Invitro and Invivo Antioxidant activity of aqueous extract of *Carica papaya* Seed

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Abstract

High concentrations of free radicals are hazardous to cells triggering damage to cells and cellular constituents in living organisms. Antioxidant molecules play a major role in controlling the free radicals produced during the metabolic processes in the body scavenging excess free radicals, thereby maintaining the redox balance. The invivo and invitro antioxidant activity of the aqueous extract of *Carica papaya* seed was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipid peroxidation inhibition assays. These were carried out using standard analytical methods. The invivo study was carried out using paracetamol to induce oxidative stress in mice. The antioxidant phytochemicals present in *Carica papaya* seed were quantified. The invivo antioxidant capacity of the extract was evaluated from the enzyme activities of superoxide dismutase (SOD) and catalase from the acute liver damage induced with paracetamol in mice.
Damage to liver was ascertained from the serum enzyme activities of alkaline phosphatase and transaminases. The median effective dose (EC50) was 402.1±3.0µg/ml, the total phenolic content was 74.1±1.1 mg/100g and the total flavonoid was 117.56±2.1 mg/100 g. The highest DPPH scavenging activity of the aqueous extract was 86.6 ±1.1 %, the highest lipid peroxidation inhibition capacity of the extract was demonstrated at the concentration of 250 µg/ml to be 74.23±2.3 %. The DPPH scavenging capacity and lipid peroxidation inhibition increased with an increase in extract concentration. *Carica papaya* seed extract as compared to the negative control, showed an increase in the activity of SOD and catalase in the acute liver damage induced with paracetamol in mice. The antioxidant effect of the Carica papaya extract was however significantly lower when compared to the stress induced mice treated with the standard drug (sylimarin). The best dose for the invivo study was shown to be 400 mg/kg body weight. This study indicates that *Carica papaya* seed extract possess antioxidant activity.

Keywords: *Carica papaya*, free radicals, antioxidant, lipid peroxidation, SOD, catalase.

Introduction

High concentrations of free radicals are hazardous in nature triggering damage of cellular constituents in living organisms. When the rate of free radical production is greater than that of its clearance, the free radical producing sites tend to undergo oxidative stress and begin to damage. Antioxidant molecules play a pivotal role in controlling the free radicals produced during the breakdown in body or through environmental factors such as tobacco, smoke and radiation, by scavenging excess free radical, thereby maintaining the redox balance (Halliwell, 2009).

Antioxidants are molecules that inhibit the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione, catalase and superoxide dismutase enzymes produced internally or the dietary antioxidants; vitamin A, C and E. Oxidative stress can be considered as either a cause or consequence of some diseases. Industrial antioxidants have diverse uses, for example as preservatives in food and cosmetics and as oxidation inhibitors in fuels, (Dabelstein *et al*., 2007).

Papaya (*Carica papaya*) belongs to the family of caricaceae and several species of caricaceae have been used as remedy against a variety of diseases (mello *et al.*, 2008; Munoz *et al*., 2010). Papaya was originally derived from the southern part of Mexico. It is a perennial plant which is distributed over the whole tropical and subtropical areas. It is one of the most consumed fruits. Papaya like other fruits such as banana or apple has been found to be a good natural source of macronutrients (carbohydrates and proteins) and micronutrients (vitamin A and vitamin C) (Peterson *et al*.; 2012). There is also a strong relationship between the intake of these antioxidant-containing plants and reduced mortality of the afore mentioned diseases (Halliwell *et al*.., 2012). Indeed, natural antioxidants warrant further scientific scrutiny given their activity against free radicals, which contribute to chronic degenerative diseases (Bray, 2010).

Medicinal plants play important roles in preventing various diseases and have received much attention from many researchers over the last few decades. Studies on the antioxidant contents of fruits and vegetables are increasing because natural antioxidant consumption has been found to be related with decreased risk for cancer and heart diseases (Temple, 2010). While much have been reported on the anti-oxidant activity of *Carica papaya* fruit flesh little or nothing is Known of the antioxidant effect of the seed. The aim of this research is focused on determining the antioxidant effect of aqueous papaya seed extracts.

Materials and Methods

Materials

Weight measurements were carried out using Adventurer (OHAUS); Concentration of extract were carried out using Griffin Water bath. Deionized water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazline-6-sulphonic acid) diammonium salt (ABTS), potassium persulfate, rutin, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), ferric chloride, sodium acetate, 2,4,6-tripyridyl-S-triazine (TPTZ), 2-thiobarbituric were of analytical grade and purchased from Sigma Chemical Co.

Sample Collection and Preparation

Fully matured and ripe *Carica papaya (C. papaya)* was purchased from one of the selected market in Minna, Niger state, Nigeria. The papaya fruit was washed under tap water cut into two and the seed were separated from the pulp. The papaya seeds were washed with water and air dried at room temperature. Milled and stored for further analysis.

Extraction of Plant Materials

Milled *C. papaya* sample were weighed (150 g) and added with 1.5 L of deionized water. Then the mixture was placed onto the shaker (HY-4 Vibrator, SEARCHTECH INSTRUMENTS) for 120 min. The sample was filtered through Whatman No. 1 filter paper and collected into another reagent bottle, while the sample residue were re-extracted using the respective extraction media.

