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Hepatoprotective Effect of Leaf Extracts of *Parkinsonia aculeata* L. Against CCl_4 Intoxication in Albino Rats

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Abstract: Hepatoprotective activity of 50% ethanolic leaf extracts of *Parkinsonia aculeata* was evaluated against carbon tetrachloride (CCl_4)-induced hepatic damage in rats. The extract at doses of 100, 200 and 300 mg kg^{-1} body weight were administered orally once daily. Hepatoprotective activity was measured based on biochemical parameters. Significantly ($p<0.05$) elevated levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and peroxide value in CCl_4-intoxicated rats were restored to normal levels in the animals treated with the extract at doses of 100, 200 and 300 mg kg^{-1} and CCl_4. The levels of total proteins, albumin, vitamins C and E appreciated significantly ($p<0.05$) in animals treated with different doses of the leaf extracts and CCl_4. The effects were dose-dependant. These results suggest the leaf extracts of this plant to have potential therapeutic and preventive efficacies, probably due to its antioxidative effect.

Key words: Hepatoprotective, *Parkinsonia aculeata*, carbon tetrachloride, vitamin C and E, transaminases, alkaline phosphatase

INTRODUCTION

In recent years, many researchers have examined the effects of plants used traditionally by indigenous healers to support treatment of liver diseases. There are no effective drugs that are available in modern medicine that confer protection to the liver against damage or help to regenerate hepatic cells (Chattopadhyay, 2003). Due to the dearth of reliable liver protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders (Chatterjee, 2000). Scientific validations are being made globally to get evidences for traditionally reported herbal plants. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes have side effects. This is one of the reasons why many researchers turn to complementary and alternative medicine (Guntupalli *et al*., 2006).

There is an increasing interest in the biochemical basis of and on the mechanism of action of protective effect of naturally occurring antioxidants in biological systems. Several plant constituents have shown antioxidant activity and are empirically proved to be useful in the clinical treatment of liver disorders (Mantle *et al*., 2000; Choi *et al*., 2002; Auddy *et al*., 2002; Wongnawa *et al*., 2006). Higher plants produce a wide array of secondary metabolites that have therapeutic and pharmaceutical applications. Flavonoids and other phenolic compounds of plant origin have roles as scavengers and inhibitors of lipid peroxidation (Formica and Regelson, 1995; Rice-Evans *et al*., 1997). CCl_4, whose catabolism produces radicals, is commonly used for induction of liver damage in rats. The radicals cause lipid peroxidation and necrosis of hepatocytes (Singh *et al*., 1998).

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Jerusalem thorn (*Parkinsonia aculeata*) is a shrubby thorny tree, similar to Kiawe (*Prosopis pallida*). It is a hardy species and valued as an ornamental or shade tree. Jerusalem thorn is widely cultivated and known to spread from its origin in North America, as far north as California, Florida, Australia and Micronesia. It is 3-10 m tall, with a green bark, smooth- branched, armed with strong spines of stipule or leaf rachis origin (Department of Natural Resources, 1998).

The leaf extracts of *Parkinsonia aculeata* are used traditionally in northern Nigeria for the treatment of hepatopathy, bacterial diseases, typhoid fever, diabetes and trypanosomiasis. The aerial parts of *P. aculeata* have been used to treat diabetes-related complications (Leite *et al*., 2007). The plant was reported to have antibacterial activity, toxic to kidney but safer to liver at subchronic administration (28 days) of 1500-3000 mg kg^{-1} body weight and with an LD_{50} greater than 3000 mg kg^{-1} body weight (Hassan *et al*., 2005). The leaves of *P. aculeata* contain tannins, flavonoids, saponins, glycosides, alkaloids, steroids and volatile oils. But cyanogenic glycosides, anthraquinones and anthraquinone glycosides have not been detected in the plant (Hassan *et al*., 2005).

To the best of our knowledge, there is no scientific report available in support of the hepatoprotective activity of *P. aculeata* leaves. Hence, to justify the herbal claims we have evaluated the hepatoprotective effect of leaves of *P. aculeata* against CCl_4-induced hepatotoxicity in rats. The hepatoprotective activity of the plant reported in this study would provide scientific evidence of its claimed medicinal properties.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade.

