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ORIGINAL ARTICLE

Antioxidant and Drug detoxification potentials of *Ficus asperifolia* Miq. Extract in CCl_4-Induced Kidney Injuries and Oxidative Damage in Wistar Rats

Oluwafemi Adeleke Ojo[1]*, Adebola Busola Ojo[2] Adwale Fadaka[1] and Basiru Olaitan Ajiboye[1]
[1] Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria
[2] Department of Biochemistry, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria.
* Email: oluwafemiadeleke08@gmail.com

ABSTRACT
Carbon tetrachloride (CCl_4) has been considered a risk factor for humans as it accumulates in body tissues, such as the liver, lungs, kidneys and reproductive organs. The aim of the present study was to evaluate the antioxidant and detoxification potentials of Ficus asperifolia (Miq.) against CCl_4-induced kidney injuries and oxidative damage in Wister rats. Thirty rats (weighing 140 - 180 g) were divided into five groups. In each treatment groups, aqueous extract of F. asperifolia (100, 200 and 400 mg/kg bw) was administered by oral gavage for 21 days before exposure to carbon tetrachloride (CCl_4) 3 mL kg^{-1}i.p. were used to test detoxification potentials of the plant extract. Antioxidant enzymes such as reduced glutathione (GSH) levels, catalase (CAT), superoxide dismutase (SOD), and levels of malondialdehyde (MDA), urea and creatinine were assessed, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were monitored and histological examination carried out. Animal exposure to the CCl_4 resulted in significant elevation in the level of MDA with concomitant depletion in the activities of kidney antioxidants when compared with control. The MDA concentration of the rats treated with 100, 200 and 400 mg/kg body weight of the extract significantly decreased ($p<0.05$) compared with the untreated CCl_4 rats. However, the creatinine concentration decreased significantly ($p<0.05$) when the CCl_4 treated animals were compared with the CCl_4 control. However, histological alterations in the kidney were ameliorated in CCl_4rats treated with F. asperifolia. In conclusion, F. asperifolia showed apparent detoxification potentials and curative effect on carbon tetrachloride induced oxidative damage.
***Keywords**: Detoxification potentials, antioxidants, carbon tetrachloride, Ficus asperifolia*

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INTRODUCTION

The use of plants in medicine is an age-long practice in various parts of the world for bothpreventive and curative effect. Today, it is estimated that about 80% of the world population relies onbotanical preparations as medicine to meet their health needs [1]. Literature has prescribed various herbs for the cure of kidney disease. *Ficus* is a genus belonging to family Moraceae; it comprises of woody trees, shrubs, vines, epiphytes, and hemiepiphyte. *Ficus asperifolia* (Miq.) is found in Nigeria, Senegal, Uganda, Tanzania, Natal (South Africa), Madagascar and Cameroon. The leaves are enormous and displayed spirally, the limb is largely oval or has a form of ellipse and the roots are most often fibrous [2]. It has shown its potential to be therapeutic in averting several diseases in various countries. Studies have established that *F. asperifolia* leaf extract possess many pharmacologicaland physiological activities such as antioxidants [3]. Carbon tetrachloride (CCl_4), which produces reactive free radicals when metabolized, has been widely used as a solvent for induction of kidney damage in animal models [4]. CCl_4 increases lipid peroxidation which leads to injuries in the heart, kidney, testis and brain [5, 6].

To the best of our knowledge there was a lack of scientific reports available in support of its traditional claim of detoxification and curative potential. So far, there has been no research reported on protective and curative effect against carbon tetrachloride induced kidney injuries and oxidative damage in rats. Hence, the present study was aimed at investigating the antioxidant and possible detoxification potential of the *Ficus asperifolia* (Miq.) aqueous leaf extract against CCl_4-induced kidney injuries in male Wistar rats.

