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Effect of aqueous extract from unripe pulp of *Carica papaya* on transaminase activities in selected rabbit tissue of normal and alloxan induced diabetic rabbit

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ABSTRACT

Aqueous extract from mature unripe pulp of *Carrica papaya* was assessed for toxicity in selected tissues (Liver, Kidney, Stomach and Small Intestine) of normal and alloxan-induced diabetic rabbits using Aspartate and Alanine amino transfarase (AST and ALT) as marker enzymes. Rabbits weighing between 1.00 – 1.5kg were grouped into two major groups Normal and Diabetic and were given aqueous extract (5.% $^{w}/_{v}$) in appropriate doses (50mg/kg, 100mg/kg and 200mg/kg) once daily at 24hr intervals for 4 weeks. The results obtained showed that administration of extract to both groups resulted in a dose dependant inhibition of the activities of Aspartate and Alanine amino transferase in tissues like Liver and Kidney. No corresponding elevation in enzyme activities were obtained in the serum. However, reduction in AST activities ($p > 0.5$) was noticed in the Liver and Kidney of normal rabbits treated with the extract at doses of 50 and 200mg/kg body weight while there was significant reduction in both AST and ALT in Diabetic rabbits treated with the dosage 100mg/kg body weight. The serum values of aspartate and alanine amino transferase (AST and ALT) were low in all the animals studied probably because the released enzymes were not getting into the serum due to inhibition of enzyme molecules insitu. The results suggest the validity of the clinical trial of unripe pulp (aqueous extract in management of diabetes mellitus.

Key words: Aspartate and alanine amino transferase, *Carica papaya.*

INTRODUCTION

It has been documented by previous workers that biochemical parameters like tissue enzyme assay can indicate tissue or cellular damage which may not be picked up by conventional histological techniques (Ngaha, 1979, Akanji, 1986). Measurement of enzyme activity in tissue and body fluids provides an excellent aid in diagnosis (Coodly, 1970). In this study, Aspartate and Alanine amino transferase activities were assayed. They were selected based on their specifications in the cell such that any change in their activities is likely to give a strong indication of cellular impairment. Transaminases form an important link between protein and carbohydrate metabolism and are widely distributed in animal tissues (King, 1965). Aspartate transaminase (AST and Alanine amino transferase (ALT) have been widely used as a diagnostic 'markers' for heart diseases (myocardiac infection) and hepatic disorder respectively (Wills, 1985).

Carica papaya is of tropical American origin throughout it is now wide spread thought tropical Africa; it belongs to the group (*Caricaceae*). The plant can be monoecious, dioecious or hermaphroditic (Purseglove, 1968 and Janick, 1988) it's hypoglycaemic effect have been reported by Emeruwa, 1982; Duke, 1984b, Olagunju *et al*, 1995; Oloyede and Akanji, 2005.

Over the last three decades, chronic disorders such as diabetes have emerged as the major causes of adult morbidity and mortality in

Caribbean Island, Egypt, India, Nigeria, China, etc. (Ayensu, 1981; Sofowora, 1986; Atsushi and Miura, 1994 and Gulliford, 1994). Effort have been made to use pharmacological means in the management of diabetes mellitus. Also the support of national attention to herbal medicine as being of great importance to the health of individuals and communities (Gulliford, 1994). Many plants are used in the treatment of diabetes mellitus e.g. Momordica charantia, *Momordica balsamina, Carica papaya, Bridellic feruginea, Veronica amggelalina* among others (Sofowora, 1986).

Despite the therapeutic effect of these plants, it is necessary to identify the possible toxic effects. The aim of this study is to demonstrate possible manifestation of toxicity of aqueous extract from matured, unripe pulp of *Carica papaya* in normal and alloxan-induced diabetic rabbits.

