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Carbon Tetrachloride-induced oxidative stress in Wistar rat brain: Neurocurative potential of *Ficus asperifolia* (Miq)

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ABSTRACT

The neurocurative potentials of *Ficus asperifolia* leaf extract against carbon tetrachloride-induced toxicity in rats was investigated. Thirty rats were grouped into five. In each treatment groups, was *F. asperifolia*extract of100, 200 and 400 mg/kg body weight was administered by oral gavage for three weeks before exposure to neurotoxin-carbon tetrachloride (CCl_4) 3 mL kg^1 i.p. were used to test neurocurative potentials of the extract. Antioxidant enzymes namely reduced glutathione (GSH) levels, catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) were assessed and histological examinations of the tissues were monitored. Results showed significant decrease in the antioxidant enzymes and an elevation in MDA. *F. asperifolia* showed a significant brain protective influence by the lowering lipid peroxidation and increasing antioxidative enzymes and glutathione. Furthermore, histological alterations were ameliorated in CCl_4-induced treated rats with *F. asperifolia*. These indicates that *F. asperifolia* possesses neurocurative potentials and can efficiently inhibit CCl_4-induced oxidative damage.

KEY WORDS: *Ficus asperifolia* Miq leaves, carbon tetrachloride, aqueous extract, neurocurative, enzymes.

1. INTRODUCTION

Carbon tetrachloride (CCl_4), a clear, colourless, volatile, heavy and non-flammable industrial liquid, reportedly used to induce free radical toxicity in different tissues of experimental animals (Khan, 2010). CCl_4 is biotransformed to tri-chloromethyl-free radical ($\cdot CCl_3$ or $\cdot CCl_3OO$) (Preethi, 2009) thus initiating thechain reaction process (Adewole, 2007; Adewole, 2012). The most prominent mechanism of CCl_4 is in the generation of radicals in tissue damage (Sahreen, 2011; Khan, 2011). This free radical and related reactive species leads to oxidative stress, which produces major interconnected changes of cellular metabolism, increases the serum marker enzymes, and lipid peroxidation (Bhadauria, 2008). In other to maneuver itself from the effects of free radicals, endogenous enzymatic and non-enzymatic systems were employed, but when the generation of free radicals is excessive, mechanisms that are more protective may be of a great importance (Tirkey, 2005). Antioxidant systems that protect the body include the antioxidant nutrients and enzymes (Sreelatha, 2009). Antioxidant have been used to study CCl_4 toxicity in other to protect tissue-induced damage by breaking the process of lipid peroxidation. Numerous crops and fruits are good sources of antioxidant, which protect body against tissue induced oxidative stress (Ogeturk, 2005). In Africa, *Ficus asperifolia* have been used as an anthelmintic and laxatives (Soforowa, 1996). *Ficus asperifolia* has been shown to possess many pharmacological and physiological activities such as antioxidants (Ojo and Akintayo, 2014). Hence, the need to evaluate the neurocurative potential of *Ficus asperifolia* (Miq) against carbon tetrachloride induced toxicity.

2. MATERIALS AND METHODS

Plant material: Fresh sample leaves were procured from local suppliers in Ado-Ekiti (Ekiti State) and authenticated at the Department of Plant Sciences, Federal University of Agricuture, Abeokuta, Nigeria and voucher specimen has been deposited. The leaves were dried and powdered with an electric grinder.

Extraction: 50 g of the powdered sample was extracted with distilled water of 500 ml for 48 hours. The filterate was measured and evaporated to dryness using a freeze dryer to obtain 12 % yield.

Experimental animals: Thirty Male rats (140 – 180 g) were maintained in the Laboratory Animal Unit of the College of Sciences, Afe Babalola University. They were fed with standard diet and water *ad libitum*. However, male rats were used for this experiment because of their constant metabolism to the female rat's physiology. Animals were acclimatize for a week. The care and handling of the rats are used follows the internationally accepted standard guidelines and were approved by Afe Babalola institutional review board.

Doses: The dose selection for extract of *F. asperifolia* used was as a result of the acute toxicity study. According to the method of Ojo (2014) with a slight modification, experimental dose of 100, 200 and 400 mg/kg that equal to 1/40, 1/20 and 1/10 of the maximum possible dose of the extract in rats that were selected.

