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Original Research Article

Effects of *Carica papaya* Seeds on Acetaminophen-Induced Hepatotoxicity in Male Wistar Rats

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ABSTRACT

Acetaminophen is an analgesic drug used commonly in clinics. However, it causes acute liver failure with continuous overdose. The present study was designed to investigate the effects of *Carica papaya* seeds on acetaminophen-induced hepatotoxicity in male Wistar rats.

Thirty-six male Wistar rats were divided into four groups: Control group (NC), positive control (PC), 1000 mg/kg and 500 mg/kg of nine animals each. The PC, 1000 mg/kg and 500 mg/kg groups were given a dose of acetaminophen (2500 mg/kg *b.w*) orally, and after day three, three rats (n=3) were taken at random from each group and serum enzymes activity levels of Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) were assessed to ascertain hepatotoxicity. Afterwards, rats were treated with *Carica papaya* seeds extract (1000 mg/kg and 500 mg/kg) for fourteen days. The rats were euthanized, plasma collected and analysed for biochemical parameters, while liver tissues were examined for histopathological changes.

There was a significant decrease ($p < 0.05$) in ALT, gamma-glutamyl transferase (ɤ-GT), globulin and reduction in Malondiadehyde.(MDA) concentrations in both 500 mg/kg and 1000 mg/kg groups compared to the PC group. However, serum albumin, total protein and superoxide dismutase (SOD) increased toward normal level in the treated groups. Histopathological studies showed improvement in the liver architecture in the 500 mg/kg group.

The increase in endogenous antioxidants and reduction in lipid peroxidation observed in this study suggests that *Carica papaya* seeds extract can be used as a natural antioxidant in handling liver injury potentiated by acetaminophen overdose.

Keywords: Acetaminophen, Hepatotoxicity, *Carica papaya Linn*, Extract.

Introduction

The liver is a vital organ that carries out essential biochemical and physiological functions, including maintaining the constant internal environment, energy production, removal of toxic substances and combating infections.[1] Exposure to several poisonous agents, including arsenic and carbon tetrachloride causes the pathogenesis of liver disease.[2] Hepatotoxicity occurs when there is the malfunctioning of the liver, due to overdose of drugs or the presence of foreign compounds.[3] Acetaminophen is an example of over-the-counter drug that is cheap, making it a common drug taken in over dose. When used at therapeutic levels, acetaminophen is safe and effective. However, a dose greater than 4 g per day is known to result in hepatotoxicity and drug-induced liver injury.[4] At toxic dose (> 4 g per day), liver conjugations is overwhelmed and reactive metabolite N-acetyl-p-benzoquinone imine (NAPQ1) increases in the cells.[5,6] The increased presence of NAPQ1 levels in cells causes a decrease in natural antioxidant stores and increase in the formation of reactive oxygen species (ROS) that attack the cellular molecules causing lipid peroxidation and hepatic necrosis.[6] This damage to the hepatocytes eventually leads to the escape and a rise in hepatocyte enzymes circulation in the blood.

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The need to use natural products as drugs for the treatment of various diseases is on the increase.[7,8] Particularly in developing countries, where the management of specific disease condition is based on ethnomedicine, due to low cost and minimal side effects.[9] In this context, *Carica papaya* belongs to *Caricaceae* family, and is a commonly consumed fruit throughout the world and used as a treatment for a broad range of diseases.[10] Several scientific studies have shown the traditional use of *Carica papaya* over a wide range of therapeutic activities such as antifungal, antibacterial, anti-inflammatory and antihypertensive.[11] These properties mainly depend on the antioxidant activity of some secondary products, phenolic compounds, vanillic acid and vitamin C present in *Carica papaya*.[12] Furthermore, the effect of *Carica papaya* seed in acetaminophen-induced nephrotoxicity.[13], carbon tetrachloride-induced hepatotoxicity [14] and hepatotoxicity of *Carica papaya* seeds in Wistar rats has been studied.[15]

However, most persons eat the fruit sweet flesh and discard the seeds, not realizing the seeds are edible and rich in phytochemicals.[13] The reason(s) for why the seeds are discarded varies, this unawareness poses enough justification for which more research needs to be carried out on *Carica papaya* seeds, so as to elucidate its therapeutic dose and its medicinal potential in ameliorating hepatic damage.

Therefore, this study was designed to study the ethnomedicinal effects of *Carica papaya* seeds on acetaminophen-induced hepatotoxicity in male Wistar rats.

Materials and Methods

Plant materials and preparation

Fresh *Carica papaya* seeds were collected from fruit vendors around September and October in Calabar Municipality, Cross River State

Nigeria. The *Carica papaya* seeds have been previously identified, authenticated and registered (Voucher specimen no.73) in the Department of Botany Herbarium, University of Calabar, Calabar.[15] The seeds were removed from the fruit and afterwards, air-dried at room temperature of 26°C for fourteen days. The seeds were blended to a fine powder using a dry Moulinex super blender and stored in airtight containers.