MATERIAL AND METHODS

Plant material

Fresh, unripe, mature fruits of *Carica papaya* were obtained from National Horticultural Research Institute (NIHORT) Ibadan, Nigeria. The fruits were peeled, seed removed and the pulp cut into small pieces, sun-dried and finely powdered with an electric grinder. The powdered material was stored in properly sealed bottles at 10^0c in the refrigerator.

Chemical and Reagents

The chemicals and reagents used were all of analytical grade and were obtained from Sigma Chemical Company, St. Louis, Mo, USA. They were prepared in double glass distilled water.

Animal grouping

Twenty-four (24) adult rabbits of both sexes weighing between 1.0kg – 1.5kg were obtained from animal breeding unit of the Department of Veterinary Physiology, University of Ibadan, Nigeria. The rabbits were maintained on normal laboratory pellet diet and water *ad libitum*. Rabbits were randomly divided into six groups of four animals each. The animals in group I to IV were normal and healthy (non-diabetic) while the animals in group V – VI were made diabetic by the administration of alloxan monohydrates. Group I received orally sterile distilled water on daily basis. This served as the control. Animals in group II – IV received aqueous extract of pulp from unripe mature fruit of *Carica papaya* (5% $^{w}/_{v}$) at different doses (50, 100 and 200mg/kg body weight). Diabetic rabbits of group V were kept as diabetic control (untreated) and were administered sterile distilled water only. Rabbits in group VI were treated with aqueous extract of pulp equivalent to 100mg/kg body weight orally. Blood glucose levels of the animals were routinely determined.

Induction of diabetes in rabbits

Animals were made diabetic by infecting them intra-peritionally with 300mg/kg body weight of alloxan monohydrate freshly dissolved as 10% $^{w}/_{v}$ solution in distilled water. 72 hours after injection of alloxan, blood glucose level of all the surviving rabbits were determined using digital one touch glucometer. Rabbits with blood glucose level above 300mg/dl were considered diabetic and were employed in this study.

Preparation and administration of extract

Aqueous extract was prepared by soaking the powdered pulp of *Carica papaya* in distilled water (5% $^{w}/_{v}$). Thereafter, the suspension was filtered and the filtrate was kept in the refrigerator at 10^0c prior to analysis. Appropriate doses were calculated and administered to the rabbits orally for 4 weeks by gastric intubation using a feeding needle. The animals were kept under observation and were closely examined for signs of restlessness, excitement, intoxication and behavioural changes.

Serum preparation

The rabbits were anaesthetised in a jar containing cotton wool soaked in chloroform vapour. When they became unconscious, they were quickly brought out of the jar. The neck area was cleared of fur and skin to expose the jugular veins. These veins were then cut sharply with sterile scalpel blade and the rabbits were held downwards and allowed to bleed into clean dry corked test tubes. These was allowed to clot and left for 10mins at room temperature for serum formation. The serum was collected using Pasteur pipette after centrifugation at 3000rpm for 10mins. The clear supernatant was kept frozen until required (Akanji, 1986).

Preparation of tissue homogenate

The rabbits were sacrificed while under anaesthesia. They were quickly dissected and the tissues of interest (liver, kidney, small intestine and stomach) were removed and transferred immediately into ice-cold 0.25M sucrose solution. The kidneys were decapsulated and the small intestine and stomach were washed clean of metabolic waste. Each tissue was cut thin with a pair of clean sterile scissors and suspended in ice-cold 0.25M sucrose solution for homogenization $1.5^{w}/_{c}$ (Akanji, 1986) using Potter-Elvejhem Teflon homogenizer running at 1000rev/min. The homogenates were kept frozen overnight before being used for protein and enzyme assays. This was to ensure the maximum release of enzymes located on the cell organelles of previously unbroken cells (Ngaha *et al*, 1989).

