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Studies on the effect of methanolic extract of *Carica papaya* stalk on hepatotoxicity induced in albino rat

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The hepatoprotective properties of methanolic extract of *Carica papaya* stalk on albino rat were evaluated. To achieve this, the phytochemical screening of the extract was carried out and carbon tetrachloride (CCl_4) (i.p) was injected into albino rats to liver damage. The rats were simultaneously given oral doses of 20, 40, 60, 80 and 100 mg/kg (p.o) of methanolic extract of *C. papaya* stalk. The effects of this extract on liver total protein concentration, alanine aminotransferase, aspartate aminotranferase, and gamma-glutamyltransferase activities of the rats were studied after 7 days period of the experiment. Administration of CCl_4 alone to rats significantly increased ($p < 0.05$) total protein concentration and the activities of the transferases studied in the serum when compared with controls which received distilled water (p.o). Simultaneous treatment of CCl_4 injection and oral administration of different doses of the *C. papaya* stalk extract significantly reduced ($p < 0.05$) total protein concentration, activities of liver transferases studied. However, the lowest significant reduction ($p < 0.05$) of total protein concentration and the transferases was observed with simultaneous administration of 100 mg/kg of the extract on the rats. This study suggests that the extract of *C. papaya* stalk possesses the phytochemicals with antioxidant properties which might be responsible for it capacity to protect the liver from oxidative damage.

Key words: Hepatotoxicity, phytochemical, liver, *Carica papaya,* rat.

INTRODUCTION

Herbal medicine, sometimes referred to as herbalism, botanical medicine or herbology, is the use of plants, in a wide variety of forms, for their therapeutic values (Vickers et al., 1999). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developed as well as developing countries, because products of higher plants origin have been shown to be effective sources of chemotherapeutic agents (Sushil et al., 1998), with little or no known side effects. *Carica papaya* is an example of a medicinal plant with numerous therapeutic values. *C. papaya* (pawpaw) belongs to the family of Caricaceae. *C. papaya* is not a tree but an herbaceous succulent plants that posses self supporting stems (Gross, 2003) with a rapid growth rate. They are usually short-lived but can produce fruit for more than 20 years. The plants are male, hermaphrodite, or female (Bruce and Peter, 2008). These plants are self pollinated (Jari, 2009). *C. papaya* leaf tea or extract has a reputation as a tumour-destroying agent (Walter, 2008). The juice has been in use on meat to make it tender (Wilson, 1994). The high level of natural self-defence compounds in the plant makes it highly resistant to insect and disease infestation (Peter, 1991).

Several investigations have been done on the therapeutic values of *C. papaya.* However, there is still limited scientific information on the hepatoprotective

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properties of *C. papaya* stalk.

Liver is the major organ in our body involved in the biotransformation of exogenous chemicals and is the target for toxic substances. During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which induce production of lipid peroxides within the hepatocytes and subsequently cause oxidative stress, cell death and ultimately result in liver disease, such as hepatocellular carcinoma, viral and alcoholic hepatitis and non-alcoholic cirrhosis (Kohen and Nyska, 2002; Vitaglione et al., 2004). All aerobic organisms including humans have antioxidant defense mechanisms that protect against oxidative damage. However, the natural antioxidant defense mechanisms can be insufficient and hence dietary intake of antioxidant components is important and recommended (Duh, 1998).

Thus this present investigation was therefore undertaken to evaluate the hepatoprotective properties of *C. papaya* stalk extracts against oxidative damage induced by carbon tetrachloride (CCl_4) in rats. This was monitored by assessing the activities of aminotranferases in serum which their elevation indicates a liver damage (Nelson and Cox, 2000).

MATERIALS AND METHODS

Chemicals

Methanol and CCl_4 were the products of BDH Chemical Company Ltd (Poole, England) and Hopkins and Williams, respectively. All reagent kits used for enzyme assays were obtained from Randox Company, United Kingdom. All other reagents used were of analytical grade.

Animals

The 21-albino rats (*Rattus novergicus*) used for this study were obtained from Ayo Ola farms Ilorin, Kwara state, Nigeria. The animals were fed with standard laboratory feeds (Bendel Feeds Ltd, Ewu, Nigeria) and tap water (*ad libitum*). This research was carried out in Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria. This was done according to the rules governing the use of laboratory animals in Nigeria (Revised Helsinki Declaration, 2008) as acceptable internationally.