Pooled extracts were concentrated using water bath at 65 ^{0}C and oven dried at 45 ^{0}C. The percentage yield (% w/w) of crude was determined by using the equation below;

$$\text{Yield (\%)} = \frac{\text{Weight}_{extract}}{\text{Weight}_{sample}} \times 100$$

Determination of Total Phenolic contents of Papaya Seed

The total phenolics (TP) were quantified spectrophotometrically using the folin-Ciocalteu's Reagent (FCR) method as described in Dubost *et al*., 2007 and Ferreira *et al*., 2007. Sample extracts (1 ml) with 5 different concentration (50-500 gmL^{-1}) were added with 4 ml of FCR reagent (previously prepared using 10-fold dilution), followed by the addition of 5mL of 7.5 % sodium carbonate solution in 100 ml of deionised water after 3 min. The mixture was shaken vigorously and incubated at room temperature in the dark for 30 min. Deionized water was used as blank. The absorbance was read at 765 nm using a visible spectrophotometer (PRIM, Secoman, Ales Gard, France). Total phenolic content was expressed as Gallic acid Equivalents (GAE), in mg/g of extract which was calculated based on the calibration curve of gallic (o.2-2.5 $\mu g\ mL^{-1}$). The calibration equation of gallic acid was:

$$\text{Scanvenging activity (\%)} = \left[1 - \frac{\text{Absorbance}_{\text{ sample }}}{\text{Absorbance}_{\text{ control}}}\right] \times 100$$

Determination of Total Flavonoid contents of *Carica papaya* seed

Aluminum chloride colorimetric method was used for flavonoids determination according to the method of (Chang, 2014). Each plant extracts (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10 % aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible

spectrophotometer (USA). The calibration curve was prepared by gallic acid solution (0-100 µg/ml) in methanol. The concentration of flavonoid was expressed in terms of µg/ml.

Determination of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging

Antioxidant Activity Assay of Extract: DPPH scavenging activity was done based on a method by (Tsai *et al*., 2009). The DPPH reagent was prepared by dissolving 7.8 mg of DPPH powder in ethanol and top up to 100 ml. Sample extracts (2 ml) at various concentrations (50, 100, 150, 200 and 250 µg/ml) were placed in test tubes with 500 µL of DPPH reagent. The reaction mixture was shaken vigorously and incubated for 30 min. at room temperature in the dark. The negative control was prepared without any addition of extract and ethanol was used as blank. The changes in the absorbance of samples were measured at 517 nm in a spectrophotometer. The DPPH radicals scavenging activity of the sample was calculated based on the following equation

$$\text{Scavenging activity (\%)} = \left[1-\frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}\right] \times 100$$

Determination of lipid peroxidation Inhibition of Extract: Lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA) 19. To 1 ml of supernatant, 0.5 ml of 30 % tricholoroacetic acid (TCA) was added followed by, 0.5 ml of 0.8 % TBA. The tubes were kept in a shaking water bath for 30 min at 80 ^{0}C. After 30 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800g for 15 min. The amount of MDA was assessed by measuring the absorbance of supernatant at 540 nm at room temperature against an appropriate blank. The percentage inhibition of Lipid peroxidation was calculated using the equation:

In vivo Antioxidant Study Animals

Mice (13.5-24.5 g) procured from ABU Zaria, Kaduna were used for the studies. The animals were housed in large polypropylene cages in a temperature controlled room (37±2 ^{0}C) and provided with standardized pellet feed and clean drinking water ad libitum

After seven days of acclimatization, the mice were divided into four groups (n=6). Treatment was done for 10 days as follows:

Group I: Positive control received the vehicle of normal saline (2 ml/kg/day)

Group II: Received normal saline (2 ml/kg/day) for 9 days and simultaneously administered paracetamol 2000mg/kg at every 72 hr.

Group III: Received *Carica papaya* extract (CPE) (200 mg/kg/day) for 9 days and simultaneously administered paracetamol at every 72 hr.

Group IV: Received CPE (400 mg/kg/day) for 9 days and simultaneously administered paracetamol 200 mg/kg at every 72 hr.

Group V: Received CPE (600 mg/kg/day) for 9 days and simultaneously administered paracetamol 200 mg/kg at every 72 hr.

Group VI: Received standard drug Silymarin (100 mg/kg/day) for 9 days and simultaneously administered paracetamol 200 mg/kg at every 72 hr.

Data (Statistical) Analysis

All experiments were carried out in triplicates and the data were analyzed using statistical packages for Social Sciences (SPSS) for Windows version 16.0 (IBM, New York, USA). All the data were expressed as mean ± standard deviation. One-way Analysis of Variance (ANOVA) was used to analyze the mean value of data and Turkey's post-hoe multiple comparison tests were carried out to assess for any significant differences among the means. A statistical probability level of $p<0.05$ was considered significant.

