



HEPATOPROTECTIVE EFFECT OF AQUEOUS LEAF EXTRACT OF *CARICA PAPAYA* LINN. AGAINST CCL₄-INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

Aqueous leaf extract of *Carica papaya* (CP) is used as medicine against some forms of liver diseases traditionally. The purpose of the study was to evaluate and determine the hepatoprotective effect of the aqueous leaf extract of CP in CCl₄ induced liver damage in rats administered subcutaneously with 75mg/kg CCl₄ after 48hours. The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the levels of serum malondialdehyde (MDA) and bilirubin were determined in rats administered with 75mg/kg CCl₄ and rats treated with 200mg/kg and 400mg/kg CP before CCl₄ administration. Results showed the extract to cause significant ($p < 0.05$), dose-dependent decrease in the elevation of serum liver enzyme markers of acute hepatocellular injury (ALT, AST, ALP), serum MDA and bilirubin. Maximum hepato-protection was offered at an oral dose of 400 mg/kg/day of the extract. The result obtained was confirmed by improvements in the CCl₄-induced hepatic histological changes. The result of phytochemical analysis showed that the CP contain appreciable amount of saponins, total phenolics, alkaloids, saponins and tannins (g/100g) 0.91 ± 0.13 , 2.25 ± 0.05 , 1.32 ± 0.48 , 0.52 ± 0.01 , and 1.56 ± 0.03 respectively. In conclusion, the results obtained in the current study justify the folkloric application of CP in the treatment of disease-related hepatic injury.

Keywords: *Carica papaya*, hepatoprotective effect, phytochemicals, histology.

INTRODUCTION

The liver is the largest organ in the body, contributing about 1/50 of the total body weight, or about 1.5 kg in the average adult human. The liver performs many different functions, yet the liver is also a discrete organ and many of its functions are interrelated with one another. This becomes especially evident in abnormalities of the liver such as hepatitis, Jaundice and Cirrhosis¹.

Carica papaya Linn. (Family: Caricaceae) is a perennial, herbaceous plant, with copious milky latex reaching to 6 – 10 meters tall. Its erect stem is about 30 cm thick and roughened with leaf scars². The unripe fruit is used traditionally for the treatment of various human and veterinary diseases including malaria, hypertension, diabetes mellitus, hypercholesterolaemia, jaundice, intestinal helminthiasis³ and for the management of sickle cell anaemia⁴. The present study was designed to investigate the effect of graded oral doses (200 and 400 mg/kg/day) and the time-course effect of the aqueous leaf extract of *Carica papaya* (CP) in CCl₄ treated rats as a way of validating its folkloric use in treatment of liver diseases. The choice of the extract dose range employed in this was based on the result obtained from the study earlier conducted.

MATERIALS AND METHODS

Plant extract

Fresh leaves of *C. papaya* were collected in May 2007 from botanical garden, Department of Biological Science, Bayero University, Kano, Nigeria. The leaves of CP was identified and authenticated by a Botanist in the

Department of Biological Sciences, Bayero University, Kano, Nigeria. The leaves were sorted to eliminate any dead matter and other unwanted particles. The leaves were air-dried for 2 weeks and then ground into fine powder using pestle and mortar. A total of 200g of the ground powder was soaked in 1 L of distilled water for 48 hours at room temperature. The mixture was filtered into 500 ml conical flask with Watman filter paper (No.1). The filtrate was dried at a temperature of 30°C for 10 hours, which weighed 20.5 g.

Experimental Animal

Young adult male Wistar rats, weighing between 120 and 150 g were used for this study. The rats were obtained from the Department of Pharmacology, Ahmadu Bello University, Zaria-Nigeria. The rats were allowed two weeks of acclimatization under standard laboratory conditions. The rats were maintained on standard rat feed (Nutri-feeds manufactured by P.S. Mandrid Plc.) and potable water *ad libitum*. The experimental rats were all handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and use of Laboratory Animals in Biomedical Research⁵.

Experimental Design

An initial study was conducted to determine the dose range of the aqueous leaves extract of *Carica papaya* to be used for the studies. Thirty five (35) rats were divided into five (5) groups of seven (7) rats each. Group I served as normal control; Group II served as CCl₄ Control, Group III served as CCl₄ + 200mg/kg/b.w *Carica papaya* leaf extract, Group IV served as CCl₄ + 400mg/kg/b.w *Carica*



papaya leaf extract, and Group V served as CCl₄ + silymarin (100mg/kg/b.w).

Experimental induction of CCl₄ hepatotoxicity

Rats were divided into five groups, each group consisting of six animals. Group I (control) animals were administered a single dose of water (1 mL/kg, p.o.) daily for 7 days and received liquid paraffin (1 mL/kg, s.c.) on day 4 and 5. Group II (CCl₄) received water (1 mL/kg body weight, p.o.) once daily for 7 days and received CCl₄: liquid paraffin (1:1, 2 mL/kg body weight, s.c.) on day 4 and 5. Test groups animals (Groups III-IV) were administered orally on a dose of 200mg/kg and 400 mg/kg of the aqueous extract, respectively, once daily. Group V received standard drug silymarin (100 mg/kg, p.o.) once daily for 7 days. The Groups III–V animals were administered simultaneously CCl₄: liquid paraffin (1:1, 2 mL/kg body weight, s.c.) on day 4 and 5 after 30 min of administration of the extract and silymarin. Animals were sacrificed 48 h after the last treatment. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out. Liver was dissected out and used for histopathological studies⁶.

