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Hepatoprotective Effect Of The Crude Leaf Extracts Of *Portulaca Oleracea* In Carbon Tetrachloride Induced Liver Injury In Rodents

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ABSTRACT

The hepatoprotective activity of *Portulaca oleracea* Linn against carbon tetrachloride (CCl4) induced liver injury in rat was investigated. Thirty (30) adult male albino wistar rats were used for the study.The rats were divided into six (6) groups (A-F) of five (5) animals each. Groups A-D received two graded doses of the aqueous extract of *Portulaca oleracea* (AEPO) and methanol extract of *Portulaca oleracea* by the oral route for seven days prior to CCl_4 injection. Group 'E' served as the CCl_4 (positive) control while Group 'F' served as baseline (normal) control group and received only distilled water. Blood was collected from all the animals for liver marker enzyme, albumin and total protein determination 24hrs post CCl_4 injection. Histopathological assessment of liver was done. Result showed significant alterations in the levels of biochemical markers of hepatic damage like ALT, ALP, AST, and albumin in positive control group when compared with the baseline control group ($p < 0.001$). However, treatment with both extracts of *Portulaca oleracea* (400 mg/kg and 800 mg/kg) significantly protected rats from injury as evident by marked reduction in liver marker enzymes and restoration of albumin levels in a dose-dependent manner ($p<0.01$, $p<0.001$). Histopathological evaluation showed that the 800mg/kg dose of AEPO protected rats from CCl_4 induced hepatotoxicity as evidence by moderate changes in liver histoarchitecture. The CCl_4-control group showed presence of vacuolation of hepatocytes and necrosis of cells. *Portulaca oleracea* extracts possess hepatoprotective potency.

KEY WORDS: *P. oleracea*, Hepatoprotection, Carbon tetrachloride, Rats, Aqueous and Methanol extracts

Hepatotoxicity implies chemical-driven liver damage. Biochemical markers are often used to indicate liver damage. Liver damage is further characterized into hepatocellular and cholestatic types. Certain medicinal agents when taken in overdose and sometimes even when introduced within therapeutic range may cause liver injury. More than 900 drugs have been implicated in causing liver injury (Friedman *et al* 2003), and it is the most common reason for drugs to be withdrawn from the market. Other chemical agents (hepatotoxins) such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Chemicals often cause sub-clinical injury to the liver which manifests only as abnormal liver enzyme tests. Carbon tetrachloride (CCl_4) is a well known hepato-destructive agent that is widely used to induce acute toxic liver injury in a range of laboratory animals (Reyes-Gordillo *et al* 2007). The hepatotoxicity of CCl_4 has been reported to be due to its biotransformation by cytochrome P 450 system to produce trichloromethyl free radical ($.CCl_3$) which readily reacts with molecular oxygen to form trichloromethyl peroxy radical (Raucy et al 1993). CCl_3OO which exert their action on lipids membranes of endoplasmic reticulum to evoke lipid peroxidation (Recknagel *et al* 1989). The changes associated with CCl_4-induced hepatic damage are similar to that of acute viral hepatitis (Rubinstein 1962). Hepatitis B continues to be a global public health problem despite large scale efforts to eliminate this chronic viral disease via education, screening and vaccination programs. It is currently estimated that 400 million people worldwide have chronic hepatitis B virus (HBV) infection (Kowdley 2004). Patients with chronic hepatitis B have a 15% to 40% risk of developing cirrhosis, liver failure, or hepatacellular carcinoma (HCC), (Lok and McMahon 2001). Fulminant hepatic failure from drug-induced hepatotoxicity may require liver transplantation.

An estimated 80% or more of the world's

population depend primarily on herbs for their health care. This dependence in medicine derived from indigenous plants is especially predominant in developing countries where modern western medicine is often unavailable or is simply too expensive (WHO 2002). *Portulaca oleracea* Linn (purslane), a member of Portulacaceae, is wide spread as a weed and has been ranked the eight most common plants in the world (Oiu *et al* 2000). *P. oleracea* has a long history of use for human food, animal feed and medicine. In Arab traditional medicine, it is used in liver and kidney disorders, as an emollient, astringent and diuretic (Ghazanfar 1994). The plant is antibacterial, antiscorbutic, depurative, diuretic and febrifuge (Chie 1984). The leaves of *P. oleracea* are a rich source of omega -3 fatty acids, which is thought to be important in preventing heart attacks and strengthening the immune system (Bown 1995). It has been described as a "power food" of the future because of its high nutritive and anti-oxidant properties (Simopoulos *et al* 1995).