Results and Discussion

Figures 1and 2 shows an increase in the free radical scavenging and lipid peroxidation inhibition of the activities of the aqueous extract of *Carica papaya* seed with respect to concentration. The standard however had a higher activity than the extracts. The extract possesse antioxidant activity, this suggests the ability of *Carica papaya* seeds to minimize oxidative damage to some vital tissues in the body (Kojie *et al*., 1998 and Weigh and *et al*., 1999). DPPH scavenging ability of papaya seed extract shown to be slightly higher than the inhibition of lipid peroxidation which could be because of the different mechanisms involved in the radical-antioxidant reactions. For example, solubility of the aqueous extracts in different testing system, substrate used and quantitation method may influence the ability of herbs to quench different radicals (Yu *et al*., 2002). The result conform with the report of (Wang *et al*., 1998) who found that some compounds with DPPH scavenging activity also are able to inhibit lipid peroxidation. As a result, it may be difficult to compare antioxidant activity based on antioxidant assay because of the different test system and the substrate to be protected (Frankel and Meyer, 2000). The major plant constituents with high level of antioxidant activity are the polyphenols, which have the ability to quench, absorb and neutralize free radicals (Duh *et al*., 1999). Their ability to scavenge free radical could be as a result of their redox properties, presence of conjugated ring structures and carboxylic group that have been shown to have inability effect on lipid peroxidation (Rice-Evans *et al*., 1995). Recent studies have shown that the aqueous extract of Carica papaya seed possess high level of phenol and flavonoid content that might account for the strong activity observed against DPPH and lipid peroxidation. These antioxidants activities may be due to the hydroxyl groups present in the aromatic ring, this help to quench free radicals (Vinson *et al*., 1998)

The serum enzymes activities of ALP, ALT and AST were lower for the standard than for the extract and the control (Figures 3, 4 & 5), suggesting the aqueous extract of *Carica papaya* seed have a lower ameliorative effect on the damaged liver compared to the standard drug. Treatment with *Carica papaya* extract at the concentration of 400 mg/kg revealed a comparable activity with the reference standard drug (Silymarin), a potent hepatoprotective drug. Administration of paracetamol at the concentration of 2000 mg/kg caused an elevation serum AST, ALT and ALP. The aqueous extracts of C*arica papaya* seed was shown to decrease serum level of AST, ALT and ALP.

The liver is an organ that play a versatile role concerned with regulation of internal chemical environment. Therefore, any form of damage inflicted by an hepatotoxic agent is of grave consequence. The most important function of the liver is for the maintenance of body homeostasis, besides it plays an important role in metabolism, detoxification, and inflammatory response (Tacke *et al*., 2009).

Paracetamol, also called acetaminophen or APAP (N-acetyl-p-aminophenol), is usually considered as a safe pain killer, though, overdoses of this agent could lead to acute liver failure and hepatic cytolysis (Michaut *et al*., 2014). Hepatotoxic drugs such as acetaminophen and D-galactosamine reduces liver functional capacity, which could lead to accumulation of waste products like ammonia in the blood (Mao *et al*., 2014). Paracetamol is known to process hepatotoxic effects in human at high doses. It has been used as a successful experimental model to evaluate the efficacy of hepatoprotective agents (Sreedevi *et al*., 2009). The mode of action on the liver is by covalent binding of its toxic metabolite, n–acetyl- p-benzoquinone-amine to the sulfhydryl group of protein resulting in cell necrosis and lipid peroxidation (Kapur *et al*., 1994).

As a result of liver injury caused by paracetamol overdose, the transport role of the hepatocytes gets disturbed leading to the leakage of the plasma membrane (Zimmerman and Seeff, 1970), thus resulting to an increase in serum enzymes levels. Administration of aqueous extract of *Carica papaya* seed at concentration of 400 mg/kg, for 10 days resulted in a significant reduction of paracetamol induced elevation of serum enzyme markers, which is comparable to the effect of silymarin, the positive control used. Silymarin is a known hepatoprotective compound obtained from silybummarianum. It is reported to have a protective effect on plasma membrane of hepatocytes (Ramellini and Meldolesi, 1976). Toxic compounds have been known to accumulate in the liver where they are detoxified (Clarke and Clarke, 1977). Liver transaminases such as AST (aspartate transaminase) or SGOT (serum glutamic oxaloacetic transaminase) and ALT (alanine transaminase) or SGPT (serum glutamic pyruvic transaminase) still remained the gold standards for the assessment of liver injury, and have been used as biomarkers of choice for decades (Howell *et al*., 2014). A study of liver function test may therefore prove useful in assessing especially the toxic effects of medicinal plants on the liver. These tests involve mainly the determination of AST and ALT (Tilkian, 1979) and any marked necrosis of the liver cells leads to a significant rise of these enzymes in the serum. The lack of this effect on these liver enzymes shows that the extract is non-toxic to the hepatocytes. SGOT, SGPT and ALP (alkaline

phospgatase) are important serum enzymes in the human liver and usually help to detect chronic liver diseases by monitoring their concentrations through their serum activity. The values were well comparable to the control group indicating that the extract is non-toxic and safe. Hepatic injury causes elevated levels of liver enzymes such as SGOT, SGPT and ALP.

The results (Figures 6 & 7) of enzymatic antioxidants, such as SOD (superoxide dismutase) and CAT (catalase) shows a decrease in enzymatic antioxidants activities, An increase in the enzymatic antioxidants (SOD and Catalase) were also observed in mice. Carica papaya seed extract treated group showed an increase in the level of SOD and catalase of the liver. The maximum protection against hepatic damage was achieved with the aqueous extract at a dose of 400 mg/kg. The analysis of the liver homogenate of mice treated with *Carica papaya* seed showed a more or less normal level of the liver having reversed to a large extent, the hepatic lesions produce by paracetamol, almost comparable to the normal control groups. Other groups of Carica papaya have been reported to be a potent adaptogen (Gupta *et al*., 2008; Perfumi and Mattioli, 2007). A high content of bioactive compounds such as rosavin, rosin, ptyrosol and garlic acid was reported in aqueous extract of Carica papaya root and stem (Mishra *et al*., 2008).