Experimental induction of brain damage: CCl_4 was dissolved in groundnut oil in the ratio 1:1 v/v. Brain oxidative damage was induced in rats at a dose of 3 mL/kg CCl_4 (Theophile, 2006).

Neurocurative activity: Thirty male rats were randomly grouped into five of six animals, each. Rats of the 1st (normal control) group and 2nd (CCl_4-intoxicated) groups received a dose of 3 mL/kg. The 3th, 4th and 5th groups were administered with the *F. asperifolia*extract. All medications were administered orally by oral gavage for three weeks. Two hours after the last dose of extract, normal control rats were given groundnut oil (3 mL/kg, SC), while rats in the 2nd to 5th groups received CCl_4 (3 mL/kg, SC). After 24 h of CCl_4 injections, blood sample (2 mL) was collected in previously 1335 abeled samples tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugating at 10,000 rpm for 5 min. Brain tissues were dissected out into two parts for determination of antioxidant status and for histopathological examination.

Assessment of CCl_4 mediated oxidative stress: Lowry (1951), alanine and aspartate aminotransferases (ALT and AST) activities were determined by Mohun and Cook (1957), Reitman, and Frankel (1957) determined Protein contents of the samples. Buege and Aust (1978) determined the lipid peroxidation level. McCord and Fridovich (1969) measured superoxide dismutase activity. Catalase was determine spectrophotometrically by Aebi (1974). Reduced glutathione level by Beutler (1963). This method is on developing a stable (yellow) color when 5',5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) mix to sulfhydryl compounds. Glutathione peroxidaseby Rotruck (1973).

Histopathology of tissues: The brains tissues from the rats were fixed with 10% formalin. The sections of the tissues were stained with hemotoxylin dye for histopathological observation.

Statistical analysis: Statistical analysis was performed usingGraphPad Prism 5 statistical package (Graph Pad Software, USA). Data were analyzed using a statistical software package (SPSS for Windows, version 20, IBM Corporation, USA) using Duncan multiple range *post-hoc* test. Values of mean ± standard deviations in triplicates were considered significantly different at $p < 0.05$.

3. RESULTS

While animals that received very high dosage did not die, other signs of toxicity were noticed 2 - 4 hours after extract administration. There were decrease locomotion, writhing, constipation and decreased in sensitivity to touch. In addition, there was decreased feed intake and prostration 15 hours after extract administration.

Administration of CCl_4 to rats showed significant elevation of brain marker enzymes (ALT, AST and ALP) in their serum after 24 hours of intoxication. Administration of the *F. asperifolia* aqueous extract at the dose used for three weeks prior to CCl_4, exhibited a significant neuroprotective activity, resulting in decreased elevated serum activities of brain marker enzymes as shown in Table 1 when compared to CCl_4- untreated rats. The toxicity induced by CCl_4 in the brain tissues was assessed by measuring the antioxidant defense enzymes like SOD, GPx and CAT, GSH (Table 2) and MDA levels (Table 3). Table 3 showed that administration of CCl_4 induced reduction in the activities of SOD, GSH, GPx and CAT enzymes when compared to the control group. Although, it increased the MDA level in brain tissues Table 4. Pre- administration with *F. asperifolia* extract to group three, group four and group five reduced the toxicity of CCl_4, evident from the observed differences shown in these groups.

Table.1. Changes in the activities of serum and Brain Marker Enzymes in CCl_4-induced rats treated with aqueous extract of *Ficus asperifolia*

Treatments	BRAIN (U/L)			SERUM (U/L)		
	AST	ALT	ALP	AST	ALT	ALP
Control	98.65 ± 1.02	85.56 ± 1.55	75.60 ± 2.25	65.54 ± 2.77	70.37 ± 1.46	68.60 ± 2.50
CCl_4 untreated	30.42± 1.02*	31.28± 1.42*	31.60 ± 2.14*	98.39 ± 2.56*	90.89 ± 2.24*	92.80 ± 4.50*
CCl_4+100mg/kg	45.60±1.05**	46.50±1.24**	43.34± 2.35**	64.13 ± 2.50**	75.48 ± 14.38**	67.10 ± 2.41**
CCl_4+200mg/kg	65.60±1.01**	64.10±1.12**	67.22± 1.46**	67.13 ± 2.10**	79.28 ± 10.12**	72.22 ± 1.80**
CCl_4+400mg/kg	75.02±1.51**	77.98±1.14**	76.55± 1.88**	70.01 ± 1.42**	86.21 ± 8.28**	75.35 ± 0.95**