A measured amount of 4.31 kg of powdered seeds was extracted by Cold maceration method using 11 L of 80% methanol for 48 h. The extract was first doubled filtered with chess cloth, then with a filter paper (whatman No 1, 24 cm). The filtrate (extract) was concentrated under reduced pressure at temperature range of 34-40°C in rotary evaporator (Model RE42A, china) to 10% volume and then to complete dryness using vacuum water bath. The extract was stored in airtight containers at -4°C. The percentage yield (7.5%) was calculated using the formula:

$$\%\, Yield = \frac{weight\ of\ extract}{amount\ of\ dry\ powder\ extracted\ (g)} \times 100$$

Acute toxicity studies

The acute toxicity test of *Carica papaya* seed extract was performed on female mice based on the OECD revised up-and-down procedure for acute toxicity testing (OECD guideline 425, 2008).[16] The mice were divided into two groups (n = 5): A control group that received distilled water and the treatment group that received the aqueous extract of *Carica papaya* seed orally at a limit dose of 2000 mg/kg. A dose of 2000 mg/kg was given to the first rat, and the rat was observed for mortality and toxic signs for the first hour, third hour and finally until 48 hours. All of the experimental animals were maintained under close observation for 14 days. The LD_{50} was predicted to be above 2000 mg/kg since three or more rats survived.

Animal handling and experimental protocol

Thirty-six male Wistar rats weighing 100-150 g were obtained from the Department of Biochemistry Experimental Research Animal House of University of Calabar, Calabar, Nigeria. Male rats used in this study was based on the assumption that, female Wister rats may cause variation in result due to changes in hormonal state. The rats were kept in standard well-ventilated cages (Wooden bottom and wire mesh top), under standard laboratory conditions (temperatures: 25-34^{o}C, humidity 30-70% and adequate light and ventilation), and allowed to acclimatize for 1 week prior to experimental procedures. The rats were given food pellets and water *ad libitum* throughout the experimental period. The experimental animals were randomly assigned to four groups of nine rats each. However, three rats (n = 3) from each group were used for preliminary studies (to ascertain hepatotoxicity) and the remaining rats (n = 6) were used in the treatment phase of the experiment (Table 1). The animal experiment was carried out according to the guidelines of the Institution (University of Calabar, Nigeria) Animal Research Ethics Committee with ethical approval number- 041BCM2710.

The induction of hepatotoxicity was done according to Mitchell.[17] The rats in groups 2, 3 and 4 (PC, 1000 mg/kg and 500 mg/kg) were administered orally with 2500 mg/kg b.w of acetaminophen reconstituted in normal saline (Table 1). After three days, three rats (n = 3) were picked at random from groups 2, 3 and 4 (PC, 1000 mg/kg and 500 mg/kg) to ascertain hepatotoxicity. The rats were sacrificed and serum levels of AST, ALT and ALP estimated. Having established that the rats were hepatotoxic, the rats (n = 6) in-groups 3 and 4 were treated with 500 mg/kg and 1000 mg/kg of the *papaya* seed extract, respectively. The extract was reconstituted in normal saline and administered orally via gastric intubation. The administration of *Carica papaya* seed extract was carried out consecutively for fourteen days.

Collection and preparation of tissues for analysis

The rats were anaesthetised over chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using hypodermic syringes and needles. Blood was collected and put into anticoagulant (EDTA) bottles. The blood sample was centrifuged with a table top centrifuge (Surgifield Centrifuge model Sm80-2 England, Uk) at 900 xg for 10 minutes to obtain plasma from cells. The plasma was gotten with sterile syringes and sent for biochemical analysis of liver function biomarkers while whole blood was used to assay for oxidative stress and antioxidant parameters. The liver was harvested and fixed in 10% buffered formalin for histological analysis.

Chemicals

All chemicals used in this study were of analytical grade. Acetaminophen manufactured by Emzor pharmaceuticals was purchased from purchased from Bez pharmacy Etta Agbor road, Calabar, Cross River State, Nigeria. Diagnostic kit for the estimation of the enzyme activity levels of AST (iμ/L) ALT(iμ/L), ALP (iμ/L), GGT, SOD, GPx (μ/L) & total serum bilirubin, conjugated bilirubin, total protein, albumin, globulin and MDA were determined using reference standard methods as obtained from Randox laboratories Ltd (55 Diamond Road, Crumlin, County Antrim, BT29 4QY,United Kingdom).

Histological analysis

The liver tissue was fixed in 10% formol saline for 24 h, after which they were processed using descending fractions of ethanol (70%, 90% absolute 1, 2, 3) for dehydration; cleared in xylene and infiltrated in paraffin wax, then embedded in paraffin and stained with haematoxylin and Eosin (H & E) for histological examination. Images of processed liver section were examined under a light microscope for morphological changes.