This study revealed that the extract is rich in phenols and flavonoid, which clearly support the antioxidant and other related pharmacological properties including hepatoprotective activity of papaya. Hence, the phenolic compounds present in Carica papaya might be responsible for its observed hepatoprotective activity.

Conclusion

The research study reveals that the aqueous extract of Carica papaya seed has low toxicity level. Liver antioxidant markers were elevated significantly, while, the serum and lipid parameters were maintained at normal levels compared to control groups. Examinations of the liver showed that extract and silymarin have a protective role over the toxicity of paracetamol. The quantitative analysis for phenol and flavonoid showed a considerable amount of the presence of these antioxidant phytochemicals which could be responsible for imparting protection to the liver of mice. Hence Carica papaya could be one of the best sources of natural hepatoprotective agents.

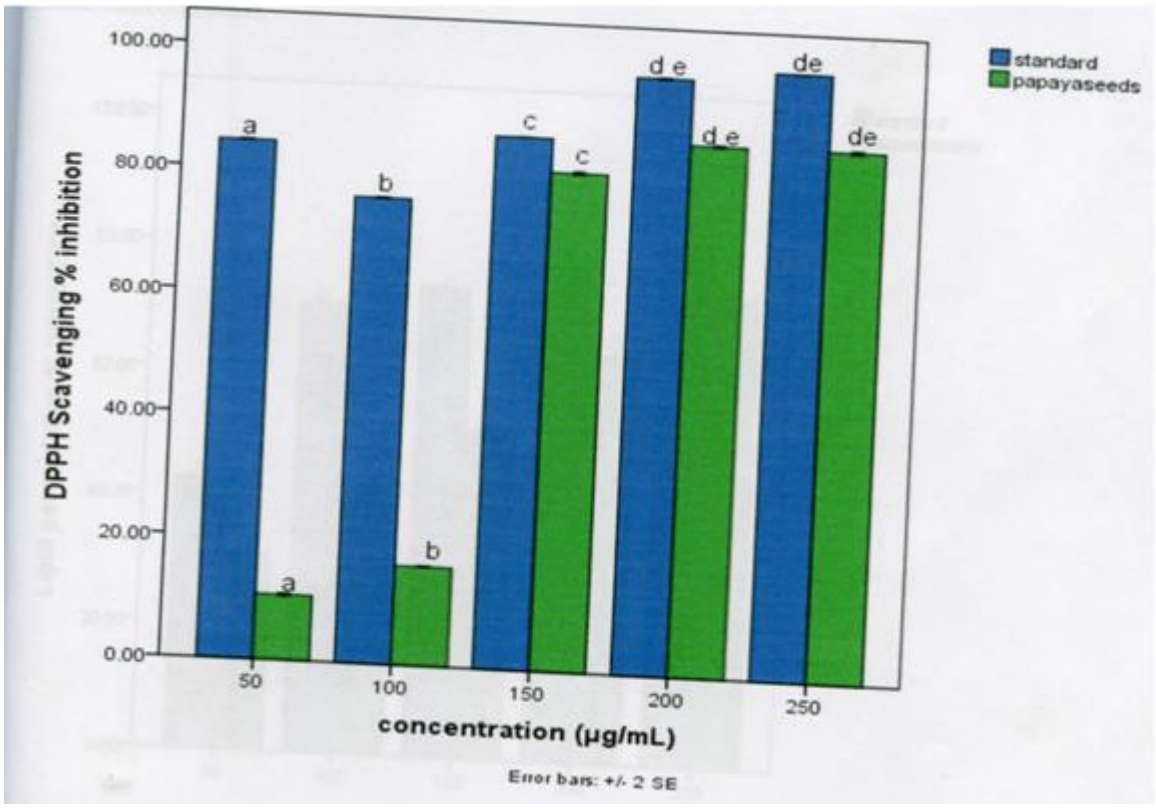

Figure1: Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity of Aqueous Extracts of *Carica papaya* Seed

