

Toxicological Evaluation of the Aqueous Extracts of Unripe Pawpaw (*Carica papaya* L.) Fruit in Experimental Rats

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Abstract

The toxicity of the aqueous extracts of unripe *Carica papaya* fruit in experimental rats was investigated. Thirty (30) mice were used for the determination of lethal dose (LD₅₀). The mice were divided into five groups and were orally administered with 5000, 2500, 1250, 625 and 312.5 mg/kg body weight of the unripe *C. papaya* extract for group 1, 2, 3, 4 and 5 respectively. Thirty (30) albino rats were also randomly divided into five groups (Group 1-5) of six rats each. Group one contained rats not treated (control), group two pawpaw extract (500mg/kg), group three (250 mg/kg), group four (125 mg/kg), group five (62.5 mg/kg). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity were determined. For LD₅₀ administration of doses for the mice in all groups caused no death or any observable signs of toxicity even after 48 hours of administration. There was slight increase in AST activity in the serum of rats given extracts of 500mg/kg and 250mg/kg. Also, there was little or no significant increase in ALT activity in 500mg/kg, 125mg/kg and 62.5mg/kg when compared to the control group. Increase in ALP activity in the serum of the rats in the groups was not significant when compared with the control. The overall results suggest non toxicity and suitability of aqueous extracts of *C. papaya* fruits for treatment of diseases.

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INTRODUCTION

Carica papaya is a member of the Caricaceae and is a dicotyledonous, polygamous, and diploid species [1] is commonly referred to as paw paw. It originated from Southern Mexico, Central America, and the northern part of South America. In Nigeria, it is also known by different local names depending on the tribe. For example, Yoruba's in the south west Nigeria, they call it "Ibepe", Hausas in the northern part of Nigeria call it "Gwanda", while the Igbos in the southern part of Nigeria call it "Okwere". The ripe fruit is edible and is usually eaten raw, without the peel and seed. The unripe green fruit (which is a rich source of vitamin A) can be eaten cooked, usually in curries, salads and stews [2]. It is now cultivated in many tropical countries such as Bangladesh, India, Indonesia, Sri Lanka, Philippines, and West Indies. The plant is native to tropical America [3] and was introduced to India in 16th century. It may be cultivated for its young leaves, shoots and fruits which are cooked as a vegetable or for its ripe fruit which is well known as a popular beverage [4] young leaves are rich in flavonoids (kaempferol and myricetin) [5], alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II) phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compounds (benzylglucosinolate) found in leaves [6]. The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender [7]. The seed is used for intestinal worms when chewed. Economically, *Carica papaya* is the most important species within the Caricaceae, being cultivated widely for consumption as a fresh fruit and for use in drinks, jams, jellies, ice-cream, pies and as dried and crystallised fruit [8,9,10]. Nutritionally, the ripe papaya fruit is a good source of calcium and an excellent source of vitamins A and C [11]. The aqueous extracts of the pawpaw fruits are taken orally for treatment of disease without consideration of toxic effect it could elicit. The present study therefore aims at evaluating the toxicity of aqueous extracts of *Carica papaya* fruits in Wistar albino rats.

MATERIALS AND METHOD

Chemicals

All chemicals and reagents used were of analytical grade and obtained from Sigma Chemical Company and used without further purification they include the following; Ethanol 100 ml, Mayer's reagent 0.5ml, Wagner's reagent 0.5ml, Distilled water, Tetraoxosulphate (VI) acid (H_2SO_4), 20% Potassium hydroxide (KOH), Fehling solution, Ferric chloride ($FeCl_3$), 10% potassium hydroxide solution, Sodium

hydroxide solution, Aluminum chloride (AlCl_3) and olive oil.

Plant Extraction

The freshly harvested *C. papaya* fruits were peeled, cut into smaller pieces and soaked with distilled water (100% w/v) for 72 h. After 72 h, the extracts were filtered with a sieve of 80 μm pore size. The filtrate was evaporated to dryness at 100 °C. The dried extract was weighed and stored at 4 °C for subsequent use.

