

THE DETERMINATION OF ASPARTATE AND ALANINE AMINOTRANSFERASE ACTIVITY IN WISTAR RATS FED WITH MODIFIED DIET OF EDIBLE BLACK *CARICA PAPAYA* L. SEEDS

¹P. I. Imoesi, ¹L. Edemhanria, ²F. A. Esekheigbe and ³K. Okoyomoh

¹Department of Chemical Sciences (Biochemistry), Samuel Adegboyega University, Ogwa, Edo State, Nigeria.

²Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

³Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria.

E-mail: imoesi.peter@yahoo.co.uk

ABSTRACT

In recent times, research done on *Carica papaya* seeds has shown its nutritive and medicinal benefits without resort to possible side effect. However, studies have shown that the consumption of *C. papaya* seeds causes vascular contraction, infertility in female rats, reduced sperm counts, sperm cell degeneration and abortifacient properties. This study was designed to determine the enzyme assay level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the serum of wistar rats fed with modified diet of *C. papaya* milled seeds to ascertain possible hepatic or myocardia defect. A total of 15 wistar rats were divided into two groups comprising of test (8) and control (7) respectively. The test group were fed with modified diet of edible black *C. papaya* seeds and the control group with grower's marsh feed for a period of 9 weeks. The determination of aspartate aminotransferase activity showed the Mean \pm SEM of aspartate aminotransferase activity in the serum of the test group as 0.38 ± 0.03 and the control group 0.19 ± 0.10 respectively. Subsequently, alanine aminotransferase activity from the obtained test and control groups showed the Mean \pm SEM of alanine aminotransferase activity in the serum as 0.86 ± 0.02 and 1.25 ± 0.03 respectively. Furthermore, there is a significant ($P < 0.05$) difference between the control and test group based on the level of aspartate and alanine aminotransferase activity in the serum. In summary, transaminases are usually used in the diagnosis of liver damage and myocardia infarction. Therefore, the increase level of aspartate and alanine aminotransferase in the serum of Wistar Strain albino rats may suggest possible damage of hepatocyte cardiac muscles associated with the consumption of diet modified fed with *C. papaya* seeds.

Keywords: *Carica papaya* seed, Aspartate Aminotransferase, Alanine Aminotransferase, Medicinal Benefits, Myocardia Infarction, Hepatocellular Damage.

INTRODUCTION

Pawpaw (*Carica papaya* L.) is a widely consumed fruit in most West Africa countries especially Nigeria. Its medicinal usefulness has made it a relevant plant among herbal practitioners (Purseglove, 1985). This interesting fruit originated from Central America with both female and male plants existing separately (Purseglove, 1985). To reveal the freshly edible black seeds in the hallowed part of the fruit, *C. papaya* is cut opened. The edible black seed contains basically, benzyl-isothiocyanate (BITC), cyanogenes, carpaine and nicotine (Marfo, Oke & Afolabi., 1986). Though, different varieties of the edible black *C. papaya* seeds contain different percentage of oil. The Florida variety of the black seed contains 25.3% oil and Senegal variety with 28.8% oil respectively (Marfo, Oke & Afolabi., 1998; Marfo, Oke & Afolabi., 1986). Furthermore, the oil is made up of 70.7% unsaturated fatty acids and protein content of the defatted seed 44.4% (Marfo, Oke & Afolabi., 1986).

More importantly, BITC present in the crushed *C. papaya* seed is believed to possess anti-helminthic intestinal parasites (Godin & Spensley, 1971). In spite of the medicinal benefits of BITC on helminthic intestinal parasites, BITC has shown to possess negative effect on vascular contraction using canine carotid artery in *in vitro* model (Chinoy, Joshi & Ghosh, 1997). Furthermore, rat feeding trial with the crushed edible black seed of *C. papaya* has shown to cause enlarged liver and kidney impairment, a possible indication that the oil may contain toxic components (Chinoy, Joshi & Ghosh, 1997). Subsequently, the oral administration of *C. papaya* seed extract inhibits ovum fertilisation, reduced sperm cell count, sperm cell degeneration and induced testicular cell lesion (Lohiya et al., 1992; Chinoy & Padman, 1996). This observation further revealed the ability of *C. papaya* seed extract via oral administration as a potent male contraceptive drug for pharmaceutical industries (Lohiya et al., 1992). Furthermore, aqueous extracts (1mg/Kg body weight for 7 or 15 days) given orally stimulates infertility and irregular oestrous cycle in female Wistar Strain rats (Oderinde, 1998). The consumption of *C. papaya* fruits is widely believed to potentiate harmful properties capable of altering pregnancy associated with its abortifacient function (Purseglove, 1985; Lohiya et.al., 1992).