Despite efforts to treat/manage liver injury with allopathic medicinal drugs, none of these agents has been able to offer dependable liver protection. On the other hand, a variety of medicinal plants have been shown to possess dependable hepatoprotective potency (Santra *et al* 1998). Therefore, there is need to search, evaluate, and scientifically validate the hepatoprotective activities of medicinal plants. The aim of this study is to evaluate the hepatoprotective potency of *Portulaca oleracea* in CCl_4 induced liver injury in rats.

MATERIALS AND METHODS

Plant collection

The plant was collected in a field at Abakpa-Nike area of Enugu State, Nigeria in the month of January, 2009. The plant was authenticated by a taxonomist at the Department of Botany, University of Nigeria Nsukka. The plant was dried under the shade to a constant weight.

Extraction of plant material

Methanol extract

The dried aerial parts of *Portulaca oleracea* were powdered with a mill grater (MS 221, Taiwan). The powder (500 g) was macerated in 2 liters of methanol for 48 hours. The extract was filtered after 48hrs with a Whatman No. 1 filter paper and the filtrate evaporated to dryness in an incubator (Gallenkamp, UK) at 60°C. The yield of the methanol extract was 11.8%. The extract (10 g) was dissolved in normal saline and made up to 100ml with the same solvent (100 mg per ml). Desired concentrations were obtained from this for the study.

Aqueous extract

The aerial parts (500 g) of freshly obtained *Portulaca oleracea* from local garden was grinded using a mill grater. The plant was then macerated for 48 hrs in 500 mls of distilled water. The extract was strained through muslin and the filtrate then filtered through Whatman No. 1 filter paper. The aqueous extract was concentrated on a rotary evaporator (Model type 349/2 Corning Ltd, England). The extractive value of the aqueous extract was 250 mg/ml.

Animals

Adult male wistar rats weighing 185-240g were obtained from the Faculty of Veterinary Medicine, University of Nigeria Nsukka. They were maintained under standard housing conditions and fed with standard rat chow (Guinea feed®, Nigeria) and provided with water *ad libitum* during the experiment. They were acclimatized for two (2) weeks before the experiment.

Acute toxicity studies

Acute toxicity studies were performed on the aqueous and methanol extracts of *Portulaca oleracea* in rats as described by Lorke (1983).

Phytochemical analysis

Test for the bioactive constituents of *Portulaca oleracea* was performed as described by Trease and Evans (1989).

Experimental design

Thirty (30) albino rats were divided into six (6) groups (A-F) of five (5) rats each. Group A-D received 400mg/kg aqueous extract of *P. oleracea* (AEPO), 800mg/kg AEPO, 400mg/kg Methanol extract of *P. oleracea* (MEPO), and 800mg/kg MEPO respectively. Group 'E'

received normal saline (5ml/kg) and served as CCl_4 control. All administration was by oral route. Group F did not receive any form of treatment whatsoever and serves as baseline (Reference) control. Daily doses of the extract were given to the various groups for seven (7) days. On the 8th day, CCl_4 in olive oil (1:1) 2ml/kg was administered to animals in groups A-E subcutaneously following a 24hrs fast (Nakade*et al*, 2002). 24 hrs post CCl4 administration, about 5mls of blood was collected from animals in all the groups by retro-orbital puncture under ether anaesthesia into a dry glass container. The blood was allowed to clot and sera separated from the cells and stored frozen until analyzed for liver enzymes, albumin, and total protein. The animals were euthanized while still under anaesthesia, and their liver excised, washed in cold saline and fixed with 10% formol saline for histopathological studies.

Statistical analysis

The results were expressed as mean ± SEM. Differences between means was determined by the student t- test. $p<0.05$ was considered significant.

RESULT

Results of acute toxicity test showed that the aqueous extract of *P oleracea* had an oral LD_{50} of 22.4 g/kg in rats while the methanol extract had an LD_{50} of 20 g/kg in rats. Preliminary phytochemical screening of the leaves revealed the presence of abundant amounts of alkaloids, steroids, proteins, moderate amounts of carbohydrates, cardiac glycosides, and flavonoids. There were trace amounts of saponins. Tannins and terpenoids were absent.

