

Original Article

The Protective Effect of *Croton zambesicus* against Carbon Tetrachloride-induced Renal Toxicity in Rats

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Received: 04.011.2018

Accepted: 26.12.2018

ABSTRACT

Background: The leaf extract of *Croton zambesicus* (CZ) is used in traditional medicine for the management of various conditions including kidney disease. The purpose of this study was to determine the protective effects of leaf extract of CZ on rat kidney toxicity induced by carbon tetrachloride.

Method: Male albino rats were divided into 6 groups of 5 rats and treated for 5 days with aqueous extract (200-400 mg/kg) and n-butanol fraction (20-40 mg/kg) of CZ. Carbon tetrachloride (2 ml/kg of 40% in olive oil) was administered subcutaneously for 3 days and the rats were treated with the extract and fraction for 3 days. Animals were euthanized on day 8 with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg). Blood sample was collected for the analysis of serum electrolytes, urea and creatinine. Kidney tissue was harvested to determine the antioxidant enzyme activity.

Results: Carbon tetrachloride produced increased serum sodium ion, chloride ion, urea and creatinine with decreased superoxide, catalase, glutathione, oxidized glutathione and an increase in malondialdehyde concentration. Treatment of rats with the aqueous leaf extract and n-butanol fraction attenuated the toxic effects of carbon tetrachloride on kidney with a significant decline in serum electrolytes, urea, creatinine and a significant increase in the concentration of antioxidant enzymes.

Conclusion: Results from this study revealed that the aqueous leaf extract and n-butanol fraction of CZ had protective effect against carbon tetrachloride-induced renal toxicity in rats, thus justifying the consideration of this plant for the management of kidney disease.

Keywords: Antioxidant Enzymes, Carbon Tetrachloride, *Croton Zambesicus*, Nephroprotective Activity, Serum Electrolytes.

IJT 2019 (1): 5-8

INTRODUCTION

Acute kidney disease is currently a global medical and public health issue with an estimated 32 % mortality [1]. The prevalence of acute toxic kidney disease in sub-Saharan Africa is 18%, which is often caused by medications, herbal products, nephrotoxins and environmental pollutants [2]. Lipid peroxidation and oxidative stress has been reported to play a major role in causing renal diseases and is also a major mechanism of kidney injury due to environmental toxins [3]. Carbon tetrachloride (CCl₄) is an environmental pollutant that causes poisoning in cases of high level exposure. It produces reactive oxygen species (ROS) that inhibits cellular antioxidant defenses, resulting in cell damages [4]. The protective effect of plant extracts on acute kidney injury has been evaluated in experimental animals using CCl₄ [5].

Medicinal plants have the potential of preventing acute and chronic diseases. The protective effect of plants is often maintained through an increased expression of antioxidants and scavenging of free radicals [6]. Antioxidants present in medicinal plants have been reported to protect against renal damage by increasing activity of endogenous antioxidant enzymes;

superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), oxidized glutathione (GPx), and reducing lipid peroxidation [7].

Croton zambesicus (CZ) Muell Arg. (*Euphorbiaceae*) is a highly valued medicinal plant in Nigeria and West African sub-Saharan region. The leaf extract is commonly used by traditional medicine practitioners in the management of several ailments, including hypertension, kidney disease and diabetes. Some pharmacologic studies on the leaves of CZ include; *anti-inflammatory, analgesic and antipyretic* [8] and *anticoagulant activities* [9]. *Vascular smooth muscle relaxant activity of a natural diterpene isolated from CZ* [10, 11] and *in vitro* antioxidant activity [12] of the leaves of this plant have also been reported. This study was carried out to determine the renal protective activity of the aqueous leaf extract and n-butanol fraction of CZ on CCl₄-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Preparation of the Aqueous Leaf Extract of *Croton Zambesicus*

The leaves of CZ were collected in October 2017 from Ilorin, Nigeria, and identified in the Botany

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Department of University of Ilorin. It was further authenticated at the Herbarium Section, Department of Pharmacognosy, University of Ilorin and a voucher specimen was deposited. The leaves were removed from the stem, shade dried for 2 weeks and reduced to fine powder, using mortar and pestle. The powdered leaves were extracted by maceration with distilled water for 24 hours and filtered. The filtrate was freeze dried with a laboratory freeze drier (Beta 1-8 LD plus; Germany) and the residue obtained was stored in a desiccator prior to use.

Preparation of the Aqueous Leaf Extract and N-Butanol Fraction of Croton Zambesicus

A 20 grams quantity of the aqueous extract was weighed and dissolved in 200 ml deionized water. This was transferred into a separating funnel with an equal volume of n-butanol. The separating funnel was shaken gently, left to stand for a few minutes then the aqueous and n-butanol fractions were collected. This procedure was repeated three times and the n-butanol fraction was concentrated over a water bath at 45-50° C.