Statistical analysis

The data was analysed using IBM SPSS statistic software version 22. The result was expressed as means ± standard error of mean (SEM) and values were calculated for each group. A one-way analysis of variance (1-ANOVA) followed by Dunnet post hoc test. Differences at $P < 0.05$ were considered significant.

Table 1: Experimental design

Group	No of Rats	Treatment
Normal control (NC)	6	0.2 mL/kg of normal saline
Acetaminophen-Induced untreated group (PC)	6	2500 mg/kg of paracetamol + 0.2 mL/kg of Normal saline
500 mg/kg treated group	6	2500 mg/kg of paracetamol + 500 mg/kg of *Carica papaya* seed extract (0.2 mL/kg of Normal saline)
1000 mg/kg treated group	6	2500 mg/kg of paracetamol + 1000 mg/kg of *Carica papaya* seed extract (0.2 mL/kg of Normal saline)

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Results and Discussion

Acetaminophen induces hepatotoxicity in rats
As presented in Figures 1a-c. The induction with acetaminophen overdose (2500 mg/kg) resulted in a significant increase ($p < 0.05$) in AST, ALT and ALP activity levels in all the acetaminophen treated groups (PC, 1000 mg/kg and 500 mg/kg) compared to the normal control (NC).

Effect of Carica papaya seeds extracts on liver enzymes activity
Figure 2 shows serum enzyme activity levels of AST, ALT and ɤ-GT after treatment with *Carica papaya* seeds exacts for 14 days. There was a significant ($p < 0.05$) reduction in AST level in the 1000 mg/kg treated group compared to the PC group (induced-untreated). While the ALT levels in the treated group (500 mg/kg and 1000 mg/kg) showed no significant ($p > 0.05$) decrease when compared to the PC and NC. However, the 500 mg/kg and 1000 mg/kg treated group showed a significant decrease ($p < 0.05$) in ɤ-GT levels.

Effect of Carica papaya seeds extracts on plasma protein biomarkers
The results showing plasma protein biomarkers after treatment with papaya seed extract are shown in Figure 3. The 500 mg/kg and 1000 mg/kg treated groups showed a significant reduction ($p < 0.05$) of total serum protein and globulin when compared to the PC group. While the 500 mg/kg group showed an increase in albumin compared to the PC group.

Effect of Carica papaya seeds extracts on MDA concentration, GPx and SOD hepatic activity
The concentration of lipid peroxidation product MDA, hepatic Gpx and SOD activity in the different groups are shown in Figure 4. The MDA level in the PC group (induced, untreated) showed a mark increase when compared to the normal control (NC), and decrease in MDA levels in the treated groups (500 mg/kg and 1000 mg/kg). The activity of glutathione peroxidase (GPx) showed no significant ($p > 0.05$) increase in both the 1000 mg/kg and 500 mg/kg treated groups in comparison to the NC and PC groups (Figure 4). Whereas, the 500 mg/kg and 1000 mg/kg treated groups showed a marked increase in superoxide dismutase activity in comparison to the PC group (Figure 4).

Effect of Carica papaya seeds extracts on histology of the liver
Figure 5 shows the result of histopathological analysis of liver tissues. After treatment, hepatocytes in the normal control (NC) showed a preserved architecture with prominent central vein and hepatocytes radiating outwards. The hepatocytes have abundant cytoplasm and a round to oval nuclei with distinct nucleoli. However, in comparison to the PC group, the hepatocytes were mildly enlarged with some displaying prominent nuclei and focal areas of piecemeal necrosis. The 500 mg/kg treated group showed a preserved architecture. While in the 1000 mg/kg treated group, the hepatocytes were enlarged with prominent nuclei and distinct nucleoli. There is marked microvesicular steatosis, and mild portal inflammatory infiltrate.

