

Anti-oxidative Effects of Butanol Seed Extract of *Parkinsonia aculeata* on Carbon Tetrachloride-Induced Liver Damage on Wistar Rats

Muhammad Bashiru AbdulRahman^{1,*}, Yusuf Gumburawa Malami², Sanusi Wara Hassan³,
Mansur Lawal³, Waliu Temitope Adanlawo⁴, Mansur Mohammed Birnin Kebbi⁴,
Kamaldeen Olalekan Sanusi⁵

¹Department of Chemical Pathology and Immunology, Faculty of Basic Clinical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria;

²Department of Science Laboratory Technology, Umaru Ali Shinkafi Polytechnic, Sokoto, Nigeria;

³Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria;

⁴Department of Chemical Pathology, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria;

⁵Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Corresponding author*
abfmhzs69@gmail.com

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Abstract

Medicinal plants have protective effect because of the presence of several compounds which have different mechanism of action. This study sought to assess the anti-oxidative effects of butanol seed extract of *Parkinsonia aculeata* on carbon tetrachloride (CCl₄)-induced liver damage on Wistar rats. The Wistar rats were put into five groups, each with six rats: Group A received a daily dosage of liquid paraffin (1ml/kg); Group B received 1ml/kg body weight of CCl₄ (30% in liquid paraffin intraperitoneal); Group C, D, and E received the seed extracts at 100, 120, and 160 mg/kg every day for two weeks. Induction of CCl₄ was three times a week for two weeks simultaneously with the extract to the last day. After sacrificed, the liver was harvested and homogenized, and used for further analyses. There was a significant increase ($p < 0.05$) in the levels of superoxide dismutase and catalase in all extract treated groups compared to positive control, except the catalase levels of group treated with 160mg/kg. Similar results was observed in vitamin C, vitamin E and glutathione in rats treated with 100 and 120mg/kg of the extract. The results of this study revealed that butanol seed extract from *P. aculeata* has antioxidant properties and can protect Wistar rats' livers from the damaging effects of CCl₄.

Keywords: antioxidant; carbon tetrachloride; liver damage; oxidative stress; *Parkinsonia aculeata*.

Abbreviations: CAT – catalase; GSH - reduced glutathione; SOD - superoxide dismutase; CCl₄ - Carbon Tetrachloride; MDA – malondialdehyde; ROS - reactive oxygen species; H₂O₂ - hydrogen peroxide.

INTRODUCTION

Antioxidant activity is an important approach to defend against liver damage. Antioxidants are chemicals that inhibit the oxidation of a molecule in the cells when present in extremely low concentrations. It has the capacity to nullify the effects of oxidation (lipid peroxidation) caused by free radicals in the liver of an organisms. The impaired electrons of free radicals are highly reactive and neutralize the harmful reactions of human metabolism (Serbinova et al., 1991). Protection of the liver against free radicals effects is provided by enzymatic antioxidants such as catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) and non-enzymatic antioxidants e.g. vitamin C and vitamin E that play a crucial role in lipid peroxidation process.

The rising amount of chemical compounds and pollution in the environment are causing an increase in

liver illnesses (Wadekar et al., 2008). Many of these chemicals cause harm to cells and molecules by producing reactive oxygen species and other free radicals. Because of its critical function in the body's metabolism of external chemical compounds, liver is one of the organs that can cause toxic reactions and is a popular subject of toxicological research (Beckman & Ames, 1998).

Carbon Tetrachloride (CCl₄) is frequently used as a chemical inducer of experimental liver cirrhosis. This toxic agent activates liver damage by forming reactive intermediates, such as trichloromethyl free radicals, via cytochrome P450-related functions in the oxidase system (Recknagel et al., 1989). The main causes of CCl₄-induced hepatic damage is related to lipid peroxidation enzymes, and generation of free radicals caused by this agent (Poli, 1993).

Medicinal plants have protective effect because of the presence of several compounds which have different

mechanism of action. Some of the components of this plant are proteins and enzymes of low molecular weight such as vitamin, flavonoids and carotenoids (Zhang & Wang, 2002). Many of these components especially phytochemical constituents exhibit hepatoprotective effects due to their antioxidant property (Madrigal-Santillán et al., 2014).

The leaf, stem bark and the seed extracts of *Parkinsonia aculeata* are used traditionally in the Northern Nigeria for the treatment of hepatopathy, bacterial diseases, typhoid fever, diabetes, malaria and trypanosomiasis (Hassan et al., 2010; Leite et al., 2007). Leaf extracts of *P. aculeata* were reported to have hepatoprotective and antioxidant activities (Hassan et al., 2005). However, there is paucity of scientific report on the seed extract. This study therefore sought to assess the anti-oxidative effects of butanol seed extract of *P. aculeata* on carbon tetrachloride-induced liver damage on Wistar rats.

