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Inhibition of Microsomal Lipid Peroxidation and Protein Oxidation by *Carica papaya* (L) Leaf against Carbon Tetrachloride-Induced Hepatic Injury in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OJS designed and wrote the first manuscript of the study. Author KK wrote and performed the histopathological protocol. Author ARA performed the statistical analysis, analyses the study and conduct the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the hepatoprotective activities of *C. papaya* leaf in Carbon tetrachloride induced hepatic damage in albino rats.
Place and Duration of Study: Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa-State, Nigeria, between April 2009 through July 2009.
Methods: Activity of total protein and bilirubin (direct and indirect bilirubin) in the serum were

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determined colourimetrically, while lipid peroxidation products, malondialdehyde (MDA), were measured in liver homogenate. Histopathological studies of the liver in both pretreated rats and controls were also, carried out. Group 1 (negative control) were fed with 100% rat feed. Groups 2-4 were pretreated with 10, 30 and 50% *C. papaya* leaf respectively, while Group 5 (normal control) received 100% rat feed. Rats in Groups (1-4), were injected with CCl_4 (0.5 ml/kg body weight in 0.5 ml olive oil) on the 29th day while rats in group 5 were not administered with CCl_4 (normal control). The results were statistically analyzed using one-way analysis of variance (ANOVA).

Results: There was significant increase ($p< 0.05$) in the levels of malondialdehyde (MDA) and bilirubin (direct and indirect) in rats Group 1 when compared with the normal control. However, there is a significant decrease in the levels of these biochemical parameters (malondialdehyde and bilirubin) in the pretreated Groups (2-4) when compared with the negative control. Also, the protein levels significantly decreased ($p< 0.05$) in the untreated rat Group 1 when compared the normal control. Pretreatment with *C. papaya* leaf significantly increased the levels of total protein in the rat Groups (2-4) when compared with the untreated Group 1. The decrease in the levels of MDA and bilirubin in the pretreated groups were concentration dependent. Rats in Group 4 that pretreated with 50% *C. papaya* leaf had the lowest values for MDA and bilirubin. Histopathology of the liver showed reduced level of injury with normal architecture in pretreated rats while; those not pretreated were presented with severe degrees of injuries.

Conclusion: Conclusively, the study reveals that *Carica papaya* may confer hepatoprotectivty to the rats exposed to carbon tetrachloride-induced liver damage.

Keywords: Histopathological; carbon tetrachloride; hepatoprotection; C. papaya leaf.

1. INTRODUCTION

Papaya is a man's common fruit and has a high nutritive value. Papaya is a polygamous species and it is difficult to identify a plant whether it is a male, female or hermaphrodite. The papaya tree belongs to a small family -*Caricaceae,* having four genera in the world. The genus *Carica Linn*. is represented by four species, of which *Carica papaya Linn* is the most widely cultivated and best known species. Papaya probably originated from Southern Mexico and Costa Rica, which was subsequently introduced as a plantation crop in Australia, Hawaii, Philippines, Sri Lanka, South Africa, India and in all tropical and subtropical regions [1]. It is grown for both commercial purpose and in home gardens. It is a tree reaching 3-10 m in height, with the habit of a palm; the fleshy stem marked by scars where leaves have fallen off, is surmounted by a terminal panache of leaves on long petioles and with 5-7 lobes [1]. The fruit bearing trees are less than 18 month old. The leaves and unripe fruits have been reported to contain milky juice in which the protein ferment Papain is presents [2,3]. It is low in calories and rich in natural vitamins and minerals. Papaya ranks among the first fruits for vitamin C, vitamin A, riboflavin, foliate, calcium, thiamine, iron, niacin, potassium and fibre [1]. Papaya has been reported also to contain broad spectrum of polychemicals including, polysaccharides, vitamins, minerals, enzymes, proteins, alkaloids, glycosides, fats and oils, lectins, saponins, flavonoids, and steroids [4-8]. It was reported to exhibit therapeutic properties against various pathological disorder including tumours [9] and immunodeficiency which was based on the free radical scavenging activity as well as normalisation of an organism's super oxide level [10]. The fruit is rich in vitamins, minerals, proteins, polysaccharides, lectins, saponins and flavonoids, and can be used in the prevention of complications of diabetes mellitus [11]. The black seeds are edible and have a sharp, spicy taste. They are sometimes ground up and used as a substitute for black pepper. In some parts of Nigeria, the young leaves of papaya are steamed and eaten like spinach. Hence, the reason for this study is to investigate the biochemical effects of *C. papaya* leaf on the carbon tetrachloride-induced liver of wistar albino rats.