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 Brain antioxidant parameters in CCl_4-induced rats treated with aqueous extract of *Ficus asperifolia*

Treatment	Brain			
	GSH	GPx	SOD	CAT
	(mg/g tissue)		(U/mg protein)	
Control	59.65 ± 0.12	52.35 ± 0.55	52.50 ± 1.21	55.47 ± 0.88
CCl_4 untreated	15.52 ± 0.15*	21.67 ± 0.36*	27.52 ± 1.48*	28.35 ± 0.58*
CCl_4 + 100 mg/kg	46.32 ± 0.35**	41.38 ± 0.40**	40.38 ± 1.30**	42.29 ± 0.41**
CCl_4 + 200 mg/kg	47.22 ± 0.55**	41.72 ± 0.45**	46.62 ± 1.44**	40.32 ± 0.37**
CCl_4 + 400 mg/kg	54.15 ± 0.67**	47.18 ± 0.33**	49.41 ± 1.57**	51.50 ± 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. Changes in the levels of lipid peroxidation in CCl_4-induced rats treated with aqueous extract of *Ficus asperifolia* (Miq) Leaves

Treatments	BRAIN (µmol MDA/mg protein)	SERUM (µmol MDA/mg protein)
Control	7.05 ± 1.02	7.82 ± 1.98
CCl_4 untreated	14.89 ± 1.05*	15.26 ± 2.61*
CCl_4 + 100 mg/kg	5.66 ± 1.04**	5.83 ± 1.06**
CCl_4 + 200 mg/kg	5.42 ± 1.25**	5.42 ± 1.15**
CCl_4 + 400 mg/kg	6.98 ± 1.02**	6.04 ± 1.03**

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 ($p < 0.05$).

Histopathological Studies: The histology of brain tissues slide of CCl_4-untreated rats showedsevere spongiosis, mild congestion, severe necrosis and hemorrhage at the meninges (Figure 1). Administrationof *F. asperifolia* extract to group three, group four and group five might have confirmed the neuroprotective activity as a significant recovery of neuronal damage, and decreased necrosis was persistent against CCl_4-induced toxicity in rats, which is similar to their control. The histological results further corroborated the biochemical findings, suggesting the useful effects of *Ficus asperifolia* in CCl_4-induced toxicity in rats.