MATERIAL AND METHODS

Chemicals

Carbontetrachloride was bought from a local chemist in Ado-Ekiti, Nigeria. Thiobarbituric acids (TBA) were bought from Aldrich Chemical Co. (Milwaukee, WI, USA). Glutathione, hydrogen peroxide, 5, 5'-dithios-bis-2-nitrobenzoic acid (DNTB) and Epinephrine bought from Sigma Chemical Co., Saint Louis, MO USA. Randox alanine aminotransferase (ALT), and aspartate aminotransferase (AST) assay kits were purchased from ABJ Chemicals, Lagos (Nigeria). Adrenaline, thiobarbituric acid (TBA), Ellman's reagent (DTNB), glutathione and bovine serum albumin (BSA) were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals were of the highest purity commercially available.

Extraction

The aqueous extract of the powered *Ficus asperifolia* Miq was air dried in the laboratory at ambient temperature (30±2°C) for 10 days, pulverized using a laboratory mechanical grinder (Christy and Norris limited, machine type 8) and the fine powders obtained stored until further use. The powdered sample (50 g) was extracted with distilled water of 500 ml for 48 h. The mixture was decanted and filtered using sterile whatman paper No 1. The filterate measured up to 425 ml and evaporated to dryness using a freeze dryer to obtain 12% yield.

Experimental animals

Male Wistar albino rats (140–180 g) were maintained in the Laboratory Animal Unit of the College of Sciences, Afe Babalola University. They were housed in metallic cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 hours of darkness and light. Male rats were used because of their constant metabolism compared to the variation in the female physiology. Animals were allowed to adapt to the laboratory environment for one week before experimentation. The care and handling of the animals were in accordance with the internationally accepted standard guidelines and were approved by Afe Babalola institutional review board.

Acute toxicity experiment

Wister rats were divided into control and test groups (6 animals each). Control group received the vehicle (normal saline) while the test groups got graded doses (1000–4000 mg/kg) of *F. asperifolia* aqueous extract orally and were observed for mortality till 48 h and the LD_{50} was calculated.

Doses

The dose selection for the aqueous extract of *F. asperifolia* was based on the acute toxicity study, which did not show any adverse effect following oral administration of doses up to 3500 mg/kg. According to modified method of (Ojo et al., 2014a), experimental oral doses of 100, 200 and 400 mg/kg of the maximum possible dose of the extract that did not cause mortalities in rats were selected.

Experimental induction of hepatic damage

CCl_4 was dissolved in groundnut oil in the ratio 1:1 v/v. Kidney injuries was induced in rats following subcutaneous (SC) injection of CCl_4 in the lower abdomen at a dose of 3 mL/kg.

Preparation of tissue homogenate for Biochemical analyses

Kidney tissues were quickly removed, washed in ice-cold, isotonic saline, and blotted individually on ash-free filter paper. The tissues were then homogenized in 0.1M 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride buffer, pH 7.4 using a Potter-Elvehjem homogenizer at 4°C, the crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 min in cold centrifuge, and the supernatant was kept at -20°C for estimation of GSH, SOD and CAT activities.

Preparation of Serum

Blood collected from the heart of the animals into plain centrifuge tubes and allowed to stand for 1 h. Serum prepared by centrifugation at 3,000 gfor 15 min in a Beckman bench centrifuge. The clear supernatant used for estimating serum enzymes.

Biochemical assays

Lipid peroxidation (LPO) was assayed by measuring thiobarbituric acid reactive substances (TBARS) as described by Varshney and Kale [7]. Catalase (CAT) activity was determinedby measuring the rate of decomposition of hydrogen peroxideat 570 nm as described by Sinha [8]. SOD activity was determined as described by Misra and Fridovich [9](1972).Reduced glutathione (GSH) level was estimated using the

method described by Beutler *et al.* [10] at 412 nm. GPx was determined by the method described by Hafeman *et al*. [11] based on the degradation of H2O2 in the presence of GSH. Glutathione-S-transferase (GST) activity was determined according to Habig *et al*. [12]. Serum ALT and AST activities were quantified spectrophotometrically using a Randox commercial assay kit.

Determination of serum urea concentration

The concentration of serum urea was determined using the method of Tietz [13] as outlined in Randox kits, UK.

Determination of serum creatinine concentration

The concentration of serum creatinine was determined using the method of Tietz [13] as outlined in Randox kits, UK.

Histopathology of tissues

The kidney from control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5µm thickness. The sections were stained with haemotoxylin and eosin dye for histopathological observation.