In spite of the medical degenerative effect of *C. papaya*, quite a number of its medical efficacy has been established. The oil extract from the black seeds may improve the viability of industries in countries where *C. papaya* is cultivated for papain production (Marfo, Oke & Afolabi, 1998). Equally, air-dried seeds administered orally, decreases dirofilaria immitis infection (Godin & Spensley, 1971; Marfo, Oke & Afolabi, 1998). Subsequently, the seed possesses anti-

inflammatory and analgesic effect, hence a possible treatment against stomach-ache and fungal infections (Ghany & Hoofnagle, 2005). More significantly, the black edible seeds possess sharp spicy taste and most often used as substitute for black pepper (Chinoy & Padman, 1996; Godin & Spensley, 1971). Based on the above remarkable benefits and possible side effects of *C. papaya* seeds, this study was aimed at investigating the possible side effect on the consumption of edible-black seeds of *C. papaya* through the determination of transaminase enzyme (aspartate and alanine aminotransferase) activities in wistar strain albino rats.

MATERIALS AND METHODS

Chemicals

Alanine and aspartate aminotransferase kits were product of Randox Laboratories Ltd. United Kingdom. Distilled water was obtained from Biochemistry Laboratory, Ambrose Alli University, Ekpoma, Edo State, Nigeria. Other chemicals used in the course of the research were of analytical grade from legitimate companies.

Sample Collection

The pawpaw was purchased in large quantity from Ohie Market, Uromi, Esan-North East Local Government Area, Edo State, Nigeria. The fruit was identified at the Herbarium Unit, Department of Botany, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The seeds were obtained through hand scrape from the opened fruit and dried with candescent bulb for 48hours before milling.

Feeds

The rats were fed through out the duration of study with grower's marsh purchased from a local distributor manufactured by Top Feed Ltd, Sapele, Delta State, Nigeria.

Experimental Animals

A total of 15 Male and Female Wistar Strain albino rats weighing between 100-150g were purchased from Physiology Department, Ladoko Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The animals were reared following approval from the Independent Ethical Committee on the Use and Care of Laboratory Animals of Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Experimental Design

The rats were housed in standard cages, and fed with grower's mash with portable drinking water for 10 days during the period of acclimatization. The rats were further grouped into two groups; group A; eight (8) tests and group B; seven (7) control respectively. The control group were fed with grower's mash and the test group were fed with modified milled *C. papaya* seeds (10g of milled *C. Papaya seeds* to 90g of grower's mash forming 100g of feed formation). Subsequently, 200g of the formulated feed was administered daily for 9 weeks and the leftover collected each day and weighed to ascertain the actual grams of feed consumed.

Assay Methods

At the end of 9 weeks, the rats were anaesthetised using a desiccator containing chloroform. The two groups were dissected and blood samples collected through cardiac puncture to sample containers without anticoagulant. Subsequently, the blood samples were centrifuged for 10 minutes at 3,000 g force and the serum was obtained from the whole blood using micropipette.

Determination of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

Spectrophotometric method was used for the assay of aspartate and alanine aminotransferase (Reitman & Frankel, 1957) with slight modification. 100 μ l (0.1ml) of the serum was added into test tubes with 500 μ l (0.5ml) of reagent 1 (buffer). The mixture was incubated for 30 minutes at 37°C. Subsequently, 500 μ l (0.5ml) of reagent 2 (2, 4- dinitrophenylhydrazine) was added and kept for 20 minutes at 25°C. The reaction was terminated with the addition of 5000 μ l (5.0ml) of 0.4Mol/L NaOH to the mixture. The blank was prepared with 500 μ l (0.5ml) of reagent 1 and 0.1 μ l (100 μ l) of distilled water. The absorbance was read at 546nm.