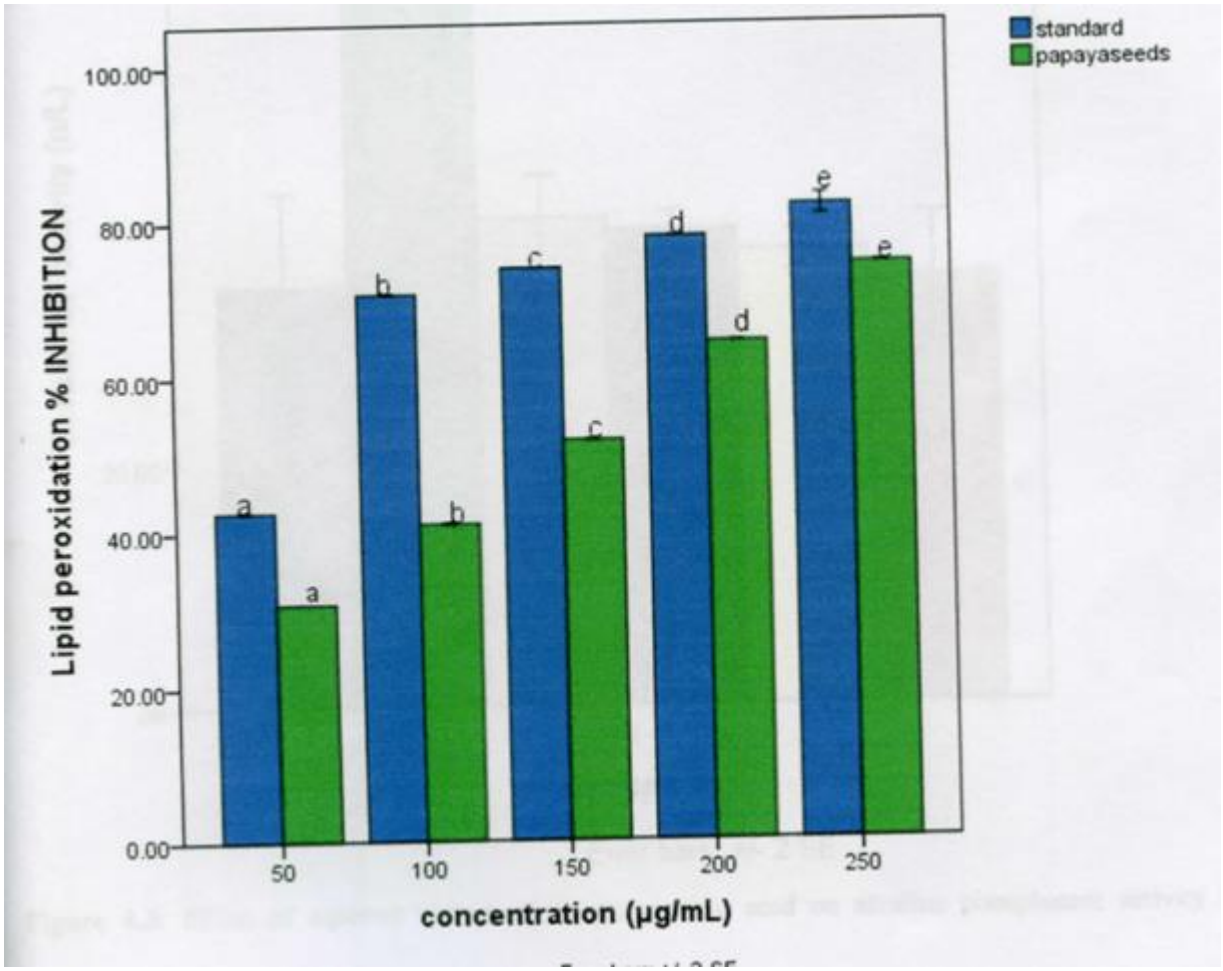

Figure 2: Inhibition of Lipid Peroxidation of Aqueous Extracts of *Carica papaya* Seed

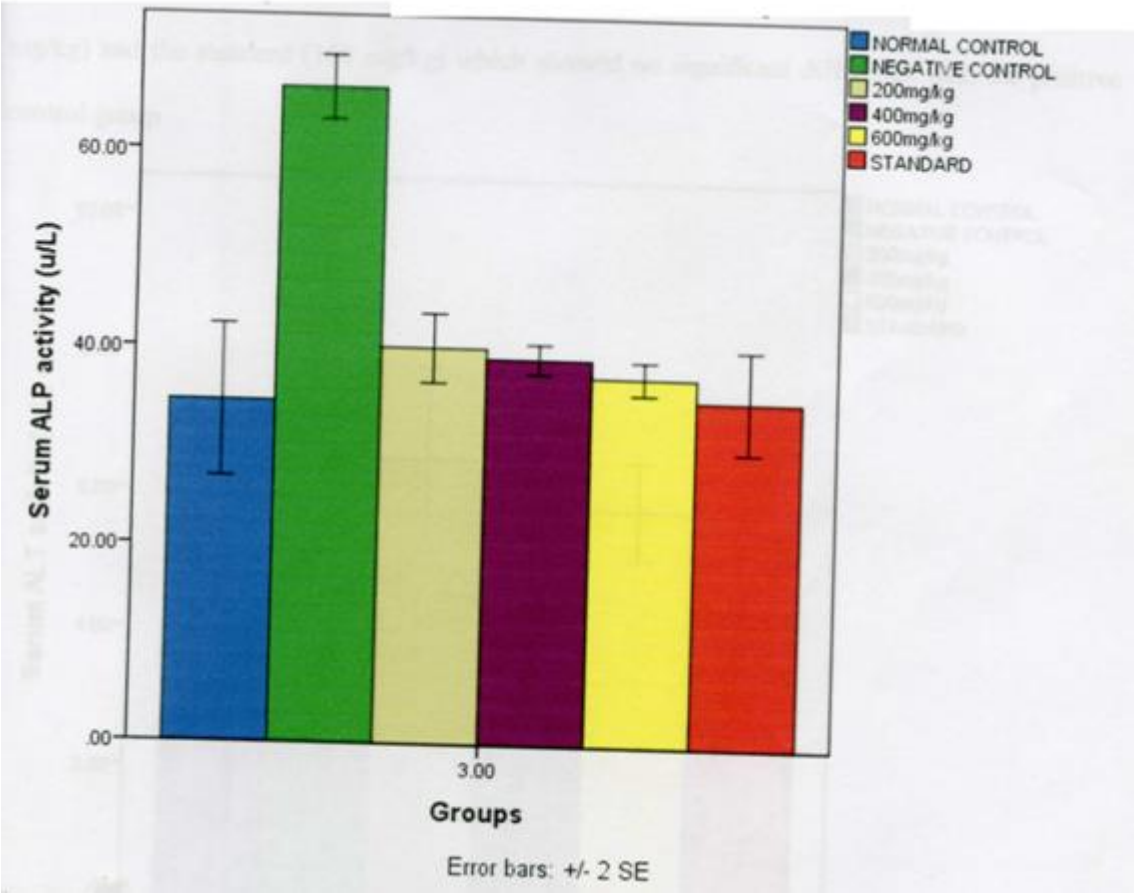

Figure 3: Effect of Aqueous Extracts of *Carica papaya* Seed on Alkaline Phosphatase Activity in Paracetamol induced hepatotoxicity in Mice