Experimental Animals

Male Albino rats (N=30), weighing 100-120 g, were obtained from the animal house of University of Ilorin were used for the study. The animals were allowed to acclimatize for 24 hours with free access to food and water *ad libitum* in the animal house of Department of Pharmacology and Toxicology, Faculty of Pharmaceutical sciences, University of Ilorin.

Ethical Considerations

Ethics clearance was obtained from the University of Ilorin's Ethics Review Committee (Reg. #: UERC/ASN/2018/1110). All experiments were carried out in accordance with the Guidelines for laboratory procedures set by the University of Ilorin's Ethics Committee on Research as well as the International Animal Care and Use Committee (IACUC) in Nigeria.

Experimental Design

The rats were randomly divided into 6 groups of 5 animals each and were treated for 5 days as follows: group I: 0.2 ml of 0.9 % normal saline orally [PO] (negative control), group II: 0.2 ml of 0.9 % normal saline PO (CCl₄ control), groups III and IV were administered CZ extract (200 & 400 mg/kg/PO), groups V and VI were administered n-butanol fraction (20 & 40 mg/kg/PO). CCl₄ was mixed with olive oil in ratio 4:6 and a dose of 2 ml/kg was administered subcutaneously for 3 days to rats in groups II-VII and then treated with aqueous leaf extract and n-butanol fraction of CZ. Animals were euthanized on day 8 with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg).

Preparation of Serum and Tissue Homogenate

Blood samples were collected through cardiac puncture and kept in plain bottles for the determination

of serum electrolytes, creatinine and urea. The kidneys were removed, weighed and placed in ice-cold 0.25 M sucrose. The kidney tissue was homogenized and centrifuged at 5,000 rpm for 15 minutes. The supernatant was transferred into Eppendorf tubes and stored at -20 °C before analysis.

Determination of Renal Function Parameters

Serum electrolytes, creatinine and urea concentrations were analyzed with Randox assay kits (Crumlin Co.; Antrim, UK) using a previously described method [13].

Determination of Renal Tissue Antioxidant Activity

Superoxide dismutase (SOD) and catalase (CAT) activities were determined using the methods described by [14]. Glutathione reductase (GSH) peroxidase (GPx) and malondialdehyde (MDA) concentrations were evaluated, using the method of Iqbal *et al.* [15].

Statistical Analysis

Data obtained were expressed as means ± standard errors and analyzed, using Graph Pad Prism Software (Version 6.0). One-way ANOVA was performed to compare the data obtained for control and treated groups. Statistical significance levels were set between p<0.05 and p<0.01.

RESULTS

Effect of Aqueous Leaf Extract of Croton Zambesicus and N-Butanol Fraction on Renal Function Parameters

Subcutaneous administration of CCl₄ to rats in group II produced a significant (p<0.05) increase in serum sodium ions, creatinine and urea concentration with a decrease in biocarbonate ions compared to those in group I (control). Treatment of rats in group V with n-butanol fraction caused a significant decrease in sodium ion (p<0.01) and urea concentration (p<0.05). In addition, the leaf extract produced a significant (p<0.05) reduction in bicarbonate and creatinine concentrations in group IV rats compared to those in group II (Table 1).

Effect of Aqueous Leaf Extract of Croton Zambesicus and N-Butanol Fraction on Antioxidant Enzymes

Subcutaneous administration of CCl₄ to group II rats also produced a significant (p<0.05) decrease in SOD and GSH concentrations compared to those in control group I. A decrease in CAT and GPx concentrations was also observed in group II. Treatment of rats in group V with N-butanol fraction reversed this effect by producing a significant (p<0.05) increase in CAT, SOD and GSH concentrations. An increase in GPx concentration was observed in group VI. In addition, CCl₄ produced a significant (p<0.05) increase in MDA concentration in group II rats and the effect was attenuated in groups IV and VI rats, treated with the leaf extract and n-butanol fraction, respectively (Table 2).

Table 1. Effect of aqueous leaf extract of *C. zambesicus* and n-butanol fraction on serum Na^+ , K^+ , Cl^- , HCO_3^- , urea and creatinine in CCl_4 -induced toxicity.

Groups	* Na^+ ppm	* K^+	* Cl^-	* HCO_3^-	**Urea	*Creatinine
I	83.12±3.75	6.34±0.20	132.40±2.61	0.69±0.08	0.48±0.08	0.11±0.06
II	104.4±2.51 [#]	5.76±0.10	144.80±5.10	0.44±0.30 [#]	1.35±0.12 [#]	0.37±0.03 [#]
III	84.98±4.20*	6.83±0.78	129.10±2.73*	0.57±0.55	0.55±0.01*	0.17±0.05
IV	95.90±4.89	6.12±0.03	130.10±4.10	0.63±0.25*	0.65±0.02*	0.06±0.00*
V	76.23±7.24**	6.34±0.29	129.00±2.81*	0.45±0.14	0.55±0.02*	0.15±0.04
VI	84.93±10.73*	6.44±1.17	130.70±2.33	0.42±0.18	0.63±0.03*	0.28±0.00

I: Control, II: CCl_4 , III: CZ 200 mg/kg + CCl_4 , IV: 400 mg/kg + CCl_4 , V: NBF 20 mg/kg + CCl_4 VI: NBF 40 mg/kg + CCl_4 . [#] $p < 0.05$ compared to Group I * $p < 0.05$, ** $p < 0.01$ compared to Group II; $n = 5$. CCl_4 : carbon tetrachloride, NBF: n-butanol fraction * expressed in mg/L, **expressed in mmol/L.