Plant source and identification

Pawpaw (*C. papaya*) stalk were obtained from Agricultural farm of Joseph Ayo Babalola University, Ikeji-Arakeji,Osun State, Nigeria and were identified at the Herbarium Section (No. DBS/H/488) of the Department of Biological Sciences of the same university.

Preparation and extraction of plant's active component

The papaya stalk was sun dried for 2 weeks after which it was blended into powdery form. 100 g of the plant powder was soaked and shaken in 500 ml of methanol for 48 h in order to obtain the methanolic extract. Extract was obtained with the use of a suction pump. The filtrate was then exposed to dryness in a water bath at 60°C.

Phytochemical screening

Phytochemical screening of *C. papaya* extract for secondary metabolites was carried out using the procedure described by Sofowora (1993).

Experimental design

Twenty-one albino rats of mixed sexes were divided into six groups, 3 in each group as follows:

(1) Group 1 received 1 ml of distilled water daily orally.
(2) Group 2 received 0.5 ml/kg body weight (i.p.) CCl_4.
(3) Group 3 received 0.5 ml/kg body weight (i.p.) CCl_4 and 20 mg/kg body weight (orally) of the *C. papaya* stalk extract simultaneously daily.
(4) Group 4 received 0.5 ml/kg body weight (i.p.) CCl_4 and 40 mg/kg body weight (orally) of the *C. papaya* stalk extract simultaneously daily.
(5) Group 5 received 0.5 ml/kg body weight (i.p.) CCl_4 and 60 mg/kg body weight (orally) of the *C. papaya* stalk extract simultaneously daily.
(6) Group 6 received 0.5 ml/kg body weight (i.p.) CCl_4 and 80 mg/kg body weight (orally) of the *C. papaya* stalk extract simultaneously daily.
(7) Group 7 received 0.5 ml/kg body weight (i.p.) CCl_4 and 100 mg/kg body weight (orally) of the *C. papaya* stalk extract simultaneously daily.

The dosage of the extracts was determined from preliminary studies in our laboratory.

After the experimental period of seven days, animals were sacrificed and venous blood was collected into sample bottles and was allowed to clot and the serum was obtained by centrifuging at 3000 rpm (Spinette – Damon/IEC bench top centrifuge) for 5 min (Ogbu and Okechukwu, 2001).

Biochemical studies

The activities of liver aminotransferases (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Gamma-glutamyl transpeptidase (GGT)) were assayed basically by colorimetric method of Reitman and Frankel (1957) while that of total protein concentration was estimated in the serum using the Biuret method (Plummer, 1978).

Statistical analysis

The statistical analysis was carried out by one way analysis of variance and Duncan Multiple Range test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Results obtained from the phytochemical screening of the methanolic extract of *C. papaya* stalk indicated the presence of alkaloids, saponins, flavonoids and steroids (Table 1). Figures 1 to 4 show the effect of the methanolic extract of *C. papaya* stalk on the activities of

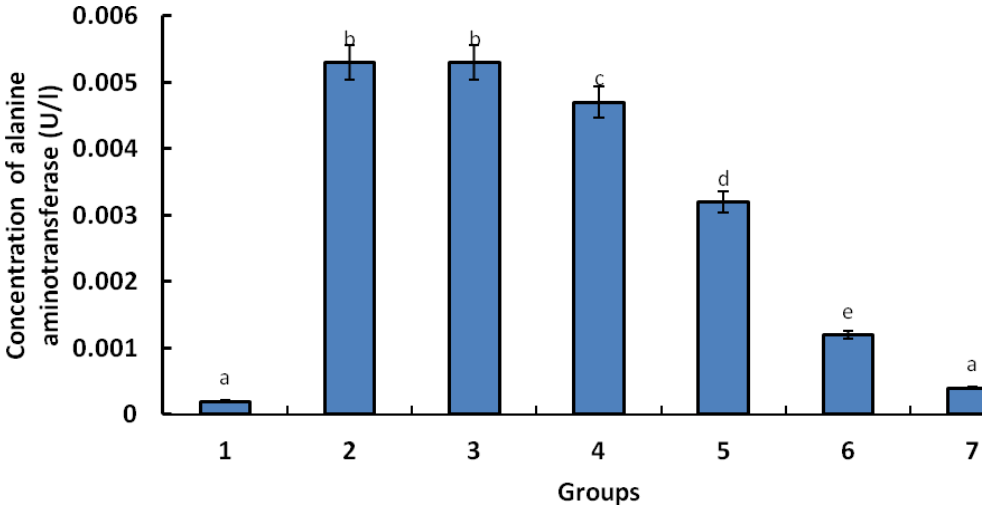

Figure 1. Effect of methanolic extract of *C. papaya* stalk on serum ALT activities of CCl_4-induced liver damage. Each value is a mean of 4 different determinations. Values with different alphabet superscripts are significantly different ($p<0.05$).