Statistical Analysis

Results were presented as mean \pm standard error of mean (SEM), ANOVA was carried out using the statistical software package SPSS for Windows (version 16). Finally the results were subjected to the t- distribution table at the corresponding degree of freedom ($P < 0.05$) to detect any significant difference between the Test and Control group respectively.

RESULTS

A total of 15 Male and Female Wistar Strain albino rats (8 as test and 7 as control) were used for the experimental analysis and the results showed a significant ($P < 0.05$) difference between the alanine aminotransferase activity of the control and test rats Table 1. Furthermore, a significant ($P < 0.05$) difference between aspartate aminotransferase activity was observed in the control and test group respectively Table 2.

Table 1: The Determination of Alanine Aminotransferase Activity (μl) in Control and Test Group of Wistar Strain Albino Rats.

Sample	Alanine aminotransferase (μl)	Alanine aminotransferase (μl)
	Control	Test
1	0.16	0.74
2	0.25	0.65
3	0.45	0.36
4	0.13	0.99
5	0.12	0.45
6	0.08	0.66
7	0.54	0.58
8	-	0.98
Mean \pm SEM	0.25\pm0.03	0.68\pm0.02

Values presented are Means \pm SEM of triplicate determination. (n=8).

Table 2: The Determination of Aspartate Aminotransferase activity (μl) in Control and Test Group of Wistar Strain Albino Rats.

Sample	Aspartate aminotransferase (μl)	Aspartate aminotransferase (μl)
	Control	Test
1	0.23	0.30
2	0.28	0.36
3	0.15	0.35
4	0.18	0.58
5	0.19	0.34
6	0.24	0.29
7	0.06	0.25
8	-	0.25
Mean \pm SEM	0.19\pm0.10	0.38\pm0.03

Values presented are Means \pm SEM of triplicate determination. (n=8).

DISCUSSION

The transfer of α -amino group of amino acids to α -keto acid is the basic role of transaminase enzymes (Marguerite, 2002). Transaminase enzymes usually are localised to all organs especially, the liver and heart respectively (Cheesbrough, 1998). Furthermore, low/high level of aspartate and alanine aminotransferase in the serum or plasma, serves as biomarker for hepatocellular or myocardia impairment (Cheesbrough, 1998). Subsequently, accelerated cell death, virus infections, hepatitis, necrosis and liver cirrhosis often leads to high level of transaminase enzymes in the bloodstream (Venkateshwarlu, Dileep, Rakesh & Sandhya, 2013). The significant increase in aspartate aminotransferase (AST) is usually associated with myocardia infarction with slight elevation in alanine aminotransferase (ALT) (Nelson & Cox, 2000). Conversely; hepatocellular disruption elevates both ALT and AST; however, high level of ALT activity is commonly observed in the liver compared with AST (Nelson & Cox, 2000).

The assay study of transaminase enzyme activities (aspartate and alanine aminotransferase) of wistar rats fed with diet modified feed of *C. papaya* milled edible black seeds showed a significant ($P < 0.05$) difference in both enzyme activities Table 1 and Table 2. The increase level of alanine and aspartate aminotransferase in the serum is usually based on conditions associated with injuries or diseases affecting the liver or heart leading to the release of hepatocellular enzymes into the bloodstream (Nelson & Cox, 2000). Further studies on ripe *C. papaya* seeds as shown mild ballooning necrosis of hepatocytes linked with prolonged dose and hepatocyte disruption due to carpaine (Paul & Ligha, 2015). This further justifies cytotoxicity of *C. papaya* seed owing to benzyl-isothiocyanate (BITC) serving as bacteriostatic, bacteriocidal and fungicidal (Paul & Ligha, 2015). The detoxification of BITC in the liver may potentiate hepatotoxic effect translating to hepatocellular impairment (Paul & Ligha, 2015; Hasim, Govardhan, Vengaiyah & Changamma, 2013).