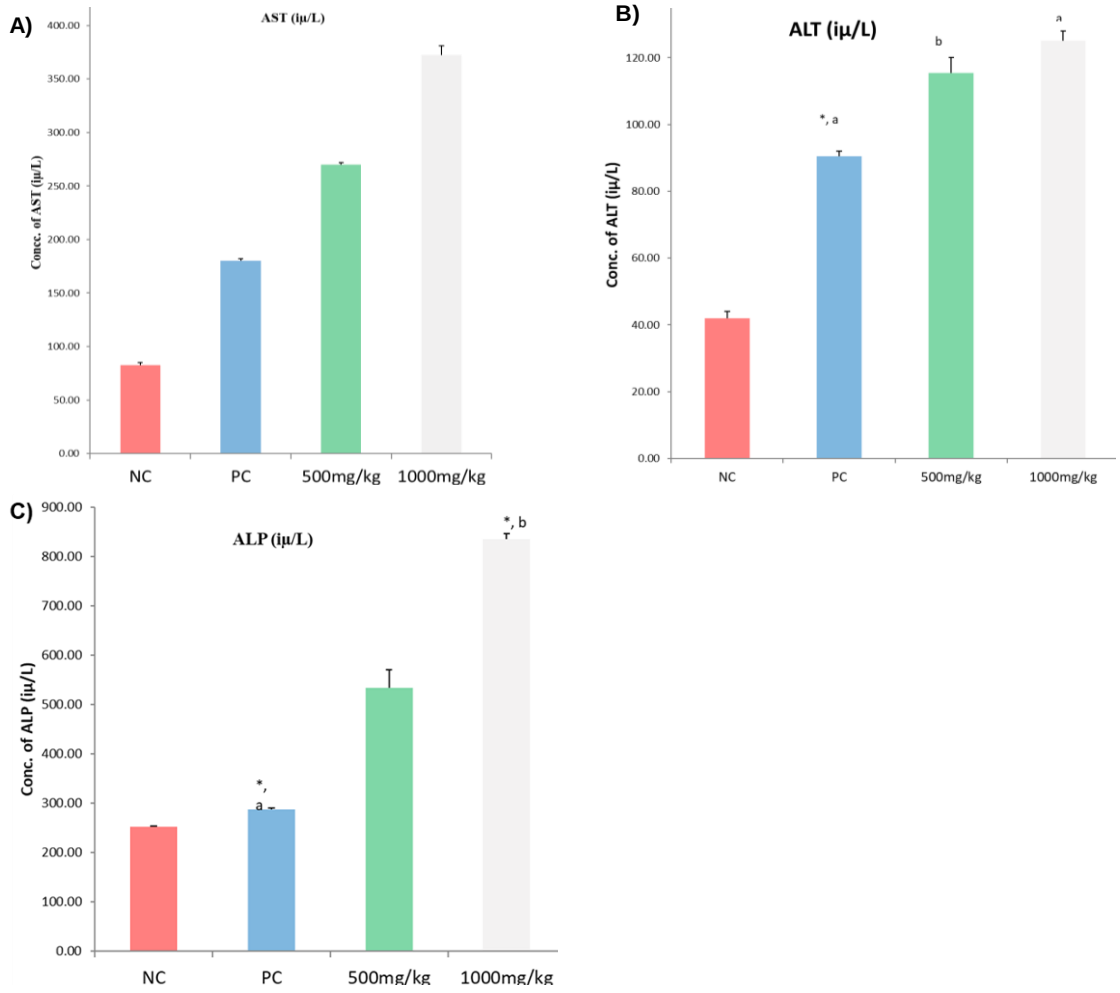

Figure 1: Effect of acetaminophen overdose on liver biomarkers (AST, ALT and ALP).
Values are expressed as mean ± SEM. n = 3. **A)** *There was significant difference between all the groups at $p < 0.05$ **B)** significantly different from NC at $p < 0.05$. a = $p < 0.05$ vs 1000 mg/kg. b = $p < 0.05$ vs PC. **C)** *Significantly different from NC at $p < 0.05$. a = $p < 0.05$ vs 500 mg/kg. b = $p < 0.05$ vs PC.

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Acetaminophen is the most commonly used over-the-counter drug with no side effects within the prescribed dose range. In clinical practice, the recommended dose is one or two tablets (500 or 1000 mg) every 4-6 h, however, patients do mistakenly exceed the recommended maximal daily dose (less than 4 g or < 150 mg/kg per day) of acetaminophen.[18] The overdose or long-term usage of acetaminophen can result in inflammation and necrosis of hepatocytes or even acute liver failure.[19,20]

The abnormally high levels of serum ALT, AST and ALP observed in the acetaminophen treated group (PC) are indications of acetaminophen-induced hepatotoxicity and damage to the hepatic cells. This observation was consistent with previous studies on experimental rats exposed to a high dose of acetaminophen (> 150 mg/kg), which resulted in hepatotoxicity with an increase in serum enzyme activities.[21-23] The measuring of serum activity levels of AST, ALP, ALT and GT has been a commonly used biomarker to ascertain liver injury.[24-26]

The reduction of AST, ALT and ɤ-GT levels after treatment with *Carica papaya* seeds may be due to restoration of hepatic functions. The decrease might have been due to the phytochemicals - saponins, tannins, flavonols and terpenoids and alkaloids present in the seeds.[13] These phytonutrients have been shown to be involved in hepatoprotection via cellular regeneration, restoration of hepatocytes function, and prevention of the leakage of intracellular enzymes by improving cell membrane integrity,[27-29] due to antioxidant and free radical scavenging properties derived from flavonoids, phenols and saponins.[30] Findings from this study is consistent with the report of Adeneye *et al*,[14] the study reported that the seeds extract of *Carica papaya* showed hepatoprotective effect from carbon tetrachloride (CCl_4), by decreasing elevated serum AST and ALT level in rats.