Acute Toxicity Test (LD₅₀)

For LD₅₀, thirty (30) mice were used. The mice were randomly divided into five groups containing six mice per group. The mice were administered with wide spread doses of 312.5mg/kg, 625mg/kg, 1250mg/kg, 2500mg/kg and 5000mg/kg body weight of extract. The behavior of the mice were examined for about 24 hours for signs of toxicity and the (LD50) value, defined as “the statistically derived single dose of a substance that can be expected to cause death in 50% of the animals in an experimental group” was determined.

Chronic Toxicity Test

Thirty (30) wistar albino rats were used. After the rats have been acclimatized for 14 days, the weights were checked during distribution to ensure a weight difference per groups. The rats were fed with the unripe pawpaw fruit extract with the exception of group 1 (control group) which was fed with distilled water only. The rats were sacrificed after 7 days of administration of the unripe pawpaw fruit extract.

Group 1 was administered with distilled water only

Group 2 received 500mg/kg of the extract

Group 3 received 250mg/kg of pawpaw extract

Group 4 received 125mg/kg of pawpaw extract

Group 5 received 62.5mg/kg of pawpaw extract

Blood Collection and Sample Preparation

Experimental period lasted for seven days after which the rats were sacrificed and collection of blood sample was done using a syringe and collection of the vital organs was also done. Blood was centrifuged at 3500rpm for 10 min to obtain plasma. Serum was also obtained from the blood sample for tests which include; alanine transaminase, aspartate transaminase, alkaline phosphate to know the level of the toxicity of the unripe *C. papaya* fruit extract on the animals.

BIOCHEMICAL ANALYSIS

Preparation of Organs Homogenate

The organs homogenate was prepared in 6.7mM potassium phosphate buffer, pH (7.4) using the Teflon homogenizer. The homogenate was centrifuged at 10,000rpm for 10 minutes at 4 °C to obtain a clear supernatant which stored at 8 °C and used for biochemical analysis.

Determination of Serum ALT (Alanine Aminotransferase)

The alanine aminotransferase in the serum was determined by the method described by Salahudeen [12] with appropriate reagent kit (Randox laboratory, LTD.UK). Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine.

Determination of Serum AST (Aspartate Aminotransferase)

The aspartate aminotransferase in the serum was determined by the method described by Burtis and Ashwood [13] with corresponding reagent kit (Randox laboratory, LTD.UK). AST is measured by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenylhydrazine. The mixture was allowed to stand for exactly 20min at (20 – 25 °C). The absorbance of the sample (Asample) were taken against the reagent blank after 5minutes at wavelength of 546nm.

Determination of Serum ALP (Alkaline Phosphate)

Alkaline phosphate acts on the AMP- buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen which was measured at 546nm. It was mix thoroughly; incubated for 15 minutes at 37 °C. The absorbance of test were taken against blank at 590nm wavelength.

STATISCAL ANALYSIS

All data were analyzed using analysis of variance (ANOVA). Significance difference between the treatment mean was determined.

RESULTS AND DISCUSSION

Acute Toxicity Study of Aqueous Extract of Unripe Pawpaw (*Carica papaya*)

Table 1 shows the effect of aqueous extract of unripe *C. papaya* on the mice. The results of the acute toxicity study indicated that at 5000mg/kg dose of the extract was not a lethal dose for the mice. Therefore, the LD₅₀ at 5000mg/kg could not be determined. For LD₅₀ administration of doses for mice in all groups, unripe *C. papaya* caused no death or any observable signs of toxicity even after 48 h of administration.