2. MATERIALS AND METHODS

2.1 Preparation of Papaya Leaf Powder

Fresh samples *of C. papaya* l*eaves* were collected from the herbal garden of Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The plant was identified and authenticated by a Taxonomist, Prof S.K. Adesina of Department of Pharmacognosy and was deposited in the herbarium with voucher no: NDUP 093. The leaves were detached from the

stems, washed twice with distilled water to remove adulterants, dried under natural conditions for two weeks, ground into powder using an electric blender and stored in airtight containers and used within duration of study.

2.2 Animals

Thirty (30) male albino rats weighing (180-190 g) were obtained from Department of Pharmacology Animal House, Niger Delta University. The animals were housed in stainless-steel cages under standard laboratory conditions of 27±2℃, relative humidity 50±15% and normal photo period (12 h dark/12 h light). They were maintained on standard rat chow and water *ad libitum* and acclimatised for a period of seven days. Permission and approval for animal studies were obtained from College of Health Sciences, Animal Ethics Committee, Niger Delta University with Ref: No. NDU/CHS/FBMS/0078.

2.3 Phytochemical Screening

In 2008, Krishna et al. [1] reported the chemical composition of *C. papaya* to include; alkaloid, α-carpaine, β-D-glucosides, β-sitosterol, papain, choline, carotene, riboflavin, vitamin C, phenyethyl- β- D- glucosides, amongst others.

2.4 Experimental Design

Thirty (30) albino rats (180-190 g) were randomly divided into five groups comprising of six rats each. Animals were fed with substances under investigation for a period of twenty eight (28) days after which they were injected with 0.5 ml/kg CCl4, dissolved in 0.5 ml olive oil. They were fed *ad libitum* with free access to water. Group 1 (negative control) were fed with 100% rat feed. Groups 2- 4 were pretreated with 10, 30 and 50% *C. papaya* leaf respectively, while Group 5 (normal control) received 100% rat feed. Rats in Groups (1-4), were injected with CCl_4 (0.5 ml/kg body weight in 0.5 ml olive oil) on the 29^{th} day while rats in Group 5 were not administered with CCl_4 (normal control).

2.5 Sample Collection

Twenty four hours after injection of CCl_4, the rats were anaesthetized in chloroform saturated chamber, sacrificed and blood samples were obtained through cardiac puncture, in non-heparinised tubes, centrifuged at 3000 rpm for 10 minutes and blood sera were then collected and stored at 4℃ prior to immediate estimation of total protein, bilirubin (direct and indirect bilirubin). The liver from both control and test animals were removed and weighed to the nearest 0.01 g. The livers were removed immediately, washed with ice-cold saline and a 10% homogenate was prepared in phosphate buffer (pH 7.0). The homogenate was centrifuged at 3000 rpm for 10 min at 4℃ and the supernatant was used for the estimation of MDA. The pieces of liver were preserved in 10% formaldehyde solution for histological study.

2.6 Biochemical Assay

Determination of total protein was by colorimetric method (Biuret method), as modified by Giodani et al. [12]. Bilirubin was estimated by colorimetric method of Jendrassik and Grof [13] while, Lipid peroxidation products MDA was according to Hunter et al. [14] as modified by Gutteridge and Wilkins [15].

2.7 Histopathological Examination

The pieces of liver were preserved in 10% formaldehyde solution for histopathological examination according to the method of Baker and Silverton [16].

2.8 Statistical Analysis

The results were statistically analysed using one-way analysis of variance (ANOVA) using SPSS version 17, followed by Tukey-Kramer Multiple Comparisons Test. P values<0.05 were considered significant.

3. RESULTS

3.1 Effect of *C. papaya* Leaf on Some Biochemical Parameters

The results of some biochemical indices shown in Table 1, indicated a significant decrease ($p< 0.05$) in the levels of total proteins in rats that were administered CCl_4 only (Group 1), when compared with rats that were pre-treated with 10, 30 and 50% *Carica papaya* (Groups 2, 3 and 4 respectively). The levels of total proteins were significantly increased ($p< 0.05$) in a dose dependent manner in rats that were pre-treated with 10, 30 and 50% *Carica papaya.* Rats administered with CCl_4 only (Group 1), showed significant increase ($p< 0.05$) in the levels of total bilirubin. However, pre-treatment with 10, 30 and 50% *Carica papaya* (Groups 2, 3 and 4 respectively) significantly decreased ($p< 0.05$) total bilirubin levels in a dose dependent manner.