Protein concentration and enzyme activities measurement

The protein concentration of serum and homogenates were determined using Biuret Method (Plummer, 1978). Method of Reitman and Frankel (1957) were employed to determine activities of Aspartate and Alanine aminotransferese. Aspartate aminotransferase activity was determined by measuring the quantity of oxaloacetate formed from Aspartate and α-ketoglutarate at 505nm, while Alanine aminotransferase activity was determined by measuring the quantity of pyruvate formed from alanine and α-ketoglutarate at 505nm. A Beckman model 21 digital UV spectrophotometer was used to measure the absorbance.

RESULTS

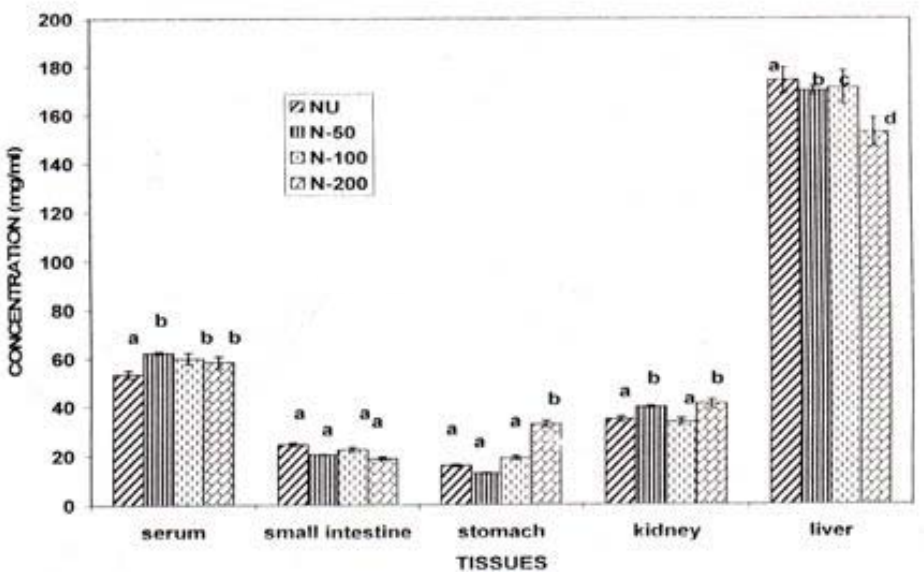
The activities of aspartate amino transferase (AST) in the various tissues of normal as well as diabetic rabbits following administration of aqueous extract of unripe pulp from *Carica papaya* are as shown in Table 1. There was a significant increase ($p > 0.05$) in enzyme activity in the small intestine and serum of normal rabbits administered 50 and 100mg/kg body weight of aqueous extract respectively. However, reductions in enzyme activity were noticed in the kidney and liver at doses of 50 and 200mg/kg body weight. In diabetic rabbits, significant reduction is as partate aminotransferase activity was observed in all tissues of animals treated with the dosage of 100mg/kg body weight.

Activities of alanine aminotransferase in selected tissues of normal as well as diabetic rabbits following administration of aqueous extract of unripe pulp from *Carica papaya* are as shown in Table 2. Significant difference ($P < 0.05$) in enzyme activity was observed in the selected tissues of normal rabbits when compared with control values. Significant reduction in serum was noticed only in animals administered 50 and 200 mg/kg body weight. In all other tissues, significant increase ($p > 0.05$) in enzyme activity was obtained with the

Table 1: Effect of oral administration of aqueous extract of Carica papaya on Aspartate transaminase activities (nM/min/mg protein) in some rabbit tissues*