Plant Material

The leaves of *P. aculeata* were collected from within Usmanu Danfodiyo University campus, Sokoto, Nigeria. The plant material was identified and authenticated taxonomically at Botany unit of the same institution. A voucher specimen of the plant was deposited for reference in the Departmental Herbarium (Botany unit). This research was conducted between June and July 2007 at Usmanu Danfodiyo University, Sokoto, Nigeria.

Preparation of Plant Extract

The collected leaves were open-air-dried under the shade, pulverized in to a moderately coarse powder (using pestle and mortar). Two hundred grams (200 g) of the powdered leaves were extracted with 50% ethanol-water (2000 mL of 1:1) at room temperature for 24 h. The extract was filtered through Whatman filter paper (No. 1) and concentrated by removing the solvents completely under reduced pressure. The yield of the extract was found to be 10% (w/w).

Animals

Albino rats (Wister strains) of either sex weighing 126-191 g were purchased 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 animal feeds (Bendel feeds and flour mills, Edo state, Nigeria) and clean tap water *ad libitum*, before and after daily administration of the plant extract between 10.30 to 11.30 h. Experiment was performed according to ethical guidelines for the investigation of experimental pain in conscious animals (Zimmerman, 1983). The standard orogastric cannula was used for oral administration of the extract.

Experimental Procedure

Carbon tetrachloride induction of hepatotoxicity was done according to reported procedures of Guntupalli *et al*. (2006) with some modifications. Animals were randomly divided into five groups of six animals. Group 1 (normal control untreated rats) received a daily dose of liquid paraffin for five days (1 mL kg^{-1} body weight, per os). Group 2 (Induction control) were administered 30% carbon tetrachloride in liquid paraffin from day two to five (1 mL kg^{-1} body weight, intra peritoneal). Groups 3-4 (test groups) were treated with a daily dose of 100, 200 and 300 mg kg^{-1} body weight (orally) of *P. aculeata* leaves for five days and CCl_4 from the second to the fifth day. All the animals were sacrificed on the 6th day. The blood collected was allowed to clot for 30 min. Serum was separated by centrifuging at 37°C for estimation of biochemical parameters.

Assessment of Hepatoprotective Activity

The activities of serum aspartate and alanine transaminases were assayed by the method (Randox assay kit) of Reitman and Frankel (1957). Alkaline phosphatase, albumin, total protein and serum total bilirubin were estimated by the methods of Rec GSCC (1972), Cheesbrough (1991), Gornall *et al*. (1949) and Varley *et al*. (1991), respectively. All these parameters were used to assess the acute hepatic damage caused by CCl_4.

Assessment of Antioxidant Activity

The liver was perfused with 0.86% cold saline to completely remove all the red blood cells. It was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) and cut into small pieces. The required amount was weighed and homogenized using a Teflon homogenizer. The homogenate was used for the estimation of non-enzymic antioxidants such as vitamin C (Urbach *et al*., 1951) and E (Baker *et al*., 1980) and lipid peroxide level (Ohkawa *et al*., 1979).

Statistical Analysis

The data are expressed as mean±standard deviation. Results were analyzed statistically by one-way Analysis of variance (ANOVA), using Graph pad Instat, Benferoni compare all columns (San Diego, USA). A value of $p<0.05$ was considered statistically significant.

RESULTS

Significant ($p<0.05$) weight gain compared to final weight after six days was observed in control group. There was no significant ($p>0.05$) loss of body weight in groups administered CCl_4 and *P. aculeata* extracts at doses of 100, 200 and 300 mg kg^{-1} body weight (Table 1). Rats treated with carbon tetrachloride exhibited increase ($p<0.05$) in the activity of hepatospecific enzymes ALP, AST and ALT and serum total bilirubin (TB) and peroxide value (PV) compared to normal control rats (Table 2). Also, serum total protein (TP), albumin (AB), vitamin C and E levels were significantly ($p<0.05$) reduced in carbon tetrachloride treated rats.