Table 1: Effect of Unripe *Carica papaya* Extract on the Mice for Determination of LD₅₀

Dose (mg/kg)	Number of Rats	Number of Deaths
5000	6	0
2500	6	0
1250	6	0
625	6	0
312.5	6	0

Table 2: Determination of Chronic Toxicity of Aqueous Extracts of Unripe *Carica papaya*

	AST (U/mg)	ALT (U/mg)	ALP (U/mg)
GRP1(control)	59.50±0.577 ^c	62.25±12.447 ^b	97.25 ± 14.245 ^a
GRP2(500mg/kg)	86.78±6.241 ^a	65.80±8.766 ^b	72.6 ± 11.491 ^b
GRP3(250mg/kg)	66.88±7.586 ^b	55.67±6.569 ^b	75.17 ± 18.250 ^b
GRP4(125mg/kg)	78.56±8.762 ^a	65.80±8.929 ^b	72.50 ± 7.976 ^b
GRP5(62.5mg/kg)	76.75±10.124 ^a	81.00 ± 18.319 ^a	69.83 ± 23.987 ^b

Specific Activities of Serum Aspartate Aminotransferase (AST)

The specific activities of AST in serum of the experimental rats are shown in Figure 1 and Table 1. The administration of the aqueous extracts in varying doses increased the level of AST in the serum of the rats. Increase in AST in 500mg/kg, 250mg/kg, 125mg/kg and 62.5mg/kg shows that it has slight effect on the liver. Prolong intake of *Carica papaya* increases AST concentration in the body. AST is an enzyme associated with the liver parenchyma cells and it catalyses the transfer of an amino group between aspartate and glutamate.

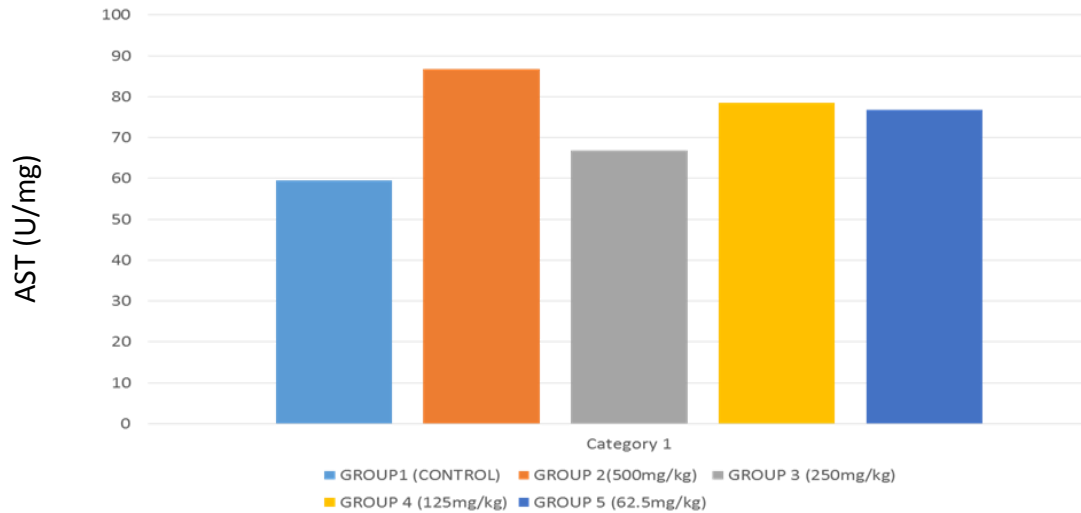


Figure 1: Specific Activities of Aspartate Aminotransferase in Serum of Experimental Rats

Specific Activities of Alanine Aminotransferase in Serum of Experimental Rats

Figure 2 and Table 1 show the level of ALT in the serum of the experimental rats. The result revealed that the level of ALT is non-dose dependent. There was a significant increase in ALT activities in groups treated with 500mg/kg, 125mg/kg and 62.5mg/kg when compared to the control group, but group 5 (62.5mg/kg) shows the highest increase in ALT. This implies that high or prolonged intake of unripe *C. papaya* caused increase of alanine aminotransferase in the body of the experimental animals but non-dose dependent.