Table 2. Effect of aqueous leaf extract of *C. zambesicus* and n-butanol fraction on antioxidant enzymes.

Group	*CAT	*SOD	*GSH	*GPx	*MDA
I	44.58±0.75	17.79±0.06	15.50±0.42	24.79±3.96	0.77±0.02
II	41.80±0.83	11.66±0.00 [#]	13.53±0.14 [#]	18.75±2.91	1.06±0.00 [#]
III	46.54±1.03	6.34±0.31	14.68±0.03	15.00±3.33	0.74±0.05*
IV	48.22±3.54	18.37±0.04*	14.32±0.24	19.79±3.13	0.69±0.02*
V	52.01±0.90*	18.34±0.01*	16.17±0.63*	19.37±2.29	0.80±0.01
VI	44.52±1.64	19.74±0.00*	16.53±0.25*	23.47±1.57	0.73±0.03*

I: Control, II: CCl_4 , III: CZ 200 mg/kg + CCl_4 , IV: 400 mg/kg + CCl_4 , V: NBF 20 mg/kg + CCl_4 , VI: NBF 40 mg/kg + CCl_4 . [#] $p < 0.05$ compared to Group I * $p < 0.05$ compared to Group II; $n = 5$. CCl_4 : carbon tetrachloride, NBF: n-butanol fraction, * expressed in U/mg protein.

DISCUSSION

The leaf extract of CZ is used by traditional medicine practitioners in the treatment of renal disease and as a food spice. The protective effect of the leaf extract of CZ was determined in CCl_4 -induced kidney injury. Toxic-acute renal injury produced by CCl_4 has been attributed to reactive oxygen species formed during its biotransformation in the endoplasmic reticula of renal cells [16]. Subcutaneous administration of CCl_4 in rats produced toxic effects in the kidneys, resulting in an increase in serum concentration of sodium ions, urea and creatinine with a decrease in bicarbonate ions. In addition, there was an increase in MDA with a decrease in concentrations of SOD, CAT, GSH and GPx. An increase in serum urea and creatinine has been attributed to reduced glomerular filtration [17]. The aqueous extract and n-butanol fraction of CZ attenuated CCl_4 -induced a reduction in glomerular filtration by restoring the concentration of electrolytes, urea and creatinine to normal levels similar to those obtained in the control group. A reduction in serum electrolytes, urea and creatinine was also reported in a previous study carried out to determine the nephroprotective effect of ethanolic root extract of CZ on gentamicin-induced kidney injury [18].

In the present study, CCl_4 treatment produced a significant increase in the concentration of MDA. Thiobarbituric acid reactive substances (TBARS) produced during oxidative stress are biomarkers of lipid peroxidation [19]. Induction of lipid peroxidation and subsequently oxidative stress was reported in animals treated with CCl_4 [20, 21]. Treatment of animals with extract and fraction of CZ produced a decrease in

concentration of MDA, indicating reduction in oxidative stress and lipid peroxidation.

The aqueous leaf extract of CZ and n-butanol fraction had protective effects on the kidneys. They both increased the concentration and activity of SOD, CAT, GSH and GPx, hence attenuating oxidative stress produced by CCl_4 . Results obtained from this study corroborates the *in vitro* antioxidant activity reported in a previous study [12]. The *in vivo* nephroprotective activity of leaf extract and fraction of CZ may be attributed to the presence of phytochemicals with antioxidant activity.

Polyphenolic compounds present in medicinal plants have been reported to have nephroprotective effect by inhibiting the production of reactive oxygen species [22]. Flavonoids, terpenoids, tannins, saponins have been found in the leaf extract and n-butanol fraction of CZ [23] while phenolic compounds including flavonoids, lignoids and proanthocyanidins are found in the volatile oil present in the leaves [24].

CONCLUSION

The results from this study demonstrated that aqueous leaf extract and n-butanol fraction had nephroprotective activity. The findings support an ethnomedicinal role of this plant in the management of kidney disease.

ACKNOWLEDGMENT

This research was partly funded by Tertiary Education Trust Fund-Institutional Based Research (TETFUND-IBR 2014-2016) grant. The authors are grateful to Mr. Shittu and Mr. Kehinde for their technical assistance during the conduction of this study.

CONFLICT OF INTEREST

The authors declare no conflicting interest in the course of conducting this study.

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