Statistical analysis

All the data are expressed in mean ± SEM. The significance of difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by the Duncan multiple range tests for analysis of biochemical data using SPSS (20.0). Values considered statistically significant at $p< 0.05$.

RESULTS

Acute toxicity experiment

All rats treated with different doses (1000–4000 mg/kg) of *F. asperifolia* extract survived during the 48 h of observation. The animals did not show visible signs of acute toxicity.

Detoxification Potentials and Antioxidant Effect of Extract of F. asperifolia Miq. Leaves Against CCL_4–Induced Kidney Injuries and Oxidative damagein Rats

Administration of CCl_4 to rats showed significant elevation of kidney marker enzymes (ALT, AST and ALP) in their serum after 24 h of intoxication. Administration of the aqueous extract of *F. asperifolia* (100, 200 and 400 mg/kg) once daily for 21 days prior to CCl_4, exhibited a significant detoxificating activity, resulting in reduction in the elevated serum activities of marker enzymes (Table 1) when compared to CCl_4- intoxicated rats.The oxidative damage caused by CCl_4 in the kidney was assessed by measuring the activities of antioxidant defense enzymes (SOD, GPx and CAT), GSH (Table 2) and the level of lipid peroxidation product (MDA) (Table 3). Results in Table 2 showed that administration of CCl_4induced significant reduction in the activities of kidney SOD, GPx and CAT enzymes with a decreased level of GSH content as compared to the normal control group. On the other hand, it increased the MDA level in kidney tissues (Table 3). Pre- administration of aqueous extract *F. asperifolia* (100, 200 and 400 mg/kg) reduced the severity of CCl_4 toxicity, as evident from the non-significant differences observed in the oxidative stress indicators and antioxidant enzyme levels in these groups.

Effects of *Ficus asperifolia* Miq leaves on serum protein, urea and creatinine in Carbon tetrachloride-induced Kidney Injuries in rats

There was a significant decrease in the levels of serum total protein in the CCl_4 untreated group when compared with the normal control group (Table 4). However, levels of this compound in the serum were significantly increased in CCl_4 treated groups with aqueous extract *F. asperifolia* (100, 200 and 400 mg/kg) when compared with the CCl_4 control group. Levels of urea and creatinine in the serum of the CCl_4 group were significantly increased when compared with the normal control group (Table 4). However, levels of serum urea and creatinine were significantly decreased in CCl_4 treated groups with aqueous extract *F. asperifolia* (100, 200 and 400 mg/kg) when compared with the CCl_4 group. The ameliorative effect of *F. asperifolia* on the levels of serum urea and creatinine was more prominent.

Histopathological Studies

Histopathological examination of the kidney sections from CCl_4untreated rats showed tubular degeneration, necrosis and severe renal cortical congestion(Figure 1).Treatment with aqueous extract of *Ficus asperifolia* (100, 200 and 400 mg/kg) confirmed the detoxification and curative activity as a significant recovery of nephron damage and decreased necrosis was evident against CCl_4 induced kidney injuries and oxidative damage in rats, which is similar to their control.

Table 1: Activities of Serum and Kidney Marker Enzymes in CCl_4-induced rats treated with aqueous extract of *Ficus asperifolia*.

Treatments	Kidney (U/L)			Serum (U/L)		
	AST	ALT	ALP	AST	ALT	ALP
Control	68.22 ± 1.02	67.41 ± 1.75	75.60 ± 3.25	10.54 ± 0.17	11.21 ± 0.26	11.40 ± 0.40
CCl_4 untreated	23.02 ± 1.08*	24.11 ± 1.22*	20.41 ± 0.23*	18.39 ± 0.46*	18.62 ± 0.12*	19.50 ± 0.20*
CCl_4+ 100 mg/kg	45.60 ± 1.75**	46.50 ± 2.11**	43.34 ± 1.88**	6.26 ± 0.20**	7.58 ± 0.18**	7.18 ± 0.41**
CCl_4+ 200 mg/kg	55.60 ± 2.11**	54.10 ± 2.02**	67.22 ± 2.02**	7.24 ± 0.30**	8.25 ± 0.12**	8.02 ± 0.70**
CCl_4+ 400 mg/kg	62.02 ± 2.04**	61.78 ± 2.14**	68.45 ± 2.18**	8.85 ± 0.22**	10.21 ± 0.25**	9.35 ± 0.85**

Values are means ± S.E.M. of 6 animals per group, CCl_4 Treated = *Ficus asperifolia* at 100 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 200 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from CCl_4 untreated ($p < 0.05$).