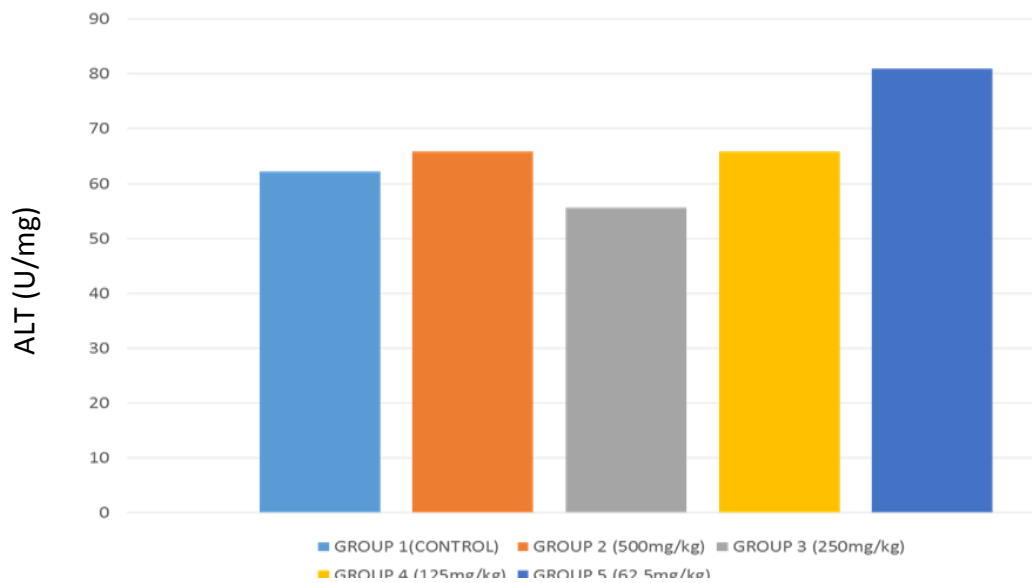


Figure 2: Specific Activities of Alanine Aminotransferase in Serum of Experimental Rats.

Specific Activities of Serum Alkaline Phosphatase

Figure 3 and Table 1 show no increase in ALP activity but it shows slight decrease in group 5 (62.5 mg/kg), this indicates that *C. papaya* when taken for a long time decreases ALP in the body of experimental animals.

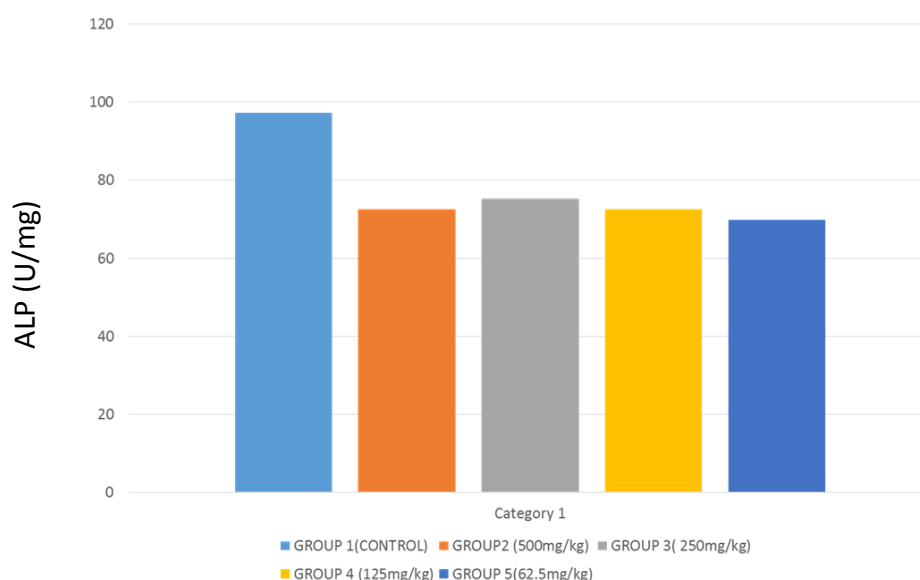


Figure 3: Specific Activities of Alkaline Phosphatase in Serum of Experimental Rats

CONCLUSION

The present study shows that *Carica papaya* fruits extract at 5000mg/kg administered orally to the mice had no acute adverse effect on the animals. Few alterations in the level of AST and ALT in the wistar rats were also observed. The unripe *C. papaya* fruits is good for human consumption because of its high nutritional and medicinal values.

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