Table 1: Comparison of analyte concentrations (mean±SEM) of treated groups with baseline control

GROUPS	ALT(IU/L)	AST(IU/L)	ALP (IU/L)	TP(g/L)	ALB(g/L)
A (400mg/kg AE+ CCl_4 2ml/kg s.c.)	180.40±0.63[a,c]	215.74±2.83[a,c]	622.40±8.55[a,c]	69.94± 0.43	42.50± 0.95[c]
B(800mg/kgAE+ CCl_4 2ml/kg s.c.)	92.28±5.45[a,c]	155.20±5.04[a,c]	393.00±32.17[b,c]	73.86± 0.45	36.38± 0.29[c]
C(400mg/kgME+ CCl_4 2ml/kg s.c.)	189.64±2.51[a,c]	265.60±1.54[a,c]	505.7±74.08[a,c]	65.42± 0.43	45.36± 0.20[c]
D(800mg/kgME+ CCl_4 2ml/kg s.c.)	116.06±5.84[a,c]	245.46±9.01[a,c]	319.14± 34.44[a,c]	69.20± 3.00	36.74± 3.25[c]
E(5mg/kg N/S + CCl_4 2ml/kg s.c.)	260.62±3.87[a]	295.78±5.63[a]	926.80±12.59[a]	68.64± 0.27	28.26±0.51[a]
F(normal control)	30.06± 1.39	88.80± 3.69	210.32± 9.00	69.16±0.18	41.36± 0.73

*p<0.05, **p<0.001, n=5 (AE= aqueous extract, ME = Methanol extract, N/S = normal Saline, and S.C = subcutaneous route). a = p < 0.001 with respect to normal control; b = p<0.05 w.r.t normal control; c = p< 0.001 w.r.t CCl_4 (positive) control.

Table 1 shows induced intoxication, as indicated by increases in serum ALT, AST, ALP, and albumin levels following carbon tetrachloride administration. When rats were exposed to the extracts of *Portulaca oleracea* (2 dose levels) prior to CCl_4injection, the rats developed various degrees of hepatic injury resulting in a rise in mean liver marker enzyme levels which were significantly higher than those of the untreated baseline group. From table 2, animals in group A had 180.40 ± 0.63 iu/l, 215.74± 2.83 iu/l, and 622.4± 8.55 iu/l for ALT, AST and ALP respectively. These were significantly higher than those of the baseline group that had 30.06± 1.39 iu/l, 88.80± 3.69 iu/l, and 210.32± 9.00 iu/l for ALT, AST, and ALP respectively ($p< 0.001$) Group 'E' that served as CCl_4 control group showed the highest increase in liver maker enzyme levels as compared with baseline control group. The CCl_4 significantly increase the mean ALT, AST, ALP and albumin levels from 30.06±1.39iu/l, 88.80±3.69iu/l, 210.32±9.00iu/l and 41.36±0.73g/l (for baseline control) to 260.62±3.87iu/l, 295.78±5.63iu/l, 926.80±12.59iu/l, and 28.26±0.5 1g/l respectively ($p<0.001$). CCL_4 did not significantly affect serum total protein ($p>0.05$).

The toxic effect of CCl_4 was controlled in the animals treated with methanol and aqueous extracts respectively as evident by the reduction in the levels of the liver marker enzymes when compared with positive control group (table 1). The 400mg/kg aqueous extract gave significant protection against CCl_4 induced toxicity. The mean level of liver enzymes of the 400 mg/kg aqueous extract treated rats were significantly lower when compared to CCl_4 control group ($p<0.001$) while the mean albumin level was significantly higher than the CCl_4 control ($p<0.001$). The methanol extract of *Portulaca oleracea* (800mg/kg) showed significant hepatoprotective potency with mean liver marker enzyme and albumin levels of 116.08±5.84 iu/l, 245.46±9.01 iu/l, 319.14±34.44 iu/l, and 36.74±3.25 g/l for ALT, AST, ALP, and albumin respectively when compared to CCl_4 control group ($p<0.001$). Total protein was not significantly affected ($p>0.05$).

Table 2: Grading of the Histological features observed on the liver sections from the various groups.