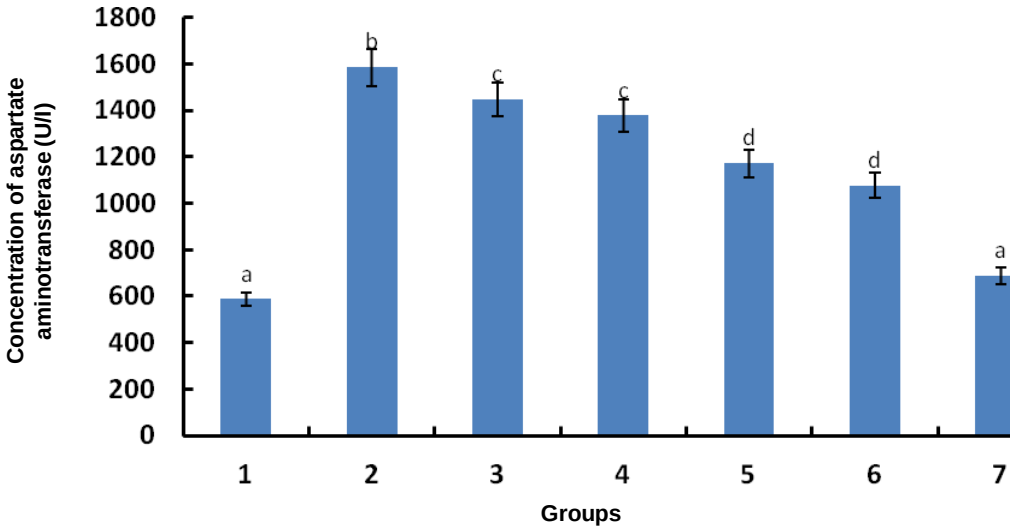

Figure 2. Effect of methanolic extract of *C. papaya*'s stalk on serum AST activities of CCl_4-induced liver damage. Each value is a mean of different determinations. Values with different alphabet superscript are significantly different ($p<0.05$).

Table 1. Phytochemical screening of *C. papaya* stalk extracts.

Phytochemical component	*C. papaya* stalk
Saponins	+
Tannins	-
Phlobatanins	-
Anthraquinones	-
Alkaloids	+
Glycosides	-
Flavonoids	+
Steroids	+

serum ALT, AST, GGT and total protein concentration in CCl_4 induced liver damage in rat.

The significant increase ($p < 0.05$) observed in the level of total protein concentrations and the activities of alanine aminotransferase, aspartate aminotranferase, and gamma-glutamyltransferase in the serum when compared with controls which received distilled water only (p.o) indicates hepatotoxicity in the rat. CCl_4 is one common hepatotoxin used in the experimental study of liver diseases (Obi et al., 1998; Ulicna et al., 2003). This might be due to the leakage from the cells through peroxidative damage of the membrane (Li et al., 2010).