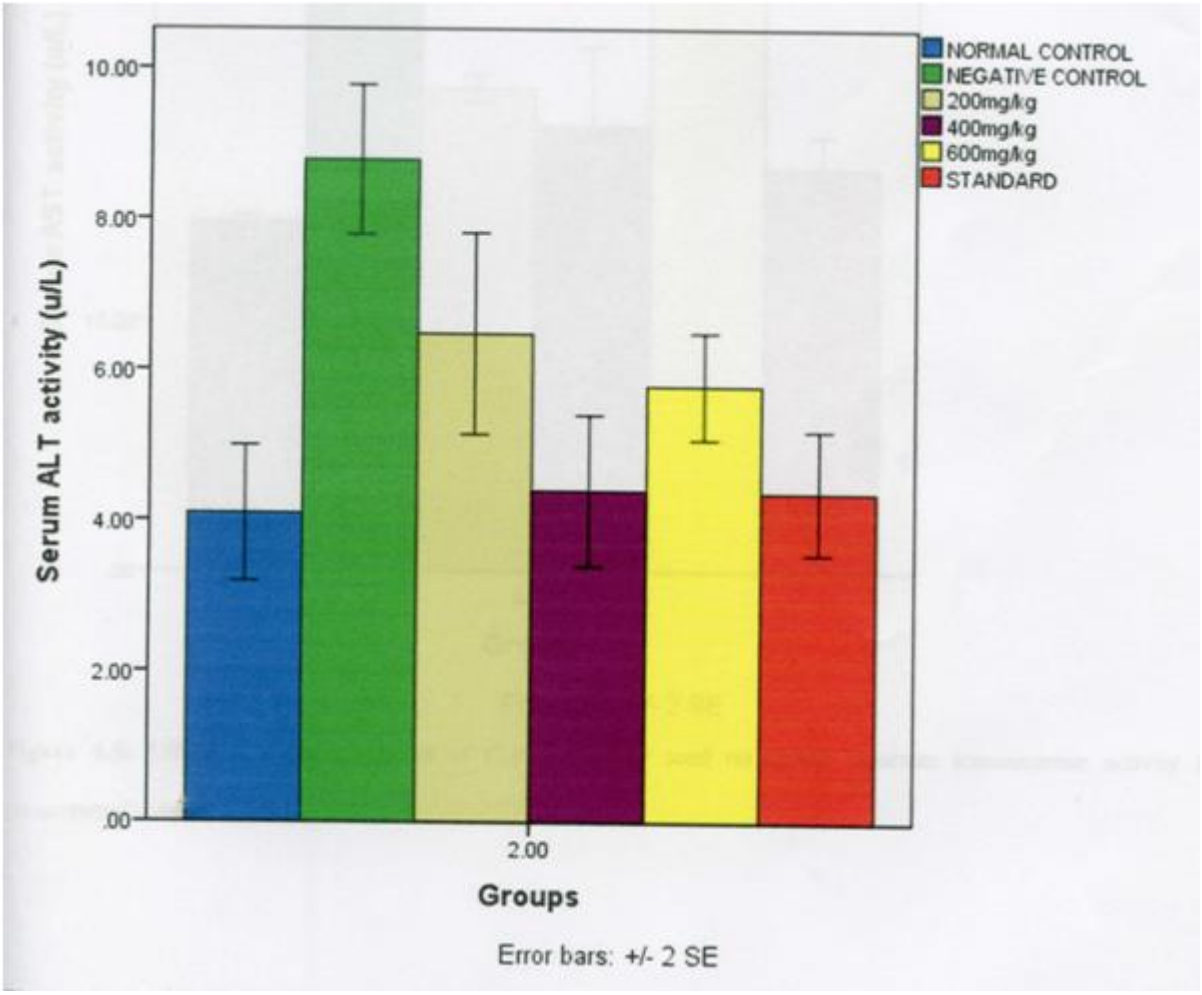

Figure 4: Effect of Aqueous Extracts of *Carica papaya* Seed on Alanine Transaminase (ALT) activity in paracetamol induced hepatotoxicity in Mice