The liver remains the primary source of most serum proteins, as the cell functions by producing albumin, fibrinogen and other coagulation factors and globulin.[23] The reduction in serum albumin protein level in the acetaminophen treated rats may be due to decrease in the number of hepatocytes, which have resulted in a decrease in albumin production. The decrease in albumin levels detected is in tandem with earlier report by Ekam and Udosen .[31] However, After treatment, an increase in albumin levels in the treated groups may be due to the hepatoprotective ability of *Carica papaya seed* extract to regenerating hepatocytes through the stimulation of RNA polymerase enzyme, and ribosomal protein synthesis in the nucleus of liver cells.[32] The restoration of albumin levels in the treated groups agrees with previous studies by Ekam and Udosen .[31]

A significant increase in globulin and total protein was observed in the acetaminophen treated group. The observed increase in total protein and globulin levels may be due to severe inflammatory response, and this agrees with the previous report, which showed that acetaminophen overdose (> 150 mg/kg), causes an increase in serum β and ɤ globulin levels.[33] Interestingly, after treatment, there was a significant decrease in total protein and globulin levels in the *Carica papaya* treated groups (500 mg/kg and 1000 mg/kg), which indicates an improvement of hepatocytes by the seeds.

It is well established that the body is endowed with an effective mechanism to counter the scavenging effect of free radicals, through endogenous antioxidants systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione.[34] The imbalance between free radical and antioxidant result in oxidative stress leading to pathological conditions.[35,36]

The depletion of GPx and SOD in the acetaminophen treated rats (PC) is in tandem with previous studies conducted by Sathya *et al*.[37] and Elisan *et al*.[38]. The depletion of cellular glutathione and superoxide dismutase stores may be due to excessive NAPQI formation after acetaminophen overdose.[39]

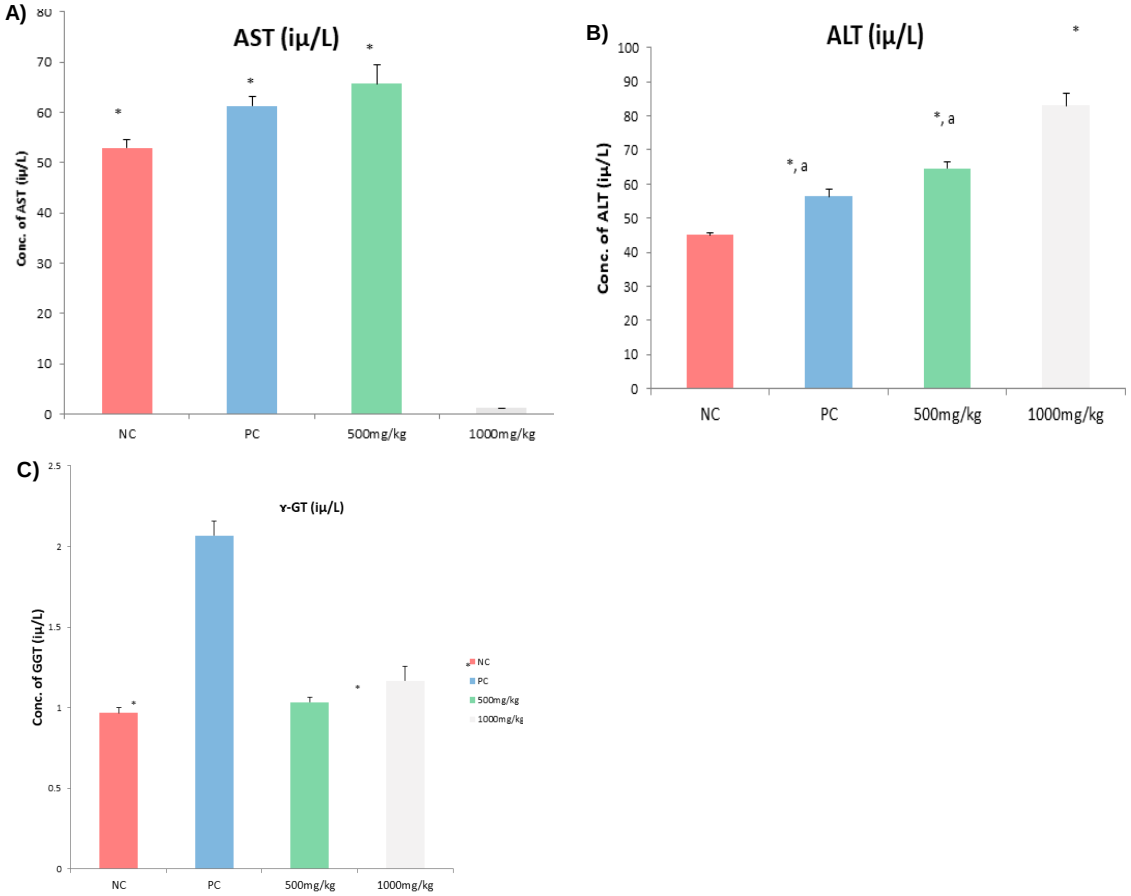

Figure 2: Effect of *Carica papaya* seeds extracts on liver enzymes activity (AST, ALT and ɤ-GT) in the different experimental groups. Values are expressed as mean ± SEM. n = 6. **A)** *Significantly different from 1000 mg/kg at $p < 0.05$. **B)** *Significantly different from NC at $p < 0.05$. a = $p < 0.05$ vs 100 mg/kg. **C)** *Significantly different from PC at $p < 0.05$.