MATERIALS AND METHODS.

Plant Collection, Identification and Storage

Parkinsonia aculeata seeds were obtained on the campus of Usmanu Danfodiyo University in Sokoto, Nigeria. The plant components were identified and taxonomically validated at Usmanu Danfodiyo University, Sokoto Botany unit. In the departmental herbarium of the Botany unit, a voucher specimen of the plant (UDUH/ANS/0038) was deposited for reference. The seeds were opened and air dried for one week in the shade before being pounded into a fine powder with a mortar and pestle and stored at room temperature until use.

Experimental Animal

Wistar rats of either sex weighing 120-200 g were obtained from animal House, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. They were kept in wire mesh cages with free access to food and water for one week to acclimatize. They were maintained on standard optimal feeds and clean tap water before and after daily administration of plant extract between 9:30 to 10:30 hours. Experiments were performed according to ethical guidelines for investigation of experimental pairs in conscious animals. The standard orogastric cannula was used for oral administration of the seed extract.

Preparation of Plant Extracts

Two hundred grams (200g) of fine powder of the plant seed were extracted with two (2) litres of methanol at room temperature overnight and filtered through Whatman No. 1 filter paper. The filtrate was concentrated to dryness using rotary evaporator and percentage yield was calculated (5.6%). The residue was dissolved in distilled water and partitioned with butanol

(saturated with water). The butanol fraction was thereafter screened for antioxidant properties.

Experimental Design

The animals were divided into 5 groups of six (6) rats.

Group A: (Normal control) Received daily dose of liquid paraffin (1ml/kg) body weight per day for 14 days.

Group B: (Positive control) Received 1ml/kg body weight of CCl₄ (30% in liquid paraffin intraperitoneal) three (3) times a week for two (2) weeks.

Group C, D and E: Received the seed extracts of *P. aculeata* at 100, 120 and 160 mg/kg per day respectively for two weeks. Induction of CCl₄ was three times a week for two weeks simultaneously with the extract to the last day.

Animals were sacrifice 24 hours after the last day. Liver was removed and rinsed in ice-cold 1.5% KCL, dried and weighed homogenized in 4x ice-cold isotonic phosphate buffer pH 7.4 and the centrifuged at 9000g for 20 minutes to obtain post mitochondria supernatant at 105,000xg for 60 minutes to obtain the microsomes cytosolic fractions of the supernatant was immediately frozen on dry ice and microsome was suspended in 0.15 sucrose solution.

Sample Preparation for the Determination of Markers of Antioxidant Activity

The liver was perfused with 0.86% cold saline to completely remove the red blood cells, it was suspended in 10% (w/v) ice-cold. 0.1cm³ phosphate buffer at pH 7.4. The liver was then cut into small pieces, some quality was weighed and homogenized. The homogenate used for the estimation of enzymatic and non-enzymatic antioxidants.

Estimation of Vitamin C

Vitamin C estimation was carried out using the method of Rutkowski *et al.* (Rutkowski et al., 1998). Briefly, 1ml of sample was measured into test tubes, 1ml of PR (50nm solution of oxalic acid) was added and mixed thoroughly at room temperature for 30 minutes. It was centrifuged at 700g for 16 minutes, supernatant was collected with pipette and used as a test sample for spectrophotometric measurements. Standard sample was prepared using 1ml of standard solution without centrifugation.

Absorbance of test sample (Ax) and standard sample (As) was measured at 700nm against the mixture of PR as the reference sample.

Concentration Cx of vitamin C (µm) in the sample was determined using the formula

$$Cx = \frac{Ax}{As} \cdot Cs$$

Where; Cs = Concentration of standard solution

Estimation of Vitamin E

Vitamin E was determined using the method of Rutkowski *et al.* (Rutkowski et al., 2005). Briefly, 0.5ml of sample was measured into test tube, 0.5ml of anhydrous ethanol were added and shaken vigorously then plugged for 1 minute. A 3ml of xylene was added again and shaken vigorously for another 1 minute, centrifuged at 1500g for 10 minutes simultaneously. A 0.25ml solution of batophenanthroline was measured into test tube II. A 15ml of the extract was collected and transferred to test tube II, the content was mixed. A 0.25ml of FeCl₃ solution was also added to test tube II, mixed then 0.25ml of H₃PO₄ solution was also added and mixed again and the absorbance was measured spectrophotometrically. 0.5ml of standard solution prepared using trolox as test sample using α tocopherol. 0.5ml of deionized water was added. Absorbance of the test (Ax) and standard (As) was measured at 539nm against blank test. Concentration of vitamin E was thereafter calculated using the formula:

Concentration of Vitamin E (Cx)

$$Cx = \frac{Ax}{As} \cdot Cs$$

Where; Cs = Concentration of Standard solution.