Furthermore, the test group justified the abortifacient properties of *C. papaya* seed associated with its ability to cause infertility, irregular oestrous cycle, reduced sperm cell count, sperm degeneration and induced testicular cell lesion (Chinoy, Patel & Chawala, 1997; Lohiya et al., 1992; Oderinde, 1998). In similar report, *C. papaya* seed extract is shown to cause antifertility, anti-implantation and abortifacient properties (Chinoy, Dilip & Harsha, 2006). Similarly, unfermented extract of *C. papaya* seeds at high dose of 150mg/Kg is reported to cause reduced palatability, treatment-induced anorexia or systemic toxicity (Abdulazeez et al., 2009). A comparative observation based on the feeding

pattern between the test and control group further justifies *C. Papaya seed* with the ability to induce anorexic symptom as observed with the test group. Conversely, the high level of fertility observed with the control group potentiates the medicinal relevance of *C. papaya* seeds as potential drug in the development of male contraceptive (Harsha, Joshi & Chinoy, 1996).

The slight significant increase in alanine aminotransferase activity Table 1 between the control and test group suggest possible hepatic or other body organ damage. Concurrently, *C. papaya* seed is known to induce mild to severe metaplasia of hepatocytes, proliferation of kupffer cells, hepatic cell cirrhosis and elevation of serum hepatic enzymes (Dikibo et al., 2012). Similar research with rat fed with *C. Papaya seed* over a period of 3weeks (acute) and 6 weeks (chronic) presented hepatic damage on histological observation. These hepatic damage ranges from hepatic infarction, pkynosis and eosinophilic cells with cellular degeneration based on increased dose and prolonged ingestion of *C. papaya* seed extract (Dikibo et al., 2012).

Simultaneously, a significant increase in aspartate aminotransferase activity is observed in the serum of the test group compared with the control group Table 2. Consequently, the notable increase in the level of aspartate aminotransferase activity in the serum (Test group Table2) indicates possible cardiac and skeletal muscle damages (Ghany & Hoofnagle, 2005). Previous studies on rat feeding trials as demonstrated the possible role of *C. papaya* seeds as causative agent of enlarged liver, kidney and heart impairment (Paul & Ligha, 2015; Dikibo et.al., 2012). Photomicrograph of liver of albino rats fed with *C. papaya* seed extracts expressed mild ballooning, necrosis of hepatocytes (2weeks). Extended trial for a period of three weeks further expressed enlarged central veins and marked generalised ballooning necrosis of hepatocytes impairment (Paul & Ligha, 2015).

In contrast to rat feeding trials, *C. papaya* seed powder showed high mortality in fingering Tilapia *Oreochromis niloticus* at LC₅₀ (9hours) (Shreeja, Thadani & Salunke, 2014; Hasim et.al., 2013). Similar research with admissible toxicant concentration of 0.018mg/L- 0.18mg/L of pawpaw seeds to tilapia showed observable erratic swimming, loss of reflex, moulting, discoloration, air gulping, loss of scale and haemorrhage directly proportional to increase in aqueous extract of pawpaw seed concentration (Ayotunde & Ofem, 2008). Further studies on leaf extract of *C. papaya* showed toxicological effect on the liver of Sprague Dawley rats with significant elevation of liver enzymes (AST, ALT and alkaline phosphatase) (Adlin et.al., 2012).

P. I. Imoesi,

Contrary to the high level of toxicological effect and severe damage of *C. papaya* seeds on organs and tissues, other reports has shown the anti-hyperglycaemic potential on rats induced with streptozotocin (Venkateshwarlu et.al., 2013). Subsequently, studies on biochemical parameters such as total protein, HDL-cholesterol, AST, ALT and ALP showed elevated activity in non-dose dependent manner. In spite of the elevated increase in the above biochemical parameters, histopathological examination of organs specifically the liver revealed no morphological alteration (Adlin et al., 2012).