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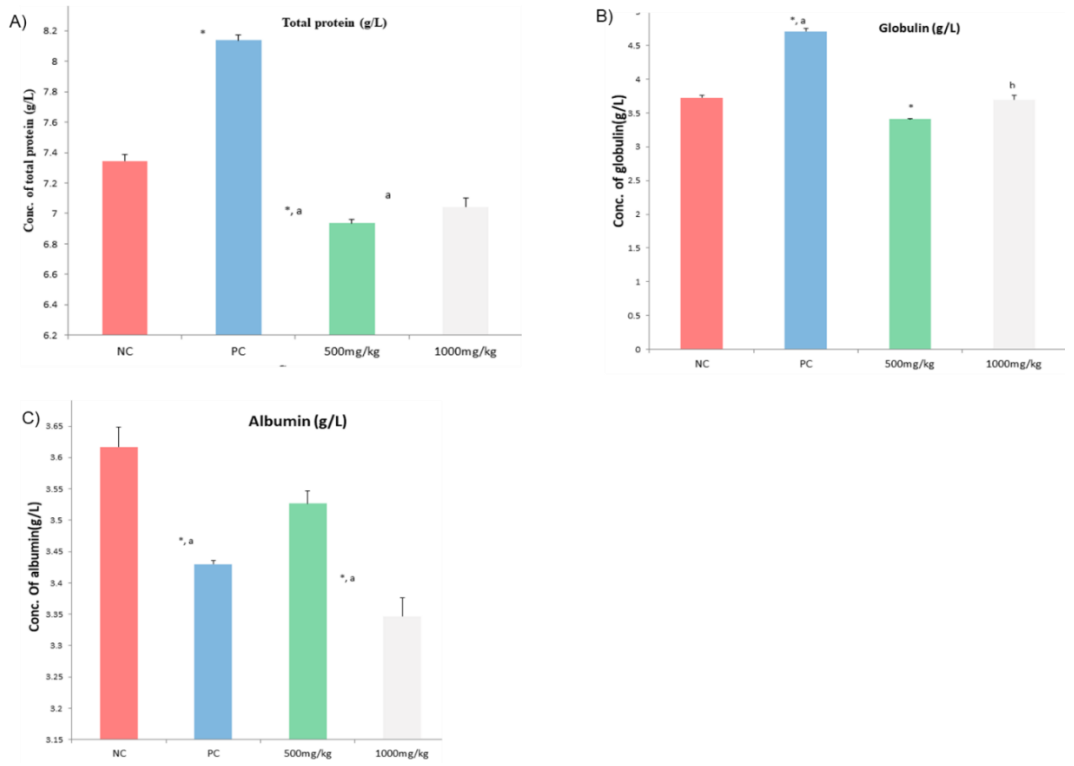

Figure 3: Effect of *Carica papaya* seeds extracts on plasma protein biomarkers (Total protein, albumin and globulin) in the different experimental groups. Values are expressed as mean ± SEM. n = 6**.** .**A)** *significantly different from NC at $p < 0.05$. a = $p < 0.05$ vs PC. **B)** *Significantly different from NC at $p < 0.05$. a = $p < 0.05$ vs 500 mg/kg. b = $p < 0.05$ vs PC. **C)** *Significantly different from NC at $p < 0.05$. a = $p < 0.05$ vs 500 mg/kg.