Table 1: Total body weights of the animals before and after treatment days

Treatments	Initial weight before treatment (g)	Final weight after treatment (g)
Control	178.80±15.70	211.40±12.30*
CCl_4	181.00±46.20	155.00±27.30
P. aculeata and CCl_4 (100 mg kg^{-1})	190.10±25.40	156.60±28.30
P. aculeata and CCl_4 (200 mg kg^{-1})	182.10±33.10	153.60±23.00
P. aculeata and CCl_4 (300 mg kg^{-1})	126.50±25.90	109.10±24.20

Values are mean±standard deviation * = Significantly different ($p<0.05$) from the initial weight after five days of treatment by using t-test, Graph Pad Instant Software (San Diego, USA)

Table 2: Effect of ethanolic leaf extracts of *Parkinsonia aculeata* on serum parameters in CCl_4 induced hepatic damage in rats

Treatments	ALP (U L^{-1})	ALT (U L^{-1})	AST (U L^{-1})	T. Bilirubin (Mg dL^{-1})	Albumin (g dL^{-1})
Control	151.20±15.20	53.68±4.74	46.90±3.13	4.57±0.23	3.73±0.64
CCl_4	286.40±21.40^c	108.16±3.68^c	107.80±18.80^c	9.74±1.54^c	1.00±0.53^c
P. aculeata + CCl_4 (100 mg kg^{-1})	163.80±15.40^e	83.20±2.99^{ec}	71.40±7.30^{ec}	4.38±0.12^e	3.93±0.70^e
P. aculeata + CCl_4 (200 mg kg^{-1})	158.40±15.10^e	72.00±1.60^{ec}	63.60±11.50^e	3.76±0.57^e	3.87±0.56^e
P. aculeata + CCl_4 (300 mg kg^{-1})	193.20±29.50^e	64.00±2.53^{ec}	56.00±4.30^e	3.61±0.61^e	4.60±0.28^e

Treatments	Total protein (g dL^{-1})	Vitamin C (mg dL^{-1})	Vitamin E (mg dL^{-1})	Peroxide value (mmol L^{-1})
Control	9.34±1.96	98.00±13.30	0.62±0.08	125.70±20.90^c
CCl_4	5.44±0.77^c	42.00±8.37^c	0.17±0.05^c	204.90±8.520^e
P. aculeata + CCl_4 (100 mg kg^{-1})	8.26±1.337^e	74.00±20.70^e	0.36±0.03^{ec}	146.30±27.40^e
P. aculeata + CCl_4 (200 mg kg^{-1})	9.22±1.66^e	78.00±14.80^e	0.42±0.03^{ec}	144.00±10.20^e
P. aculeata + CCl_4 (300 mg kg^{-1})	7.74±0.76^e	88.00±12.7^e	0.53±0.06^e	140.00±14.00^e

Values are mean±standard deviation. ALT= Alanine amino transferase, AST= Aspartate amino transferase, ALP = Alkaline phosphatase, T = Total, e = Significant Vs CCl_4, c = Significant Vs control, ec = Significant Vs control and CCl_4 by using analysis of variance (n = 6), Benferroni compare all columns, Graph Pad Instat Sofware (San Diego, USA). A p-value of <0.05 was considered statistically significant

The liver damage was evaluated by the measurement of serum levels of ALP, AST, ALT, TB, AB and TP. Rats that were orally administered leaf extracts of *P. aculeata* (100, 200 and 300 mg kg^{-1}) and CCl_4 showed significant ($p<0.05$) decrease in the serum enzymes activities, TB and peroxide value and increase in the levels of total protein, albumin, vitamins C and E were observed compared to CCl_4 treated group (Table 2). The CCl_4 intoxication resulted in a state of liver injury in rats as manifested by the significant increase in the serum enzyme activities, total bilirubin and peroxide values.

DISCUSSION

The animals in the control group in this study show significant ($p<0.05$) increase in body weight at the end of the study. The weight gain could have resulted from proper feed utilization and conversion. A non significant ($p>0.05$) loss of body weight in groups administered CCl_4 and *P. aculeata* extracts at doses of 100, 200 and 300 mg kg^{-1} body weight were observed.

Attention has been focused on the role of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemical to highly reactive compounds such as free radicals, carbenes and nitrenes (Gupta *et al*., 2004). The toxic metabolite CCl_3 radical of CCl_4 is produced by the action of cytochrome P_{450} which further reacts with oxygen to give trichloromethyl peroxy radicals. This radical binds covalently to macromolecules and causes peroxidative degradation of lipid membrane of the adipose tissue (Avijeet *et al*., 2008). In this view, the reduction in the levels of hepatospecific enzymes by the leaf extracts of *P. aculeata* is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. This effect suggests probably that serum levels of hepatospecific enzymes return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes.