Estimation of Reduced Glutathione and Enzymatic Assay of Superoxide Dismutase and Catalase

The colorimetric assay for catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) was carried out using commercial kits (Radox Lab) according to the manufacturer's protocol.

Malondialdehyde (MDA) Determination

Tissue supernatant (150 μ l) was diluted to 500 μ l with double deionized water. Two hundred and fifty (250) μ l of 1.34% thiobarbituric acid was added to all the test tubes followed by addition of an equal volume (250 μ l) of 4% trichloroacetic acid (TCA). The resulting mixture was shaken and incubated for 30 minutes in a water bath temperature greater than 90^oC. The test tubes were allowed to cool at room temperature and the absorbance of the complex formed was read at wave length of 532nm (Hartman, 1983). The absorbance was extrapolated from a standard curve generated by using a standard (1,1,3,3-tetraethoxy propane). The result was expressed as nanomoles of MDA per cubic centimeter of supernatant.

RESULTS

Estimation of non-enzymatic antioxidants.

There was a significant increase ($p < 0.05$) in the levels of vitamin C, vitamin E and GSH in rats treated with 100 and 120mg/kg of the extract. However, it was observed that the higher the dose, the lower the values (Table 1).

Table 1. Non-enzymatic antioxidant properties of rats administered butanol seed extracts of *Parkinsonia aculeata* and carbon tetrachloride.

Group	Vitamin C (umol/L)	Vitamin E (mg/dL)	GSH (mg/ml)
A	121.07 \pm 24.62	96.88 \pm 27.87	22.90 \pm 1.24
B	110.46 \pm 21.34 ^{α}	89.23 \pm 7.61 ^{α}	14.84 \pm 5.35 ^{α}
C	156.91 \pm 14.94 ^{$\alpha\beta$}	173.36 \pm 5.88 ^{$\alpha\beta$}	48.15 \pm 2.00 ^{$\alpha\beta$}
D	130.88 \pm 7.17 ^{$\alpha\beta$}	107.29 \pm 8.79 ^{$\alpha\beta$}	27.44 \pm 1.41 ^{$\alpha\beta$}
E	111.59 \pm 25.97 ^{α}	103.87 \pm 3.04 ^{β}	20.37 \pm 1.46 ^{β}

Values are expressed as Mean \pm SEM (n = 5) α = significantly ($p < 0.05$) different vs A, β = significantly ($p < 0.05$) different vs B, by using the analysis of variance and LSD multiple comparison test on SPSS software (IBM Corp.USA, 2011). GSH: Reduced Glutathione.

Group A: Liquid paraffin treated group, Group B: 30% CCl₄ treated group, Group C: Administered 100mg/kg of butanol extract + CCl₄, Group D: Administered 120mg/kg of butanol extract + CCl₄, Group E: Administered 160mg/kg of butanol extract + CCl₄

Estimation of enzymatic antioxidant markers

A significant increase ($p < 0.05$) was observed in the levels of antioxidant enzymes such as SOD and CAT in all treatment groups compared to positive control, except the CAT levels of group treated with 160mg/kg of the extract. Moreover, the levels of lipid peroxidation marker (MDA) was only significantly lower ($p < 0.05$) in the group treated with 160mg/kg of the extract (Table 2) compared to positive control.

Table 2. Enzymatic antioxidant properties of rats administered butanol seed extracts of *Parkinsonia aculeata* and carbon tetrachloride.

Group	SOD (u/ml)	CAT (u/ml)	MDA (umol/L)
A	14.30 \pm 6.01	288.88 \pm 43.09	130.34 \pm 32.93
B	8.06 \pm 6.38 ^{α}	279.14 \pm 20.62 ^{α}	195.94 \pm 58.65 ^{α}
C	44.53 \pm 21.40 ^{$\alpha\beta$}	410.06 \pm 23.47 ^{$\alpha\beta$}	217.17 \pm 99.33 ^{$\alpha\beta$}
D	89.94 \pm 25.44 ^{$\alpha\beta$}	352.65 \pm 21.31 ^{$\alpha\beta$}	222.22 \pm 8.79 ^{$\alpha\beta$}
E	56.93 \pm 35.72 ^{$\alpha\beta$}	276.44 \pm 8.95 ^{α}	96.15 \pm 5.25 ^{$\alpha\beta$}

Values are expressed as Mean \pm SEM (n = 5) α = significantly ($p < 0.05$) different vs A, β = significantly ($p < 0.05$) different vs B, by using the analysis of variance and LSD multiple comparison test on SPSS software (IBM Corp.USA, 2011).