The levels of direct and indirect bilirubin significantly increased ($p< 0.05$) in rat that were administered CCl_4 only, when compared with the rats in Group 5 (normal control). Rats that were pre-treated with 10, 30 and 50% *Carica papaya* showed significant decrease ($p< 0.05$) in the levels of direct and indirect bilirubin, when compared with rats that were administered with CCl_4 only. Malondialdehyde (MDA) levels increased significantly ($p< 0.05$) in rats injected with CCl_4 only, when compared to rats that were not administered with CCl_4 (Groups 5)). However there was significant decrease ($p< 0.05$) in the levels of MDA in rats that were pre-treated with 10, 30 and 50% *Carica papaya* (Groups 2, 3 and 4 respectively), when compared with rats that were treated with CCl_4 only.

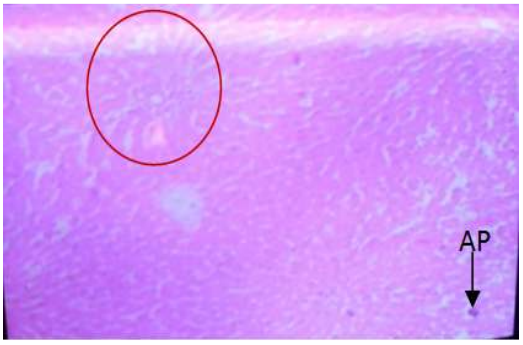

Plate 1. Liver slide of rats administered 100% Feed + CCl_4: (negative control) Portal triaditis (mild) (red circle) and apoptosis (AP) of individual liver cells. H&E 100X

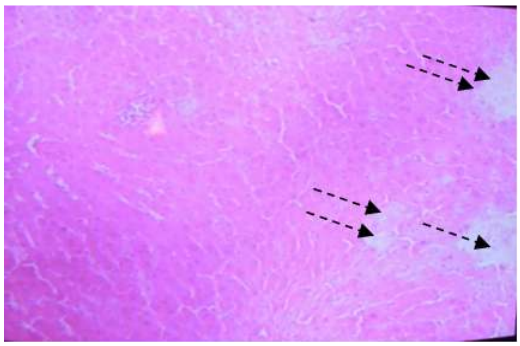

Plate 2. Liver slide of rats administered 10% *Carica papaya* + CCl_4: Mild microvesicular steatosis (dash arrows) of the liver. H&E 100X

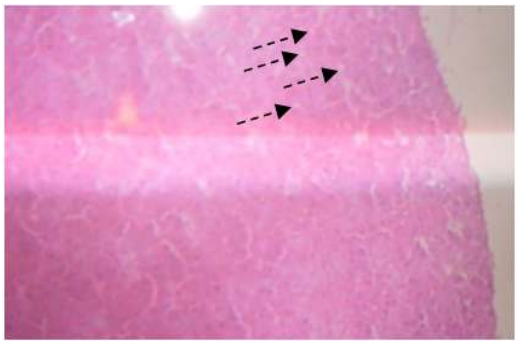

Plate 3. Liver slide of rats administered 30% *Carica papaya* +CCl_4: Normal liver architecture with mild microvesicular steatosis (dash arrows). H&E 100X

Table 1. The effects of *C. papaya* leaf on some biochemical parameters in CCl_4 induced hepatotoxicity

Group	Treatments	Total bilirubin (µmol/L)	Direct bilirubin (µmol/L)	Indirect bilirubin (µmol/L)	Total protein (g/L)	MDA µmol/L × 10^{-5}
1	100% FEED + CCl_4	26.13^{a}±0.20	14.76^{a}±0.20	11.37^{a}±0.20	40.25^{a}±0.20	4.50^{a}±0.01
2	90% FEED + 10% *C. papaya* leaf+ CCl_4	13.52^{b}±0.10	7.38^{b}±0.10	6.14^{b}±0.10	52.96^{b}±0.10	3.50^{b}±0.01
3	70% FEED +30% *C. papaya* leaf+ CCl_4	10.50^{c}±0.20	5.88^{c}±0.20	4.62^{c}±0.20	56.86^{c}±0.10	3.20^{c}±0.01
4	50% FEED + 50% *C. papaya* + CCl_4	9.90^{d}±0.10	5.30^{d}±0.10	4.60^{c}±0.10	63.48^{d}±0.10	2.70^{d}±0.01
5	100% FEED - CCl_4 (General Control)	9.20^{d}±0.10	5.20^{d}±0.10	4.20^{c}±0.10	72.76^{e}±0.10	2.60^{e}±0.01

Values are mean±S. D for 6 replicates (n= 6)
Means with different superscripts are significantly different at the 0.05 levels in the columns

3.2 Histopathological Examination

To ascertain the effects of various treatments on the organs of the rats, the histopathological examinations of liver of rats were carried out. Tissue slides of liver of rats in test and control groups were prepared and the results are as shown.