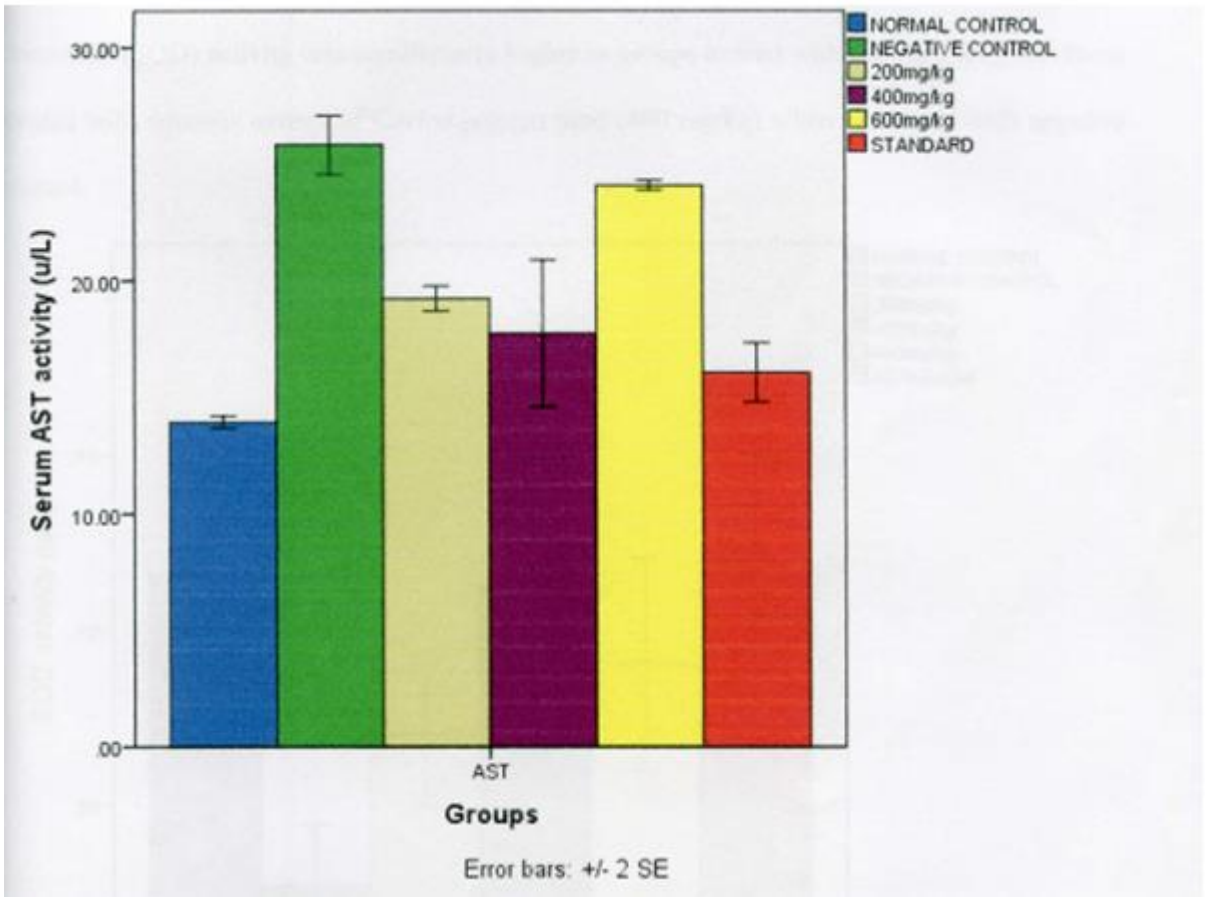

Figure 5: Effect of Aqueous Extracts of *Carica papaya* Seed on Aspaartate Transaminase (AST) activity in paracetamol induced hepatotoxicity in Mice