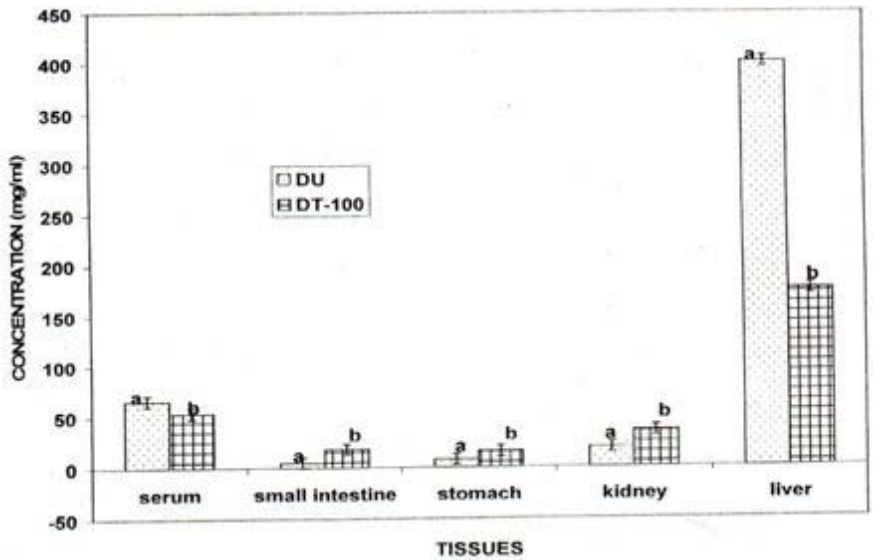
Group	Dose (mg/kg)	Serum	Small intestine	Stomach	Kidney	Liver
Normal untreated rabbits (control)	_	0.14-±0.03^{a}	2.86±0.25^{a}	0.46±0.03^{a}	0.47±0.01^{a}	5.00±1.99^{a}
Normal treated rabbits	II 50	0.17±0.01^{b}	3.71±1.11^{bc}	0.77±0.15^{b}	0.37±0.02^{b}	1.26±0.02^{b}
	III 100	0.14±0.01^{a}	2.43±0.63^{b}	0.82±0.23^{b}	0.39±0.07^{bcf}	4.07±0.59^{a}
	IV 200	0.16±0.01^{a}	3.25±0.19^{c}	0.17±0.01^{c}	0.34±0.01^{c}	4.17±0.77^{a}
V. Diabetic untreated rabbit	_	0.43±0.10^{c}	21.12±3.24^{d}	12.60±4.08^{d}	10.43±1.94^{d}	10.84±0.87^{c}
VI. Diabetic treated rabbits	100	0.30±0.01^{d}	2.54±0.01^{b}	0.34±0.01^{e}	0.39±0.01^{f}	7.98±1.55^{d}

*Results are means of four determinations ± SEM. Values with different notations are significantly different ($p<0.05$)



Results are means of four determinations ± SEM. Values carrying different notations are significantly different ($p<0.05$).
N = -50, -100 and -200: normal rabbits administered 50, 100 and 200mg/kg b.wt. respectively.
NU = Normal untreated rabbits

Fig. 1: Bar chart showing the effect of oral administration of aqueous extract of *Carica papaya* on total protein concentration (mg/ml) of some tissues of normal rabbits



Results are means of four determinations ± SEM. Values carrying different notations are significantly different ($p<0.05$).
DU = Diabetic untreated rabbits DT = Diabetic rabbits administered 100mg/kg b.wt. of extract daily

Fig. 2: Bar chart showing the effect of oral administration of aqueous extract of *Carica papaya* on total protein concentration (mg/ml) of alloxan-induced diabetic rabbits

dosage of 50mg/kg body weight. For diabetic rabbits administered 100mg/kg body weight of aqueous extract, significant reduction in alanine aminotransferase activity in serum and liver was obtained as opposed to increase in enzyme activity noticed in small intestine, stomach and kidney (Table 2).

The effect of oral administration of aqueous extract of unripe pulp of *Carica papaya* on total protein concentration of selected tissues of normal rabbit is as shown in Fig. 1. There was significant difference ($p < 0.05$) in the serum of rabbits treated with the extract when compared with control values. Significant reduction in protein concentration was however observed in the liver of normal animals administered 200mg/kg body weight of unripe pulp as opposed to increase protein concentration observed in the kidney. Fig 2 shows the effect of aqueous extract of unripe pulp of *Carica papaya* on total protein concentration of some tissues in alloxan-induced diabetic rabbits. Protein, decreased significantly ($p < 0.05$) in serum and liver of animals treated with 100mg/kg body weight of extract while increased concentration in protein was observed in small intestine, stomach and kidney when compared with control (untreated diabetic rabbits).