Generally, BI TC has shown to have negative effect on vascular contraction using a canine carotid artery *in vitro* model (Chinoy, Joshi & Ghosh, 1997). In spite of the quantity of the milled *C. papaya* seed (10g) to 90g of grower's marsh feed in the formulated feed, a significant increase of transaminase enzymes was observed in the serum of the test group. However, the direct consumption of *C. papaya* seed may pose severe health risk such as liver, heart and kidney impairment.

CONCLUSION

This study showed the health implication of *C. papaya* consumption through the determination of transaminase enzyme activities in the serum of albino rats. The significant level of aspartate and alanine aminotransferase in the serum suggest high level of liver and heart damage. It is clearly seen that the use of *C. papaya* seed as a medicinal herb may present potential health risks compared to its medicinal benefits. However, other parameters such as haematological indices, kidney function tests and total protein may be determined in future to ascertain a holistic conclusion on the effect of *C. papaya* seed consumption.

REFERENCES

- Abdulazeez, A., Mansurah, Ameh, D. A., Sani, I., Joseph, A. & Ambal, S. F. (2009). Effect of fermented and unfermented seed extracts of *Carica papaya* on pre-implantation embryo development in female Wistar rats (*Rattus norvegicus*). *Scientific Research and Eassy*, 4(10), 1080- 1084.
- Adlin, A., Noor, R. A., Siti, Z. H., Badrul, A. R. & Raja, H. R. et al., (2012). Repeated dose 28 days oral toxicity study of *Carica papaya* L. leaf extract in Sprague Dawley rats. *Molecules*, 17, 4326- 4342.
- Ayotunde, E. O. & Ofem, B. O. (2008). Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to Nile Tilapia *Oreochromis niloticus* (Linn 1757), Fingerlings. *Advance in Environmental Biology*, 2(3), 101- 107.

- Cheesbrough, M (1998). Clinical chemistry tests In: *District Laboratory Practice in Tropical Countries (Part 1)*. (pp. 358-362). Cambridge: University Press.
- Chinoy, N. J., Dilip, T. & Harsha, J. (2006). Effect of *Carica papaya* seed extract on female rat ovaries and uteri. *Phytother. Res.*, 9(3), 165- 169.
- Chinoy, N. J., Joshi, H. & Ghosh, P. (1997). Toxicity relation response of female albino rats treated with benzene and alcoholic papaya seed extracts. *Indian Journal of Environment and Toxicology*, 7(2), 62-64.
- Chiony, N. J. & Padman. S. (1996). Antifertility investigations of the benzene extracts of *Carica papaya* seeds in male albino rats. *Journal of Medicinal and Aromatic Plant Sciences*, 18(13), 489-894.
- Chiony, N.J., Patel, K.C. & Chawala, S. (1997). Reversible effects of aqueous extracts of papaya seed on microenvironment and sperm metabolism of cauda epididymis of rat. *Journal of Medicine and Aromatic Plant Sciences*, 19(3), 770- 723.
- Dikibo, E., Okpe, A. C., Turray, A. A., Onodagu, B.O., Ogbodo, L. A. & Oyadonghan, G. P. (2012). The effect of *Carica papaya* seeds on the histology of the liver in Wistar rats. *International Journal of Herbs and Pharmacological Research*, 1(2), 48- 54.
- Ghany, M. & Hoofnagle, J. H. (2005). Approach to the patient with liver disease. In: Dennis, L. K. (Ed.), *Principles of Internal Medicine*. (16th ed.). (pp. 1814-1815). New York: MacGraw-Hall.
- Godin, N.J. & Spensley, P.C. (1971). Oils and oilseeds, crop and product digest. *Journal of Food Sciences*, 43(1), 255- 256.
- Harsha, Joshi & Chinoy, N. J. (1996). Reversible antifertility effects of benzene extract of papaya seed on female rats. *Phytotherapy Research*, 10(4), 327- 328.
- Hasim, B. S., Govardhan, N. A., Vengaiyah, V. & Changamma, C. (2013). Impact of *Carica papaya* Linn. Seed extraction on some marker enzymes in male albino rats. *Int. J. Pharm. Sci. Rev*, 22(2), 214- 217.
- Lohiya, N. K., Goyal, R. B., Jayaprakash, D., Sharma, S. Kumar, M. & Ansari, A.S. (1992). Induction of reversible antifertility with a crude ethanol extract

P. I. Imoesi,

of *Carica papaya* seeds in albino male rats. *International Journal of Pharmacognosy*, 30(4), 308- 320.