Also, necrosis of the liver leads to elevation of the serum marker enzymes which are released from the liver in to blood (Ashok Shenoy *et al*., 2002). AST, ALT and ALP are considered markers for liver function (Tolman and Rej, 1999; Hilaly *et al*., 2004) ALT is located primarily in the cytosol of hepatocytes. This enzyme is considered a more sensitive marker of hepatocellular damage than AST. AST is found in the cytoplasm and mitochondria in different tissues, chiefly in the heart, skeletal muscles, liver, kidneys, pancreas and erythrocytes (Aniagu *et al*., 2004). From our results, increases of serum ALP, ALT and AST in rats treated with only CCl_4 are indication of liver necrotic injury and cholestasis (Achliya *et al*., 2004; Hassan *et al*., 2005). The increase of AST in the CCl_4 treated group

may be due to the release of enzyme from the cells of the damaged organ, or to change in the membrane permeability of the cells. Determination of plasma proteins like albumin can act as a criterion for assessing synthetic capacity of the liver, since nearly all of them are synthesized in hepatocytes. In the present study, decrease in plasma proteins and albumin as seen in only CCl_4 treated rats may reflect chronic damage. Elevation of enzymatic activity caused by CCl_4 correlates with the high level of serum bilirubin as observed in this research work. The aqueous ethanolic extract of the plant has induced suppression of ALP activity and depletion of bilirubin. This suggests that the extract restores biliary function in injured rat liver (CCl_4 induced) and thus preserves the functional integrity of the liver tissue. Therefore, administration of 50% ethanolic extracts revealed hepatoprotective activity of leaves of *P. aculeata* against the toxic effect of CCl_4.

The plant kingdom appears to be an important resource of phytochemicals with antioxidant and hepatoprotective properties. For example *Hypericum* specie exhibit antioxidant properties *in vitro* probably owing to flavones and polyphenols (Niko *et al*., 2007). Sanmugapriya and Venkataraman (2006) reported significant hepatoprotective activity of seeds of *Strychnos potatorum* Linn. with presence of triterpenes, steroids, polyphenolic and saponins. Leaf extracts of *Momordica dioica* Roxb. have been reported to have antioxidant and hepatoprotective activities (Avijeet *et al*., 2008). Also leaf extract of *Cistus laurifolius* possesses protective activity against acetaminophen induced hepatotoxicity in mice (Küpeli *et al*., 2006).

Preliminary phytochemical analysis of the leaves of *P. aculeata* show the presence of tannins, flavonoids, saponins, glycosides, alkaloids, steroids and volatile oils (Hassan *et al*., 2005). Compounds like triterpenes, steroids, flavonoids and polyphenolics, alkaloids have been reported to have hepatoprotective and antioxidant activities (Di Carlo *et al*., 1999; Ahmed *et al*., 2000; Kumar *et al*., 2004; Sanmugapriya and Venkataraman, 2006). Increased level of lipid peroxide value and decrease of vitamin C and D levels in the liver homogenate of CCl_4 intoxicated rats is an indication of excessive free radical formation. The significant decrease of lipid peroxide value and increases of vitamin C and D levels in the liver homogenate of rats pre-administered *P. aculeata* extract (100 to 300 mg kg^{-1} body weight) and CCl_4 indicates anti-lipid peroxidative effect of *P. aculeata*. This exerts a beneficial action against pathological alterations caused by the generated free radical (CCl_3). Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation.

The present study has demonstrated that aqueous ethanolic extract of *P. aculeata* had therapeutic and preventive efficacies in CCl_4 induced hepatotoxicity in rats. Hence, it is possible that the mechanism of hepatoprotection by *P. aculeata* leaves may be due to its antioxidant action and to the presence of tannins, flavonoids, saponins, glycosides, alkaloids, steroids and volatile oils in the extract. Further studies are recommended to elucidate the mechanisms of the hepatoprotective action of this plant and identification of its active agent(s).

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