Determination of Markers of Liver Damage

The rats were sacrificed 48h post administration of CCl₄ at a dose of 75mg/kg under anaesthesia using petroleum ether. The separated serum was estimated for various biochemical parameters; aspartate aminotransferase (AST)⁷, alanine transaminase (ALT)⁸, serum alkaline phosphatase (ALP)⁸, malondialdehyde (MDA)⁹ and total bilirubin¹⁰.

Histological Studies

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol 80%, cleared in xylene and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H and E) dye for photomicroscopic observation, including cell necrosis and fatty change.

Quantitative determination of Phytochemicals

Quantitative determination of phytochemicals was carried out for total phenolics¹¹, flavonoids¹², alkaloids¹¹ tannins¹³, and saponins¹⁴.

Statistical analysis

All results were expressed as means ± SD for each group. Data were analyzed statistically with student's t-test using SPSS programme. P values of less than 0.05 (P < 0.05) were considered significant.

RESULTS AND DISCUSSION

The quantitative phytochemical analysis of the aqueous leaf extracts of *Carica papaya*, revealed the presence of some potent bioactive compounds (Tables 1). However, the presence of these phytochemicals conferred the hepatoprotective and anti-inflammatory effects of *Carica papaya* observed in the CCl₄ induced liver damaged groups. As the medicinal effect of the plants is due to the phytochemicals present.

Carbon tetrachloride (CCl₄) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. It is actively metabolized in the body tissues to its highly reactive halogenated metabolites (CCl₃ and ·Cl) and its metabolic activation is accompanied by the release of reactive oxygen species (ROS)¹⁵. The reactive metabolites and the free radicals released subsequently result in the induction of lipid peroxidation leading to array of organ toxicities such as hepatotoxicity, nephrotoxicity, neurotoxicity, cardiotoxicity and hematotoxicity^{16, 17}.

In detecting liver damage by CCl₄ the determination of enzyme levels such as AST, ALT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver¹⁸. Serum ALP and bilirubin levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure.

Table 1: Quantitative phytochemical constituents (g/100g) of aqueous extract of the leaf of *Carica papaya*

SAMPLE	Flavonoids (g/100g)	Alkaloids (g/100g)	Saponins (g/100g)	Total Phenolics (g/100g)	Tannins (g/100g)
LEAF	0.52 ± 0.01	1.32 ± 0.48	0.91 ± 0.13	2.25 ± 0.05	1.56 ± 0.03

Values are presented as mean ± Standard Deviation. All values are of triplicate determination

Table 2: Effect of *Carica papaya* aqueous leaf extract on some biochemical parameters of liver in rats

GROUPS	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	MDA (µM)	Total Bilirubin (mg/dl)
I	36 ± 7.67	58 ± 7.14	144 ± 9.65	0.12 ± 0.05	0.54 ± 0.07
II	135 ± 26.37 ^a	157 ± 28.35 ^a	421 ± 27.58 ^a	0.40 ± 0.10 ^a	2.25 ± 0.33 ^a
III	115 ± 10.21 ^{a,b}	139 ± 13.10 ^a	350 ± 13.11 ^{a,b}	0.33 ± 0.01 ^{a,b}	1.84 ± 0.16 ^a
IV	103 ± 9.00 ^{a,b}	117 ± 11.04 ^{a,b}	300 ± 14.01 ^{a,b}	0.29 ± 0.10 ^{a,b}	1.43 ± 0.15 ^{a,b}
V	88 ± 11.10 ^{a,b}	101 ± 12.81 ^{a,b}	200 ± 19.22 ^{a,b}	0.25 ± 0.11 ^{a,b}	0.97 ± 0.05 ^{a,b}

Values are presented as mean ± Standard Deviation. ^a represents a highly significant at p<0.05 when compared to Group I while ^b represent highly significant at p<0.05 when compared to Group II.



In this study, acute liver damage was induced by CCl_4 at a single intraperitoneal dose of 75mg/kg. Administration of CCl_4 caused a significant ($P < 0.05$) elevation of enzyme levels; AST, ALT, ALP and total bilirubin when compared to control (Table 2). There was a significant ($P < 0.05$) restoration of these enzyme levels and total bilirubin on administration of the leaf extract in a dose dependent manner and also by silymarin at a dose of 100 mg/kg (Table 2). Although no there is no significant difference between the bilirubin levels of group II rats and that of CCl_4 -induced group. The decreased levels of serum liver enzymes in CCl_4 -induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This agrees with the fact that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes¹⁹.

The result also showed a significant ($P < 0.05$) increased in MDA level in CCl_4 induced liver damage groups (Table 2). This signifies increased production of reactive oxygen species. This agrees with the previous findings that CCl_4 -induced liver damage is associated with the release of reactive oxygen species¹⁵. But administration of 200 and 400mg/kg/b.w of CP decrease the level of MDA to near normal (Table 2).