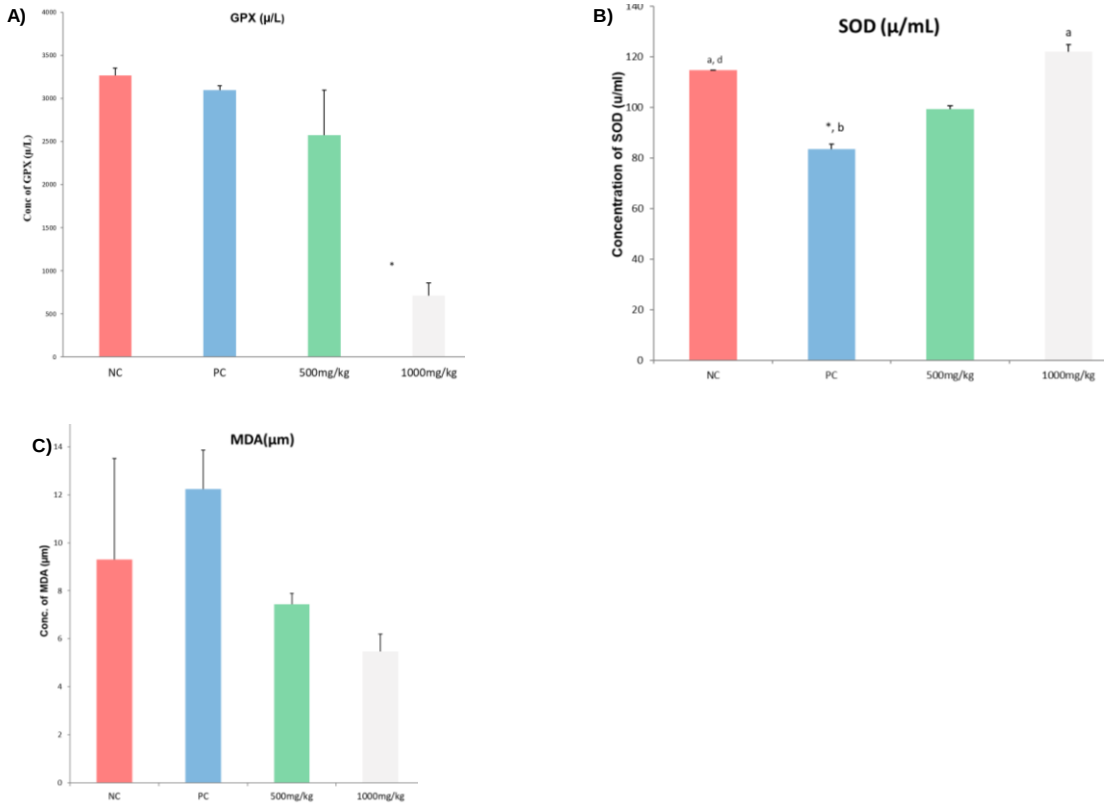

Figure 4: Effect of *Carica papaya* seeds extracts on MDA concentration, GPx and SOD hepatic activity level in the different experimental groups. All values are expressed as mean ± SEM. N = 6. **A)** *significantly different from PC at $p < 0.05$. **B)** *Significantly different from NC at $p < 0.05$. a = $p < 0.05$ vs 500 mg/kg. d = $p < 0.05$ vs 1000 mg/kg. **C)** There was no significant difference between the groups at $p < 0.05$.

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However, the extract was able to restore the GPx activity level close to normal in the 500 mg/kg group and SOD in the 1000 mg/kg and 500 mg/kg groups. Active phytochemicals in *Carica papaya* seeds may be responsible for increasing endogenous antioxidant, by acting as free radical scavenger against harmful reactive oxygen species. These results corroborate earlier findings of Olufunsho *et al*.[40] and Dadkhah *et al*.[41]

The elevation of MDA is usually considered an important biomarker of oxidative stress and lipid peroxidation in liver tissue.[42] Previous report has shown that administering high dose of acetaminophen to rats, positively correlated with increased lipid peroxidation, due to direct oxidation of unsaturated fatty acids in the cell membrane.[43-45] However, no significant increase of MDA concentrations in the acetaminophen treated group (PC) was observed in this study. After treatment with *Carica papaya* seed extract, a decrease in MDA levels was observed in both the 500 mg/kg and 1000 mg/kg groups.

This reduction may be due to the mopping of hydroxyl radical that initiate a chain reaction of lipid peroxidation,[45] by phenolic compounds such as *p*-hydroxybenzoic and vanillic acid present in the *Carica papaya* seeds. These phenolic compounds in *Carica papaya* seeds have been reported to have strong antioxidant activity that scavenge and chelates oxidative molecules inside the cell.[46,47]

Another essential consideration in checking the efficacy of a potential therapeutic agent is on the histopathology of the tissue involved. The 500 mg/kg treated group showed a preserved architecture through the restoration of the damaged liver tissue. The effect observed may be due to the antioxidant activity of the plants. Previous studies on medicinal plants with hepatoprotective properties have been shown to mediate their protection via antioxidant and free radical scavenging activities due to the high concentration of flavonoids, phenolics and other active compounds they contain.[28,48] This is in agreement with the findings of this study. The reason why the hepatocytes in the 1000 mg/kg treated group were still enlarged and inflamed is still unclear.