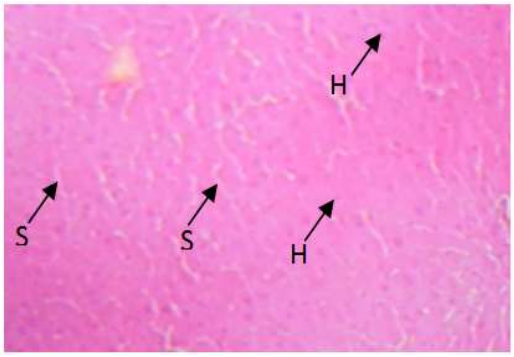

Plate 4. Liver slide of rats administered 50% *Carica papaya* + CCl_4: Normal liver architecture. Hepatocyte (H), Sinusoid (S) H&E 100X

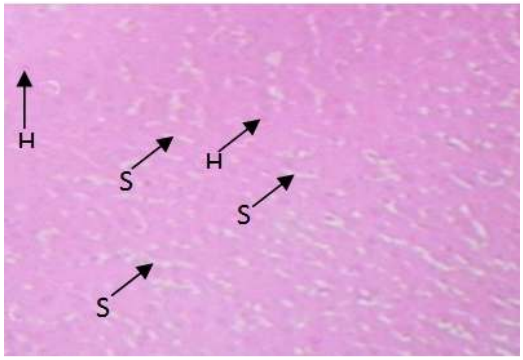

Plate 5. Liver slide of rats administered 30% *Carica papaya* – CCl_4 (normal control): Normal liver architecture. Hepatocyte (H), Sinusoid (S) H&E 100X

4. DISCUSSION

Hepatoprotective activity of *Carica papaya* was investigated using biochemical indices such as total protein, direct and indirect bilirubin and lipid peroxidation products. The results of this study suggests that rat groups that were pretreated with *Carica papaya* prior to administration of CCl_4 showed a significant decrease in hepatotoxicity when compared with rats in Group 1 (negative control). The results showed a significant decrease in the levels of total bilirubin (direct and indirect) and malondialdehyde (MDA), in the serum. The estimation of these biochemical indices in the serum was reported to be a useful quantitative marker for the extent and type of hepatocellular damage [17]. The results of this study further agree with the earlier observation of Babalola et al. [18], that the terpenoid fraction of *V. amygdalina* leaf extract ameliorates carbon tetrachloride induced hepatotoxicity in rats. Krishna et al. [1] earlier reported the chemical composition of *Carica papaya* to include; alkaloid, α-carpaine, β-D-glucosides, β-sitosterol, papain, choline, carotene, riboflavin, vitamin C, phenyethyl- β- D- glucosides, amongst others. These compounds have been reported to have the potentials to function as antioxidants by scavenging the superoxide anion, hydroxyl radical and peroxyl radical or quenching singlet oxygen thus inhibiting lipid peroxidation in biological system [19]. *C. papaya* leaf was also reported to exhibit potential supportive role on oxidative inflammatory damage in cirrhosis caused by hepatitis C virus [20]. It was also suggested by Sallie et al. [21] that the reduction of serum bilirubin levels in pretreated rat groups (Groups 2, 3 and 4 in Tables 1), may provide scientific justification for its use by herbalists as a treatment for jaundice being most sensitive markers employed in the diagnosis of hepatic damage because they are released into circulation after cellular damage. The results also showed an increased serum total protein levels in rats pretreated with *Carica papaya* (Table 1), which could be an indication that the synthetic function of the liver was improved. A decrease in serum total proteins was earlier reported as indications of hepatotoxicity [22]. Histopathology of the liver cells reveled portal triaditis (mild) and apoptosis of individual cells in untreated rat Group 1, indicating some levels of cellular damage due to toxicity, hence supporting the biochemical results. Mild microvesicular steatosis of individual liver cells with normal architecture was also observed in rats pretreated with 10% *Carica papaya*. However, normal liver architecture with mild microvesicular steastosis especially beneath the capsular area was seen in rats pretreated with 30% *Carica papaya*. The livers of rats pretreated with 50% *Carica papaya* and normal control groups showed normal architecture and did not indicate any damages as reported in Plates 4 and 5 respectively. This is in agreement with the reports by Aravind et al. [23] that *C. papaya* leaf could reduce the risk of disease caused by free radical activities and high cholesterol in the blood [24]. Thus, the study

reveals that *Carica papaya* may confer hepatoprotectivty to the rats exposed to carbon tetrachloride-induced liver damage.

5. CONCLUSION

Conclusively, the study suggests that *Carica papaya* may possess hepatoprotectivty to the rats exposed to carbon tetrachloride-induced liver damage.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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