Table 2: Changes in the levels of Kidney antioxidant parameters in CCl_4-induced rats treated with aqueous extract of *Ficus asperifolia*.

Treatment	Kidney			
	GSH (mg /g tissue)	GPx	SOD	CAT (U/mg protein)
Control	45.12 ± 0.12	42.35 ± 0.42	42.60 ± 0.31	45.23 ± 0.58
CCl_4 untreated	25.12 ± 0.19*	21.17 ± 0.26*	24.02 ± 0.18*	25.44 ± 0.48*
CCl_4 + 100 mg/kg	36.42 ± 0.65**	35.48 ± 0.10**	35.38 ± 0.10**	36.23 ± 0.21**
CCl_4 + 200 mg/kg	38.42 ± 0.25**	35.92 ± 0.25**	37.72 ± 0.22**	38.12 ± 0.17**
CCl_4 + 400 mg/kg	40.05 ± 0.37**	39.28 ± 0.13**	39.21 ± 0.47**	41.51 ± 0.25**

Values are means ± S.E.M. of 6 animals per group, CCl_4 Treated = *Ficus asperifolia* at 100 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 200 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from CCl_4 untreated ($p < 0.05$).

Table 3: Levels of lipid peroxidation in CCl_4-induced rats treated with aqueous extract of *Ficus asperifolia* Miq. Leaves

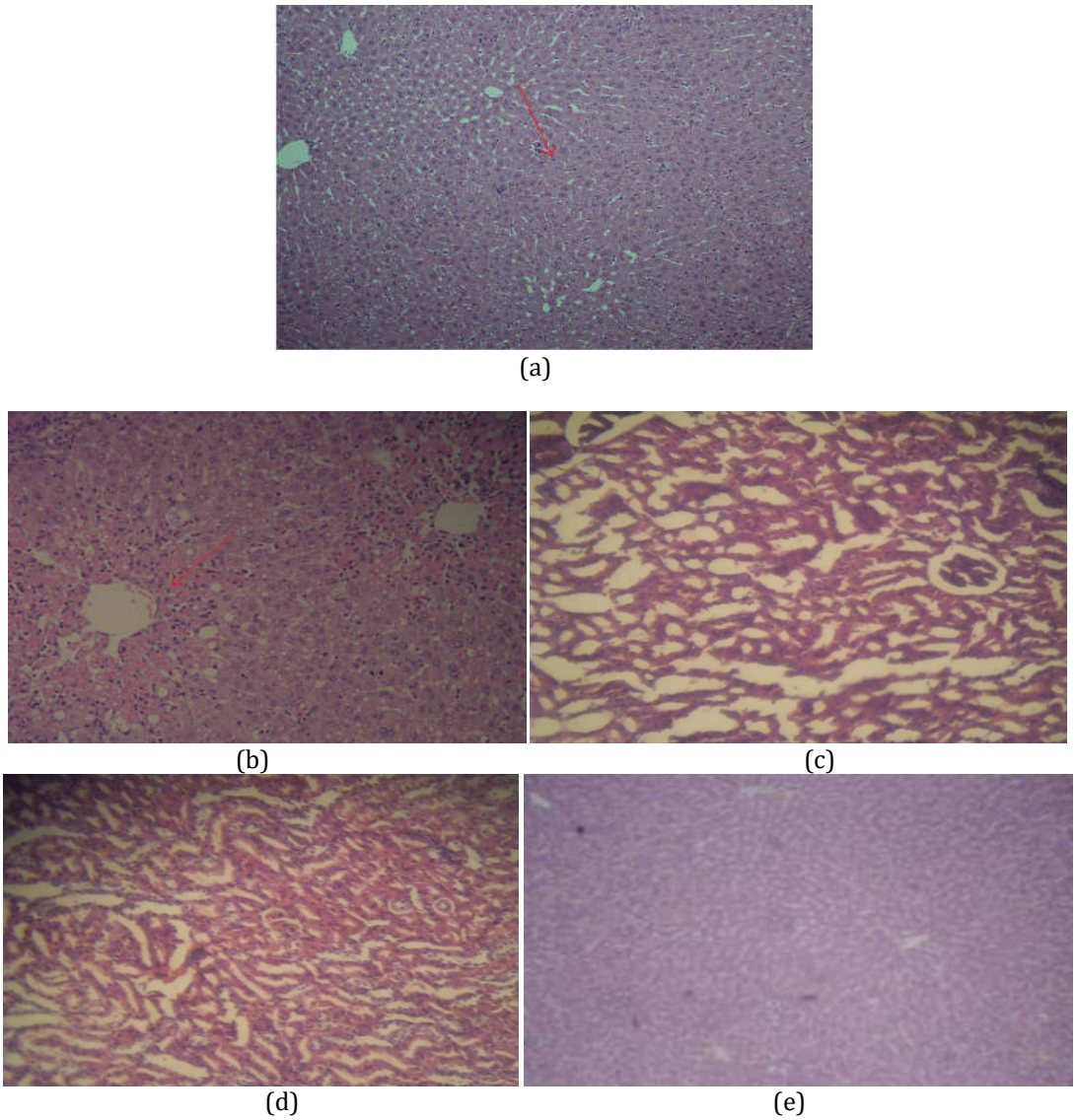
Treatments	Kidney (µmol MDA/mg protein)	Serum (µmol MDA/mg protein)
Control	9.52 ± 0.02	9.82 ± 0.08
CCl_4 untreated	12.22 ± 0.45*	12.58 ± 0.41*
CCl_4 + 100 mg/kg	6.86 ± 0.14**	6.83 ± 0.76**
CCl_4 + 200 mg/kg	7.72 ± 0.65**	7.82 ± 0.65**
CCl_4 + 400 mg/kg	8.48 ± 0.42**	8.04 ± 0.40**