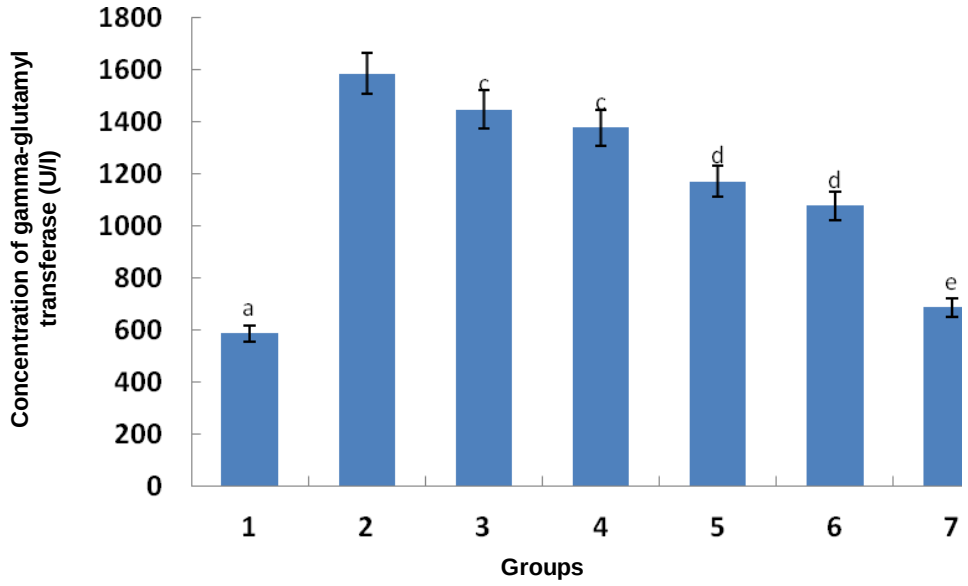

Figure 3. Effect of methanolic extract of *C. papaya* stalk on the serum GGT activities of CCl_4-induced liver damage. Each value is a mean of 4 different determinations. Values with different alphabet superscripts are significantly different ($p<0.05$).

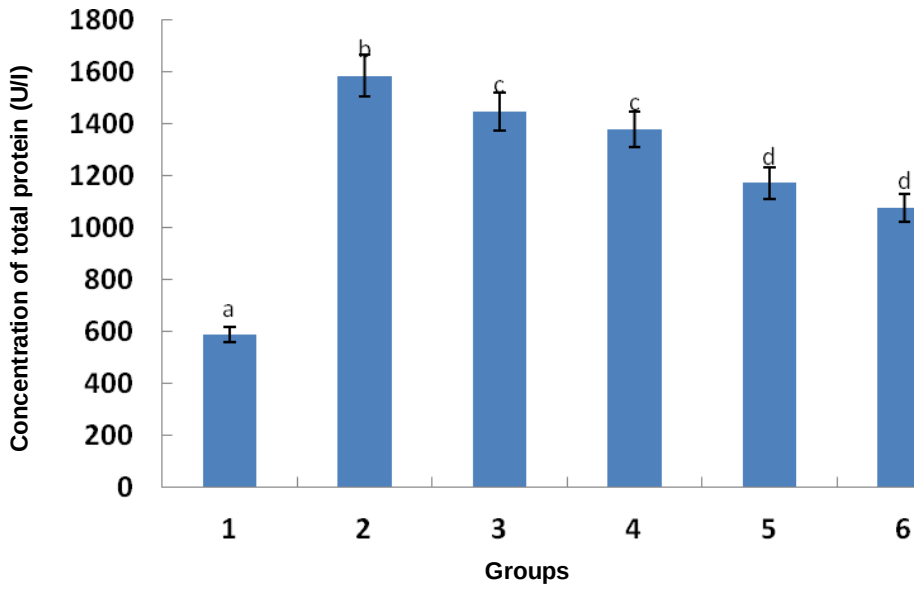

Figure 4. Effect of methanolic extract of *C. papaya* stalk on serum total protein level of CCl_4-induced liver damage in rats. Each value is a mean of 4 different determinations. Values with different alphabet superscripts are significantly different.

The monitoring of the leakage of liver enzymes into the serum has proven to be a very useful tool in assessing liver damage (Nelson and Cox, 2000).

The significant reduction ($p < 0.05$) observed in the level of total protein concentrations and the activities of the transferases in the serum after simultaneous treatment of CCl_4 injection and administration of different oral doses of 20, 40, 60, 80 and 100 mg/kg of the *C. papaya* stalk extract (Figures 1 to 4) suggest that the extract must have protected the liver from the injurious effects of CCl_4. This attribute might be as a result of the presence of some phytochemicals in the extract (Table 1). Phytochemicals such as steroids, flavonoids, alkaloids, saponins have been reported to have antioxidant properties capable of preventing hepatotoxicity that are associated with chronic diseases (Havsteen, 2002). Earlier

an extract of *Actaea racemosa* with similar phytochemical components has been reported to possess hepatoprotective properties against CCl_4 induced liver damage (Iniaghe et al., 2012). These findings therefore suggest that the methanolic extract of *C. papaya* stalk possesses phytochemical components with antioxidant properties which might be responsible for hepatoprotective effect it demonstrated against CCl_4-induced liver damage.

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