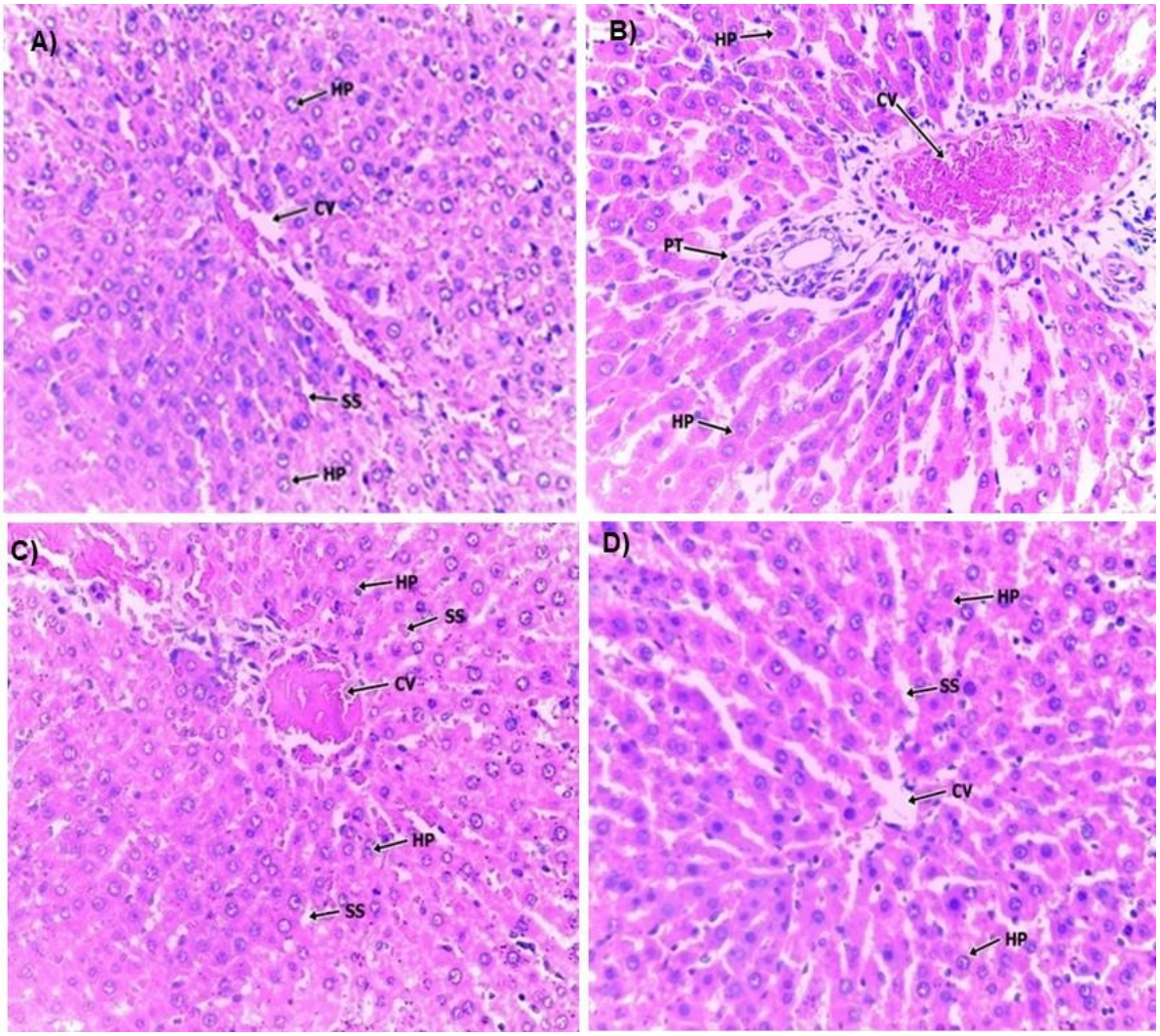

Figure 5: Photomicrograph of liver architecture after treatment with *Carica papaya* seed extract- **A**) Normal control (NC) centrobular area (x400). This section of the liver show prominent central vein with plates of hepatocytes radiating outward. The hepatocytes have abundant cytoplasm and a round to oval nuclei with distinct nucleoli. **B)** Positive control (induced, not treated) centrobular area (x400). The hepatocytes are mildly enlarged with some displaying prominent nuclei and nucleoli and having dispersed chromatin pattern. There are focal areas of piecemeal necrosis. **C**) Centrobular area (x400) of the liver after treatment with 500 mg/kg of *Carica papaya* seeds shows a preserved architecture. The central vein is congested and plates of prominent hepatocytes radiate outward. **D)** Centrobular area (x400) of the liver after treatment with 1000 mg/kg of *Carica papaya* seeds shows hepatocytes are enlarged with prominent nuclei and distinct nucleoli. There is marked microvascular steatosis and mild portal inflammatory infiltrate. The liver tissue was stained with H & E = Haematoxylin and Eosin. (Cv - central vein, Hp - hepatocytes, SS - sinusoidal spaces)

Conclusion

Collectively, data from this study suggest that the combined presence of phytochemicals (saponins, tannins, flavonols, glycosides, terpenoids, alkaloids, phenols (p-hydroxybenzoic and vanillic acids) in the seeds of *Carica papaya*,[12,13] showed an ameliorative effect in the liver tissue by increasing endogenous antioxidant and suppressing oxidative stress. Therefore, *Carica papaya* seeds can be used as a natural antioxidant in handling liver injury potentiated by acetaminophen overdose. However, the mechanism of action of the actual bioactive compound responsible for its hepatoprotective function needs to be researched.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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