Values are means ± S.E.M. of 6 animals per group, CCl_4 Treated = *Ficus asperifolia* at 100 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 200 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from CCl_4 untreated ($p < 0.05$).

Table 4: Levels of Total protein, Urea and Creatinine in the serum of control and experimental groups of rats.

Treatments	Protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	9.8 ± 0.55	0.98 ± 0.01	0.94 ± 0.21
CCl_4 untreated	3.47 ± 0.22*	2.24 ± 0.24*	2.58 ± 0.14*
CCl_4 + 100 mg/kg	6.45 ± 0.31**	0.64 ± 0.25**	0.58 ± 0.13**
CCl_4 + 200 mg/kg	7.89 ± 0.25**	0.70 ± 0.32**	0.75 ± 0.21**
CCl_4 + 400 mg/kg	8.50 ± 0.22**	0.84 ± 0.45**	0.86 ± 0.30**

Values are means ± S.E.M. of 6 animals per group, CCl_4 Treated = *Ficus asperifolia* at 100 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 200 mg/kg, CCl_4 Treated = *Ficus asperifolia* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from CCl_4 untreated ($p < 0.05$).



(a) (b) (c) (d) (e)

Figure 1: Histology of Kidney tissues. (a) Kidney section of normal control rat (normal architecture), (b) kidney section of CCl_4-treated rats showing massive tubular degeneration, necrosis and severe renal cortical congestion,(c) kidney section of rats treated with $CCl_4$and 100 mg/kg of *F. asperifolia* shows lesser degeneration and absence of cell necrosis, rectified tubular degeneration, (d) kidney section of ratstreatedwith$CCl_4$and 200 mg/kg of *F. asperifolia* showing absence of cell necrosis and slight congestion in renal cortical, and (e) kidney section of rats treated withCCl_4 and 400 mg/kg of *F. asperifolia* showing repairing of tubular degeneration.