GROUPS	Oedema	Vacuolation	Inflammatory cells	Necrosis
A (400mg/kg AE+ CCl_4 2ml/kg s.c.)	-	++	++	-
B (800mg/kg AE+ CCl_4 2ml/kg s.c.)	-	+	-	-
C (400mg/kg ME+ CCl_4 2ml/kg s.c.)	+	+++	++	-
D (800mg/kg ME+ CCl_4 2ml/kg s.c.)	-	++	+	-
E (5mg/kg N/S + CCl_4 2ml/kg s.c.)	++	++++	+++	+++
F (Baseline control)	-	-	-	-

Key: Absence (-); Mild (+); Moderate (++); Severe (+++)

Histological evaluation of liver of the animals confirmed the hepatoprotective effect of both extracts of *Portulaca oleracea* against CCl_4 induced intoxication. Liver sections of the CCl_4 control showed marked dearrangement in hepatocyle architecture evidenced by the presence of vacuolation, inflammatory cells at the peri-vascular and peri-central areas, necrosis and oedema (fig.2). The drug treatment almost normalized these effects in the histoarchitecture of liver (fig 3.) in a dose dependent manner. The normal control showed normal hepatocytes (fig. 1).

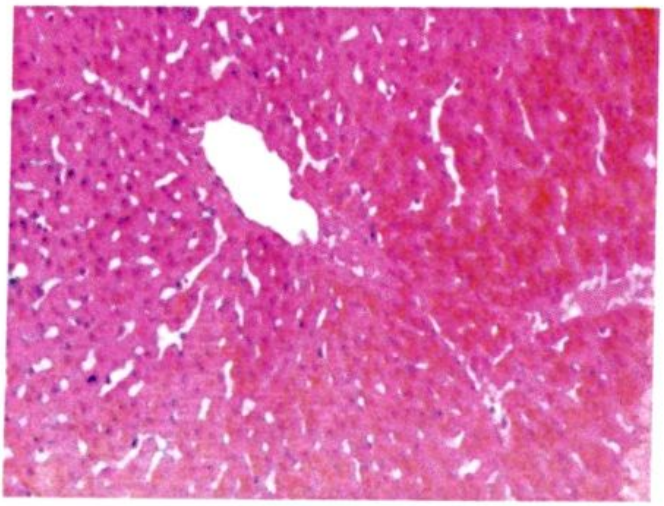

Figure 1: Liver histology of the normal control group (H & E x 200) showing normal histoarchitecture

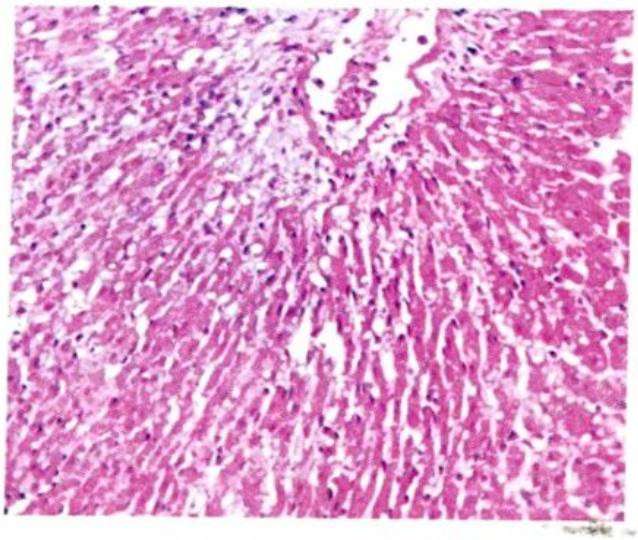

Figure 2: Liver histology of the CCl4 control group (H & E x 200) showing vacuolated and oedematous hepatocytes, necrosis and infiltration of inflammatory cells.

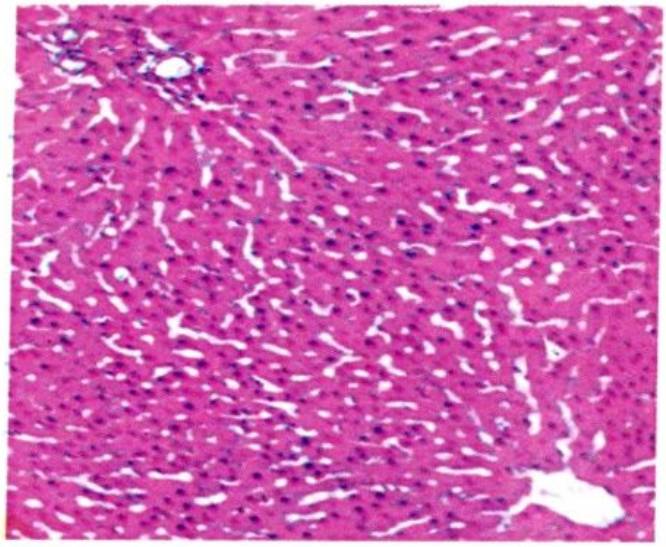

Figure 3: Liver histoarchiture of the high dose aqueous extract treated group (H&Ex200) showing intact tissue parenchyma.