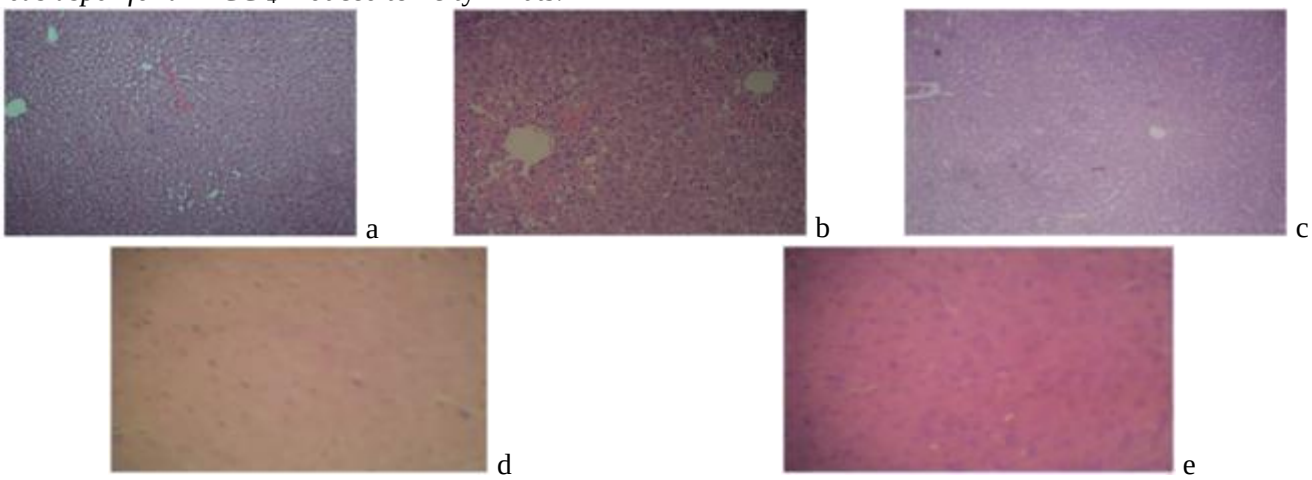

Figure.1.Histological changes of brain tissues. (a) Brain section of normal rat shows veins surrounded by hepatic cord of cells(normal architecture), (b) Brain section of CCl_4-treated rats showing mild spongiosis, severe congestion (indicated by arrow), hemorrhage at the meninges (c) Brain section of rats treated with CCl_4 and 100 mg/kg of *F. asperifolia* shows lesser spongiosis and absence of cell necrosis, (d) Brain section of rats with CCl_4 and 200 mg/kg of *F. asperifolia* showing absence of ballooning, inflammatory cells, and regeneration of neurons toward near normal brain architecture (e) Brain section of rats with CCl_4 and 400 mg/kg of *F. asperifolia* showing repairing of neuronal damage

DISCUSSION

Oxidative stress is involved in many neurological diseases. Gupta, (2003) reported that free radical increases during seizures (Gupta, 2003), suggesting their importance in the pathogenesis of epileptic seizures (Patel, 2004). Similarly, increase in MDA levels and a reduction in thiol groups of animals that had CCl_4-induced damage was observed. It was well demonstrated that production of ROS, including superoxide anions and hydrogenperoxide,

increases in the brains of animals subjected to seizures (Sudha, 2001; Rodrigues, 2012). Hence, weinvestigate the protective influence of the *Ficus asperifolia* against CCl_4-induced toxicity in rats. However, results show that extract possesses protective action against oxidative induced damage by the potent toxin CCl_4.

However, administration of the extract up to 4000 mg/kg did produce other signs of toxicity, which includes decrease locomotion, writhing, constipation and decreased in sensitivity to touch. Therefore, *F. asperifolia*leaf extract can be considered as highly safe (Ojo et.al, 2013).

Futhermore, enzymes (ALT and AST) and the histological changes were used to evaluate the *F. asperifolia* neurocurative potentials. Consistent with reported studies, results confirmed that CCl_4 exposure damaged the brain, as seen by the elevation in the serum aminotransferase activities and morphological changes seen in the brain sections (Renugadevi and Prabu, 2010). Reduction of these enzymes with *F. asperifolia* could be due to the stabilization of plasma membrane and repair of brain damage caused by CCl_4 similar to that reported by (Ojo et.al, 2014a).

The effect of *F. asperifolia* on the brain toxicity by CCl_4 as determined by the antioxidant defense enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione reduced and peroxidase (GSH and GPx). Pre-treatment with *F. asperifolia* extracts significantly ($p < 0.05$) ameliorated the tissue parameters with significant ($p< 0.05$) increase to CCl_4untreated group (Table 2). Furthermore, it can be confirmed by measuring malonaldehyde levels. The MDA levels were significantly ($p<0.05$) elevated in CCl_4 group than with normal group (Table 3). Administration with *F. asperifolia* significantly ($p<0.05$) decreased the levels to CCl_4 untreated group suggesting that *F. asperifolia* has antioxidant activity as reported by (Ojo and Akintayo, 2014).

Histological changes reveal that CCl_4 caused abnormal ultrastructural in the brain tissue, including spongiform necrosis, nuclear vacuolization pycnosis and lymphocytic inflammatory changes. Sequel to histopathological observation in *F. asperifolia* treated CCl_4-intoxicated rats; CCl_4 has recovered the observed pathological impairments by CCl_4 to be significant, which indicates that F. asperifolia is capable of preventing the neuronal toxicity.

The findings elucidate the neurocurative potentials of *Ficus asperifolia* (Miq) leaf extract on the brain. The possible mechanism of neuro-protection may be attributed to presence of phyto-constituents in the leaves of this plant (Ojo, 2014b).

4. CONCLUSION

In conclusion, *F. asperifolia* exhibited a significant curative action against CCl_4-induced neurotoxicity in rats. The leaf extract also shows neurocurative potential; it protected the rat brain against CCl_4-induced neurotoxicity and enhanced the antioxidant status of the brain.

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Competing Interests: The authors declare no conflict of interest.

Animal Rights: The institutional and international guide for the care and use of laboratory animals was followed. See the 'materials and methods' part for details.

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