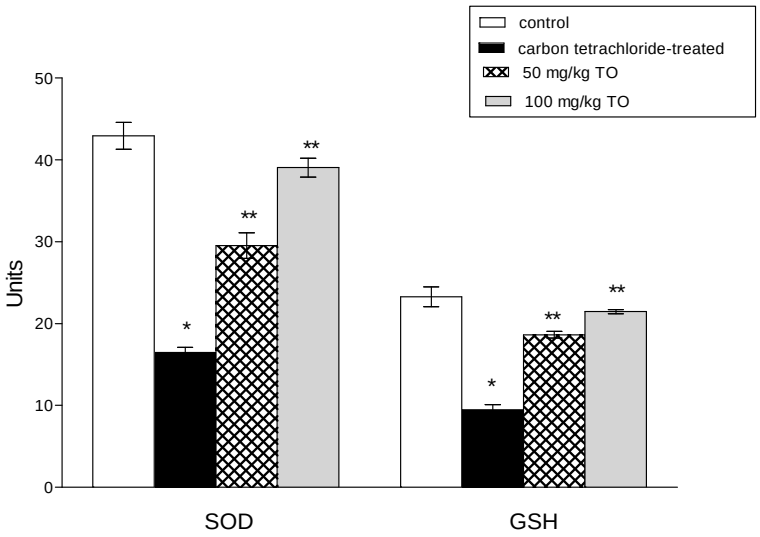

Fig. 3. Effect of *Telfairia occiadentalis* extract on hepatic level of SOD (U/mg protein) and GSH µg/mg protein) during CCl_4 induced oxidative stress in rats
*Values are mean±SEM. * Group 2 (CCl_4 treated rats) compared with group 1 (control rats). ** Groups 3 and 4 (Telfairia occidentalis (TO) treated rats) compared with group 2 (CCl_4 treated rats)*

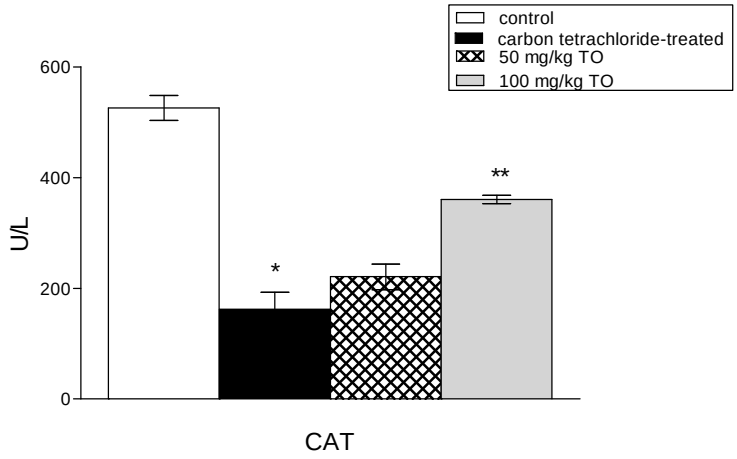

Fig. 4. Effect of *Telfairia occiadentalis* extract on hepatic level of catalase (U/mg protein) during CCl_4 induced oxidative stress in rats
*Values are mean±SEM. * Group 2 (CCl_4 treated rats) compared with group 1 (control rats). ** Groups 3 and 4 (Telfairia occidentalis (TO) treated rats) compared with group 2 (CCl_4 treated rats)*

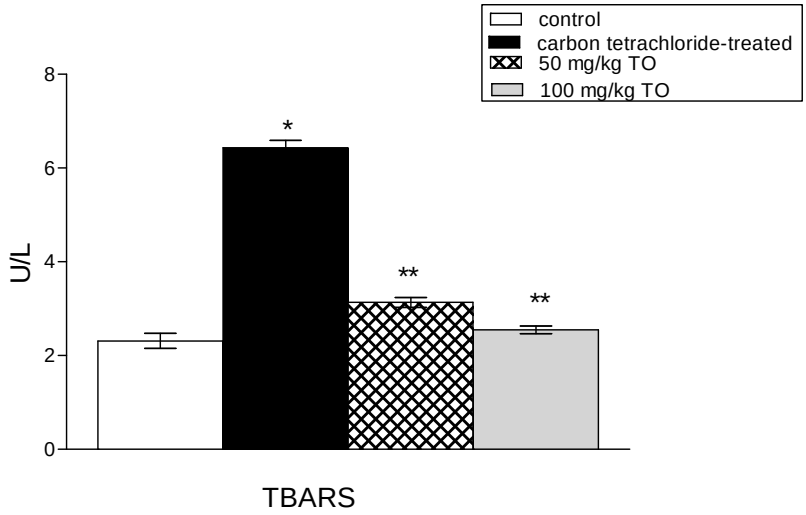

Fig. 5. Effect of *Telfairia occiadentalis* extract on hepatic level of thiobarbituric acid reactive substance (TBARS) (nM/mg protein) during CCl_4 induced oxidative stress in rats

*Values are mean±SEM. * Group 2 (CCl_4 treated rats) compared with group 1 (control rats). ** Groups 3 and 4 (Telfairia occidentalis (TO) treated rats) compared with group 2 (CCl_4 treated rats)*

3.8 Effect of Treatment with CCl_4 on the Levels of Thiobarbituric acid Reactive Substance

Rats treated with CCl_4 showed significant increase in Lipid peroxidation level (LPO) of liver homogenates when compared to rats in control group as shown in above Fig. 5. Pretreatment with *Telfairia occiadentalis* at two different doses (50 mg/kg and 100 mg/kg) significantly reduced the LPO level when compared with CCl_4 treated rats.

4. DISCUSSION

The change in dietary habits and chemoprevention show considerable effective strategy against oxidative stress and are the main focus of area of research these days [22]. Oxidative stress form when there is imbalance between free radical generating and scavenging systems has been implicated in the pathogenesis of wide range of disorders, including neurodegerative disorders, cardiovascular diseases, cancer, and ageing [23]. Several epidemiological studies suggest the importance of a high consumption of secondary plant products widely distributed in fruit and vegetables in ameliorating the effects of oxidative stress [14,15]. The current investigation was undertaken to evaluate the possible protective effect of *T. occiadentalis* 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 $ABTS^{\bullet+}$ and DPPH radical scavenging assay are among the common applied methods. In the present study, *T. occiadentalis* 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. occiadentalis*. Based on our results, *T. occiadentalis* showed significant radical scavenging activity in a concentration dependent manner.

Antioxidant potential of *T. occiadentalis* extract against CCl_4 induced liver damaged was also evaluated in this study. In many studies, carbon tetrachloride (CCl_4) was used as a toxic substance to induce liver damage in rats [24,25], this is due to biotransformation of CCl_4 by Cytochrome P_{450} 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 CCl_4 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 CCl_4 administration. The results obtained in the present study regarding the effect of CCl_4 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 extracts 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 CCl_4 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 CCl_4 leads to the formation of highly unstable free radicals ($\bullet CCl_3$ or $\bullet CCl_3O_2$) which initiates peroxidation [34]. Extracts of *T. occidentalis* showed ability to prevent CCl_4 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 CCl_4 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 50 mg/ml and 100 mg/ml prior to CCl_4-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.

5. CONCLUSION

It must be mentioned that all altered biochemical profiles due to CCl_4 exposure is reversed towards normalization by *T. occidentalis* extracts. The contents of the extracts 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* extracts may be due to presence of some phenolic components that have membrane stabilizing effects. These phenolic compounds may directly combine with free radicals and inactivate them which may suppress the intracellular concentration of free radicals.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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