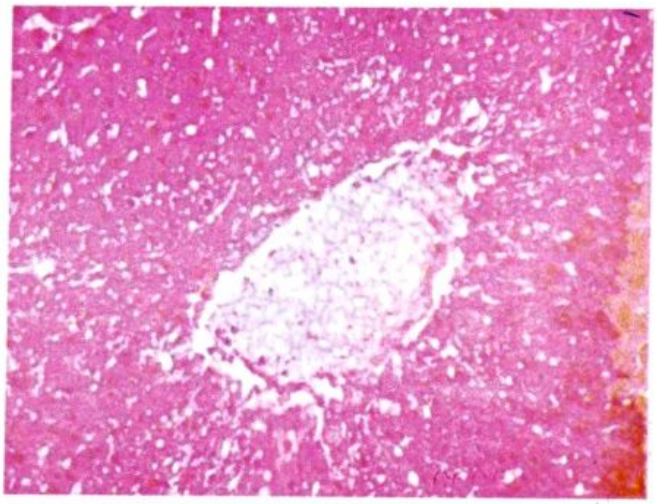

Figure 4: Liver micrograph of the methanol extract treated group (x 200) showing hydropic change of the hepatocytes

DISCUSSION

Portulaca oleracea is an edible weed, which has been used as an analgesic, anti-inflammatory, and antioxidant agent (Zakaria *et al* 1998). It is also used in liver and kidney disorders (Ghazanfar 1994). *P oleracea* contains flavonoids which modulates enzyme activities and affects the behaviour of many cell systems (Ezekwe *et al* 1999). Our findings show that both extracts have the potency to normalize the elevated liver marker enzyme levels and maintain the synthetic function of the liver when compared with the positive control group. This indicates restoration of the normal functional

status of the liver. The significant elevations in the liver marker enzymes such as ALT, AST, and ALP, as well as decrease in albumin levels in CCl_4 control when compared with normal control suggest liver injury, since these are reliable indices of liver toxicity (Omoniyi and Mathew 2006). Albumin is produced entirely by the liver and constitutes about 60% of total serum protein. In liver damage, the synthetic capacity of the liver is reduced and consequently syntheses of albumin, clotting factors, and so on are affected. The oral LD_{50} of the *P. oleracea* extracts in rat is 22.4 g/kg and 20 g/kg for the aqueous and methanol extracts respectively. Therefore, 800 mg/kg used is much lower than the LD_{50} and gives a high safety margin. The mechanism of CCl_4 induced liver injury involves oxidative stress. Injury is through the free radical (CCl_3 and CCl_3OO) of its metabolism which may cause lipid peroxidation and subsequent injury (Sies, 1997). The antioxidant property of *P. oleracea* has been validated (Simopoulos *et al*, 1995). Therefore, the protective potency of the *P. oleracea* extracts could be due to antioxidant effects. Preliminary phytochemical analysis shows that *P. oleracea* contains abundant amounts of alkaloids, steroids, proteins, moderate amounts of carbohydrates, cardiac glycosides, and flavonoids and trace amounts of saponins. Flavonoids have been suggested to act as antioxidants by free radical scavenging activity (Baek *et al*, 1996; Takeoka and Dao, 2003). Also, saponins and alkaloids are known to possess hepatoprotective activity (Tran *et al*, 2001; Vijyan *et al*, 2003). Therefore, it is likely that the hepatoprotective effect of the extracts of *Portulaca oleracea* is due to single or combined effect of these bioactive substances. Histological studies also confirmed the hepatoprotective effect of both extracts of *P. oleracea* against CCl_4 induced intoxication evident from the maintenance of the histoarchitecture of the liver. The protective effects due to treatment with *P. oleracea* extract strongly indicated the possibility of the extract being able to prevent and/or mitigate any leakages of marker enzymes into circulation, enabling rapid regeneration of the liver to its original size, structure and function; preserves the integrity of the plasma membranes and hence restores these enzyme levels.

In the light of these findings it is concluded that the extracts of *P oleracea* have hepatoprotective effect in CCl_4 induced hepatotoxicity. In order to find out which chemical in *P oleracea* is responsible for its effects, a chromatographic analysis of *P oleracea* should be performed and it ingredients studied in the CCl_4 induced hepatotoxicity model.

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