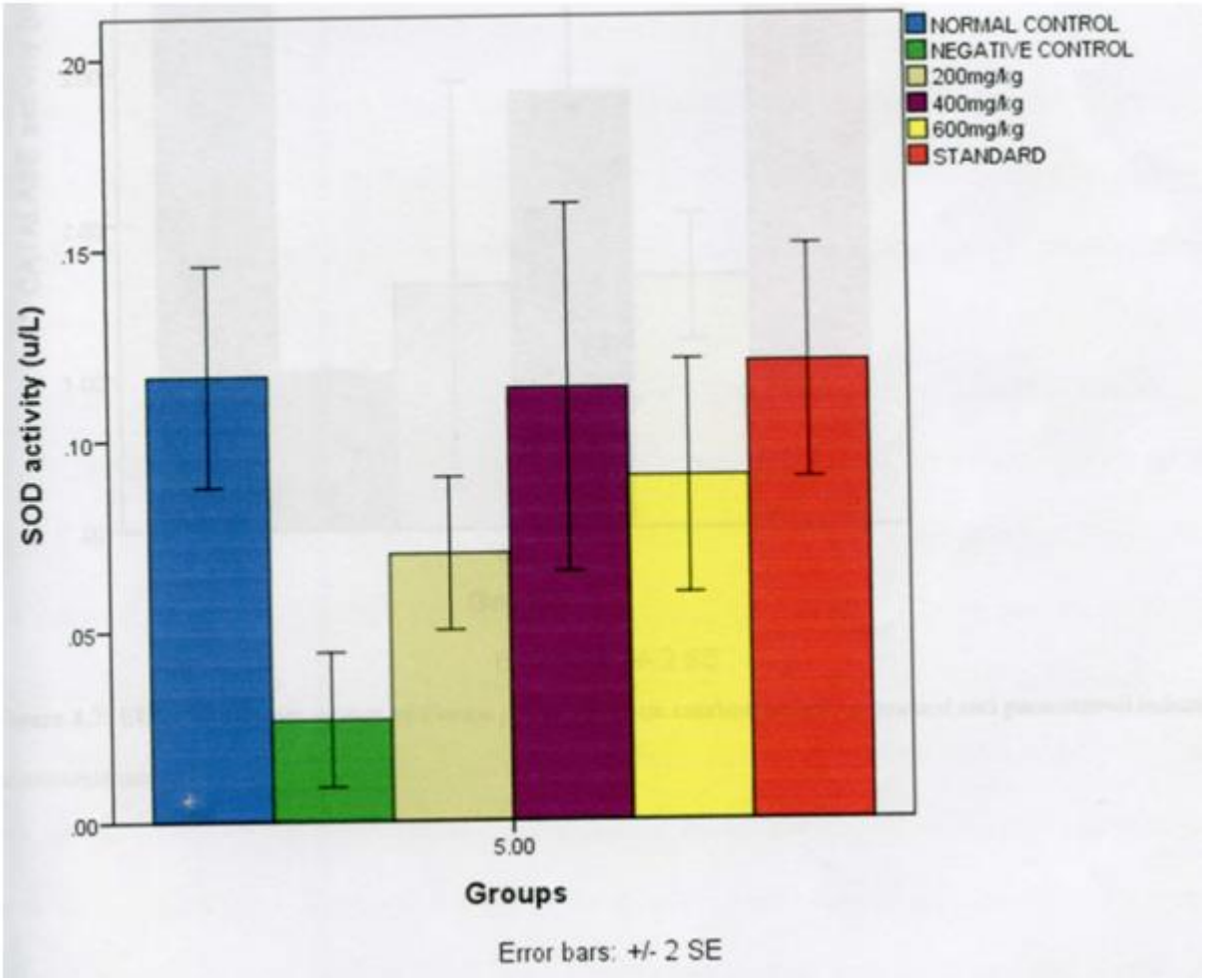

Figure 6: Effect of Aqueous Extracts of *Carica papaya* Seed on Super Oxide Dismutase (SOD) activity in paracetamol induced hepatotoxicity in Mice

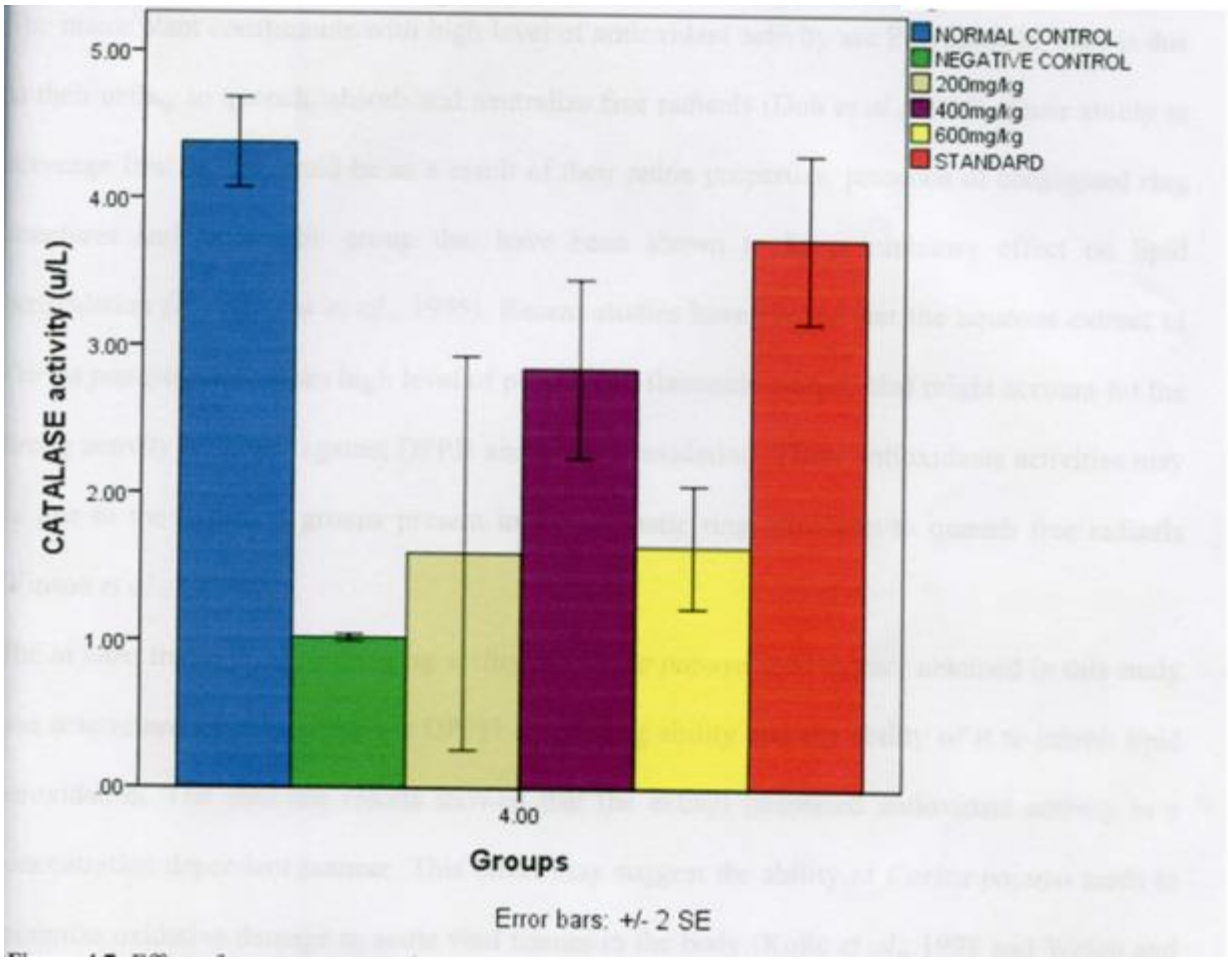

Figure 7: Effect of Aqueous Extracts of *Carica papaya* Seed on Catalase Activity in paracetamol induced hepatotoxicity in Mice

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