DISCUSSION

Chemicals such as environmental toxicants and clinically useful drugs can cause severe cellular damages in different organs of our body through the metabolic activation to highly reactive substances such as free radicals. CCl_4 is one of such extensively studied environmental toxicant [14]. The phytochemical study of *Ficus asperifolia* leaf extracts revealed the plant is a reservoir of bioactive compounds. It contains presence of polyphenol-rich compounds [15].Polyphenols have been suggested to decrease oxidativestress in humans.Studies reveal that, the carbonyl groups present in phenolic compounds were responsible for antioxidant activity [16]. Ojo *et al*. [15] revealed that the *Ficus asperifolia* contain pharmacologically active substance (s) such as alkaloids, glycosides, saponins, tannins, and phenolic compounds, which are responsible for the antioxidant activity.

However, oral administration of *F. asperifolia* extract in doses up to 4000 mg/kg did not produce any symptom of acute toxicity and none of rats died during 48 hours of observation. Accordingly, it is suggested that oral LD_{50} of the tested extract was higher than 4000 mg/kg b.wt. Therefore, *F. asperifolia*

plant can be categorized as highly safe since substances possessing LD_{50} higher than 50 mg/kg are non-toxic [17].

Furthermore, rats intoxicated with CCl_4 developed significant kidney damage as manifested by a significant increase in the serum activities of ALT, AST and ALP that are indicators of oxidative damage and loss of functional integrity. Pre-treatment of rats with extracts of *F. asperifolia* in doses of 100, 200 and 400 mg/kg effectively protected rats against CCl_4-induced kidney damage, resulting in reduction in serum activities of kidney marker enzymes when compared to the CCl_4-intoxicated control rats. Decrease in the level of these enzymes with *F. asperifolia* is an indication of the stabilization of plasma membrane as well as repair of kidney damage caused by CCl_4 similar to that reported by [18, 19].

Administration of CCl_4 resulted in a significant increase in the renal content of MDA indicating increased lipid peroxidation which implicates the renal oxidative damage. However, CCl_4 caused a significant decrease in the activities of GPx, GSH, SOD and CAT. A decrease in the level of antioxidant enzymes and an increase in lipid peroxidation level were recorded after CCl_4 intoxication [19]. Damage to renal tissue observed in this study may be resulted from the increase in lipid peroxidation and decrease of antioxidant enzymes in the kidney following exposure to CCl_4. Decrease in SOD, CAT, and GPx was significant in the CCl_4untreated group indicating that CCl_4 induced kidney injuries and oxidative damage. In treatment groups, *Ficus asperifolia* showed detoxification potentials in decreasing the MDA level in animals exposed to CCl_4. Aqueous *Ficus asperifolia* extract worked as an antioxidant [20] and increased the level of non-enzymatic antioxidant GSH, enzymatic antioxidants CAT, SOD, and GPx, and the AST and ALT in animals exposed to CCl_4. However, *Ficus asperifolia* extract reduces the oxidative damage in the rats, due to its high reactive oxygen species scavenging ability and protecting the antioxidant enzymes from being denatured.

Chawla, [21] postulated that increased levels of serum urea and creatinine is linked to kidney disease. In this study, the CCl_4 untreated group showed a significant increase in serum urea and creatinine that might suggest the inability of the kidney to excrete these products, indicating an impairment of kidney functions. These effects could be attributed to the changes in the threshold of tubular re-absorption, renal blood flow, and glomerular filtration rate [22].The obtained results in the current study showed that the aqueous extract of *Ficus asperifolia* had a detoxification potentials effect against CCl_4 induced kidney damage.

Histological studies of the kidney tissue reveal that CCl_4 intoxication caused abnormal ultra-structural changes in the kidney tissue including tubular degeneration, necrosis and severe renal cortical congestion. However, *Ficus asperifolia* treated CCl_4 induced kidney injuries in rats; the observed pathological impairments by CCl_4 have been recovered significantly which indicates that *Ficus asperifolia* is capable of preventing the kidney damage induced by CCl_4. Therefore, it may be suggested that *Ficus asperifolia* might inhibit CCl_4 induced kidney injuries and oxidative damage.

CONCLUSION

In conclusion, the aqueous extracts of *Ficus asperifolia* exhibited detoxification potentials and curative effects against CCl_4 induced kidney injuries and oxidative damage in rats which could be attributed to its antioxidant constituents.

DECLARATIONS OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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