Table 2: Effect of oral administration of aqueous extract of Carica papaya on alanine transaminase activities (nM/mg protein/min) in some rabbit tissues*

Group	Dose (mg/kg)	Serum	Small intestine	Stomach	Kidney	Liver
Normal untreated rabbits (control)	_	0.09±0.01a	1.55±0.23a	0.52±0.02a	0.44±0.02a	0.94±0.01a
Normal treated rabbits	II 50	0.02±0.01b	1.71±0.43b	0.57±0.01b	0.50±0.02b	1.09±0.02b
	III 100	0.18±0.04c	1.47±0.74bc	0.52±0.01c	0.61±0.01c	1.12±0.02b
	IV 200	0.02±0.03b	1.88±0.24b	0.22±0.01d	0.60±0.01c	1.04±0.01b
V. Diabetic untreated rabbit	_	0.07±0.01d	1.02±0.31c	0.31±0.01e	0.33±0.01d	0.37±0.01c
VI. Diabetic treated rabbits	100	0.01±0.0001b	1.13±0.22c	0.64±0.01f	0.64±0.02e	0.10±0.01d

*Results are means of four determinations ± SEM. Values with different notations are significantly different ($p<0.05$)

DISCUSSION

In the present study, it was observed that Aspartate amino transferase (AST) activities in the liver of normal and diabetic rabbits were considerably reduced when compared with control values (Table 1). The decrease in activities of the enzyme could possibly be due to inhibition or inactivation of the enzymes insitu or leakage of enzyme into extracellular space. Aspartate transaminase is associated with the mitochondria and cytoplasm, alteration in its activity implies an alteration in the cytosolic content.

The mitochondrion is regarded as the power house of the cell and exposure of this organelle to assault of any form could imply cell impairment or even death. No corresponding elevation of aspertate transaminase activity was observed in the sera of the animals studied. This shows that there is no leakage from the organs into the blood. High serum levels of aspartate transaminase here been used as indicator for some forms of hepatic disease (Subbarao and Gupta, 1976). Elevated aspartate transaminase activity in the small intestine of normal rabbits administered aqueous extract may indicate increase in enzyme synthesis.

Significant reduction observed in alanine transaminase activities in the kidney of diabetic animals treated with aqueous extract (100mg/kg) (Table 2) when compared with control values might be explained in terms of the recretic activities in these organs (Amino and Giese, 1976). There may be an alteration of endothelial permeability (Brucechwatt, 1993) leading to the escape of abnormal quantities

of the enzyme into the extra cellular space. Elevated activities in the small intestine, stomach and kidney of diabetic animals treated with aqueous extract (100mg/kg) however signifies recovery. The serum values of aspartate and alanine transaminases (AST, ALT) were low in all the animals studied (Table 1 & 2) because the released enzymes were not getting into the serum probably due to inhibition of enzyme molecules insitu.

The high protein concentration observed in the liver of untreated diabetic group (Fig 2) suggests that there is an increased supply of amino acids for gluconeogenesis because in the absence of insulin, less protein synthesis occurs. In diabetes, the rate at which amino acids are catabolised to carbondioxide and water has increased. In addition, more amino acids are converted to glucose in the liver.

The decrease in protein concentration observed in the liver of diabetic animals treated with aqueous extract 100mg/kg unripe pulp (Fig 2) is similar to what is observed in normal control values (Fig 1). The result revealed the anti-diabetic effect of the extract which suggests that aqueous extract from unripe pulp may be repairing b cells in the pancreas and also stimulates production of insulin and inhibition of glucagons secretion. Hence there is increase in protein synthesis. In general, these results suggest the safe use of unripe pulp extract (aqueous) in management of diabetes mellitus.

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