The result can be further confirmed with histopathological studies. The histopathological examination clearly reveals the peri-portal fatty change characterized by vacuolated hepatocytes around the portal tract from rat treated with 75mg/kg CCl_4 (Figure 2). The CCl_4 also showed to produce extensive vascular degenerative changes and centrilobular necrosis in hepatocytes of those rats treated with 75mg/kg CCl_4 only (Figure 2). But treatment with 200mg/kg and 400mg/kg doses of *Carica papaya* extract showed that the hepatic cells are similar to those of normal control group, with group IV, showing maximum hepato-protective effect, in contrast to group which received CCl_4 only (Figure 3). This is also nearly similar to group that received 100mg/kg silymarin (Figure 4).

It has been reported that decreasing the metabolic activation of carbon tetrachloride, prevention of generation of reactive oxygen species and scavenging of generated free radicals or by combination of these are important mechanisms in the protection against CCl_4 -induced hepatic lesion^{20, 21}. Thus, based on the result obtained, it is suggested that the extract may exert its hepato-protective effects either by decreasing the metabolic activation of carbon tetrachloride, or by acting as a chain-breaking antioxidant for scavenging free radicals or by a synergistic combination of these effects.

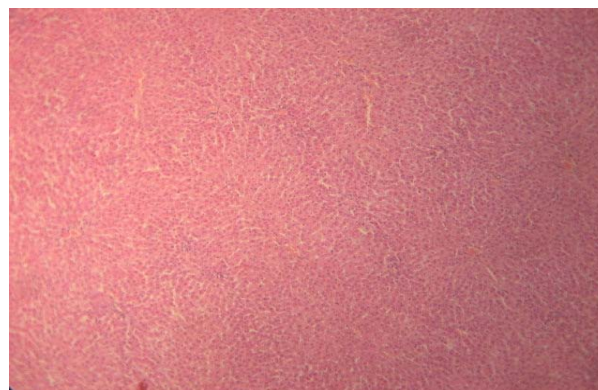


Figure 1: A representative section of an untreated control rat liver.

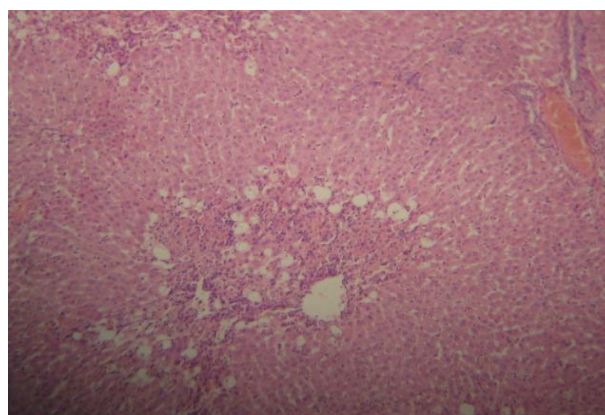


Figure 2: Liver section of CCl_4 treated group showing massive fatty changes and necrosis

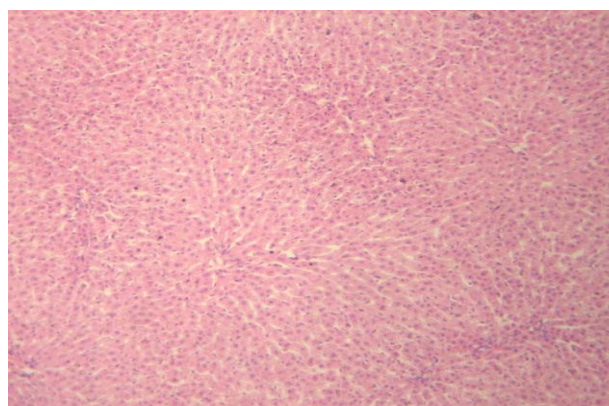


Figure 3: Liver section of CCl_4 + 400mg/kg/b.w aqueous CP leaf extract treated group

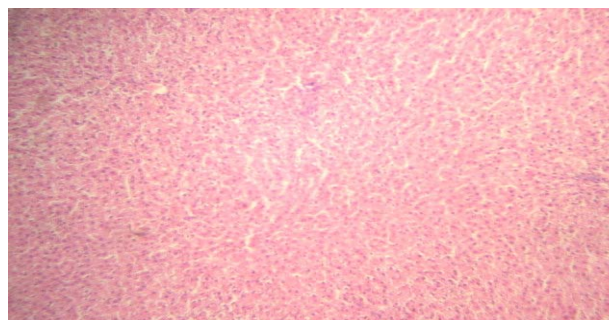


Figure 4: Liver section of treated CCl_4 + 100mg/kg/b.w Silymarin

CONCLUSION

Thus, in conclusion, *Carica papaya* aqueous leaf extract can be considered to be an effective hepato-protective agent as it brings near to normalcy the damage caused by CCl₄ to hepatic function. Hence the extract can be used in polyherbal formulations to provide a synergistic effect with other hepato-protective drugs and thereby preventing the process of initiation and progress of hepato-cellular diseases.

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