Group A: Liquid paraffin treated group, Group B: 30% CCl₄ treated group, Group C: Administered 100mg/kg of butanol extract + CCl₄, Group D: Administered 120mg/kg of butanol extract + CCl₄, Group E: Administered 160mg/kg of butanol extract + CCl₄, MDA: Malondialdehyde, CAT: Catalase; SOD: Superoxide dismutatse.

DISCUSSION

Free radicals play a role in the aetiology of a variety of diseases (Covacci et al., 2001). They are easily produced in the body through regular metabolic pathways. Antioxidants, on the other hand, can neutralise free radicals and thereby prevent disease. Natural antioxidants derived from plant sources have been linked to a lower risk of chronic disease due to their ability to stop free radical growth in the biological system (Covacci et al., 2001). Antioxidant activity, or the prevention of free radical formation, is critical in the

prevention of CCl₄-induced hepatopathy (Venukumar & Latha, 2002).

To avoid and neutralise free radical-induced damage, the body possesses an excellent defence mechanism. Catalase, superoxide dismutase, and glutathione peroxidase are examples of endogenous antioxidant enzymes that help with this. These enzymes form a mutually beneficial defence team against reactive oxygen species (ROS) (Hewawasam et al., 2003). In CCl₄ induced hepatotoxicity, the balance between ROS production and antioxidant defences may be lost hence oxidative stress result, which through a series of event regulate cellular functions leading to hepatic necrosis. From the result of this study, rats administered CCl₄ showed significant changes (P<0.05) on the liver function and antioxidant parameters when compared with normal control group. The reduced activity of antioxidant enzymes observed point out the hepatic damage as the rats administered with CCl₄. The seed extract had indicated reversed changes in liver function and antioxidant parameters when compared with CCl₄ treated group.

In terms of non-enzymatic antioxidants, GSH is a significant predictor of tissue sensitivity to oxidative damage, and hepatic GSH depletion has been linked to increased toxicity to compounds such as CCl₄ (Kidd, 1997). In the present study, decrease in hepatic tissue of GSH level was observed in CCl₄ treated groups. Moreover, the findings showed significant decrease in the levels of vitamin C and vitamin E in CCl₄ treated group when compared to the normal control group. This is in agreement with the findings of Narasimhanaidu and Ponnain, (2006) that indicated that condition of severe oxidative stress may also lead to the depletion of protective physiological moieties such as GSH, vitamin C and vitamin E in rats (Kamalakkannan & Prince, 2006). Glutathione through its significant reducing power contributes to the recycling of other antioxidants such as vitamin C and E that have become oxidized (Kidd, 1997). Interestingly, the reduced levels of GSH, vitamin C and vitamin E caused by CCl₄ was attenuated by the butanol seed extract of *P. aculeata*.

Lipid peroxidation is a measure of membrane damage and changes in the structure and function of cellular membranes, and it was found to be higher in the CCl₄-treated group. The observed increase in MDA levels of CCl₄ groups suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanism to prevent formation of excessive free radicals (Achliya et al., 2004). This is also in agreement with findings of Moscrella et al. (1994) and Patrick-Iwuanyanwu et al. (2007). The treatment with 160mg/kg of the extract was however able to reduce the MDA levels, which suggests a hepatoprotective effect.

Similarly, the activities of SOD and CAT was observed to increase as result of the CCl₄ administration.

SODs are the first line of defence against harm caused by ROS. These proteins catalyse the dismutation of superoxide anion free radical (O₂⁻) into molecular oxygen and hydrogen peroxide (H₂O₂), lowering the level of O₂⁻, which can harm cells at high levels. (Yasui & Baba, 2006). Catalase is an antioxidant enzyme that acts as a catalyst for the conversion of hydrogen peroxide to oxygen to oxygen and water. It prevents the effect of H₂O₂ that is present intracellular (Nandi et al., 2019). The effect of CCl₄ on the antioxidant enzymes in this study was significantly reversed by the treatment with the butanol seed extract of *P. aculeata*. The presence of phenolic compounds in the plant might have scavenged the free radicals, thus offering the hepatoprotection.

CONCLUSIONS

Findings from this study showed that butanol seed extract of *P. aculeata* exhibit anti-oxidative property which could rescue the liver of Wistar rats from the toxic effects of CCl₄. Moreover, *P. aculeata* at minimal concentration is recommended as optimal effects was recorded at a low dose (100mg/kg) used in this study.

Competing interests: The authors declare that there are no competing interests.

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