Marfo, E. K., Oke, O. L. & Afolabi, O. A. (1986). Chemical composition of papaya (*Carica papaya*) seeds. *Food Chemistry*, 22(4), 259- 266.

Marfo, E. K., Oke, O.L. & Afolabi O. A. (1998). Nutritional evaluation of pawpaw (*Carica papaya*) and flamboyant (Denolix) seed oils. *Nutritional Report International*, 37(2), 303- 310.

Marguerite, W. C. (2002). Amino acid metabolism. In Thomas M. Devlin (Ed.), *Textbook of biochemistry with clinical correlations*. (5th ed.). (pp. 780-783). New York: Wiley-Liss.

Nelson, D. L. & Cox, M. M. (2000). *Principle of Biochemistry*. (4th ed.). (p. 183). New York: W. H. Freeman and Company.

Oderinde, O. (1998). Abortifacient properties of *Carica papaya* (Linn) seeds in female Sprague-Dawley rats. *Niger Postgrad Medicinal Journal*, 1(2), 163-172.

Paul, C. W. & Ligha, A. E. (2015). Methanol extract of ripe *Carica papaya* seed is not friendly with histology of albino wistar rats liver. *Asian Journal of Medical Sciences*, 7(2), 17- 21.

Purseglove, J. W. (1985). *Tropical crops: Monocotyledons*. (4th ed.). (p. 45- 51). London: Longman Press.

Reitman, S. & Frankel, S. (1957). Glutamic-Pyruvate transaminase assay by colorimetric method. *Am. J. Clin. Path*, 28- 56.

Shreeja, J., Thadani & Salunke, S. P. (2014). Effect of *Carica papaya* seed powder on liver and muscle of commonly cultured catfish, *Clarius batrachus*. *Cibtech Journal of Zoology*, 3(1), 86- 92.

Venkteswarlu, E. Dileep, P., Rakesh, K. R. & Sandhya, P. (2013). Evaluation of anti-diabetic activity of *Carica papaya* seeds on streptozotocin-induced type II diabetic rats. *Journal of Advanced Scientific Research*, 4(2), 38-41.

Reference to this paper should be made as follows: P. I. Imoesi, et al., (2015), The Determination of Aspartate and Alanine Aminotransferase activity in Wistar Rats Fed with Modified Diet of Edible Black *Carica Papaya* I. Seeds. *J. of Biological Science and Bioconservation*, Vol. 7, No. 2, Pp. 30 – 40.

Biographical Notes:

P. I. Imoesi: Assistant Lecturer, Department of Chemical Sciences (Biochemistry Option), College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa-Ehor Road, Edo State. B.Sc. Biochemistry- Ambrose Alli University, Ekpoma Edo State and M.Sc. Molecular Biology from the University of Hertfordshire, England, United Kingdom.

L. Edemhanria: Assistant Lecturer, Department of Chemical Sciences (Biochemistry Option), College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa-Ehor Road, Edo State. B.Sc. Biochemistry- Ambrose Alli University, Ekpoma, Edo State and M.Sc. Biotechnology from the University of Bedfordshire, England, United Kingdom.

F. A. Esekheigbe: Senior Lecturer, Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State. B.Sc./ M.Sc. Biochemistry- University of Benin. Ph.D. Ambrose Alli University, Ekpoma Edo State.

K. Okoyomoh: Assistant Lecturer, Science Laboratory Technology (Biochemistry Option), School of Applied Science, Federal Polytechnic Nasarawa. B.Sc. Biochemistry- Ambrose Alli University, Ekpoma, Edo State and M.Sc. Biochemistry Ahmadu Bello University, Zaria.
