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Acid phosphatase level in selected tissues of alloxan induced diabetic rabbits following administration of aqueous extract from unripe pulp of *carica papaya*

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ABSTRACT

The *in vivo* effect of oral administration of aqueous extract of unripe pulp *Carica papaya* on acid phosphatase activity in normal and alloxan induced diabetic rabbits was investigated. Both normal and diabetic rabbits were administered 50mg, 100mg and 200mg per kg body weight of the extract consecutively for two weeks. The effects of the aqueous extract of unripe pulp of *Carica papaya* in test animals were compared with control group which received distilled water alone. Acid phosphatase is a membrane bound marker enzyme. Kidney and liver levels of Acid phosphatase (ACP) increased in a dose related manner in normal rabbits. This may be due to *de novo* synthesis of enzyme molecules in these organs. Reduction of Acid phosphatase (ACP) activity in kidney of diabetic rabbits is due to leakage of enzyme through altered lysosomal membrane and the release of its component into extracellular environment. The reduced activity of this membrane bound enzyme suggest altered membrane structure and function. Since there is no corresponding increase in Serum enzyme activities, initiation of disease is not established. This observation confirms the validity of the therapeutic use of the extract in the management of diabetes mellitus.

Key words: Acid phosphatase *Carica papaya,* de novo synthesis *invivo.*

INTRODUCTION

Acid phosphatase is a lysosomal enzymes (Collins and Lewis, 1971) which has been found in different body tissues and fluids. It has been found to have multiple forms (isoenzymes). Five isoenzymes have been identified in normal serum (Grunding *et al,* 1965, Avila *et al,* 1989). In the identification of diseased organs, different inhibition of acid prostate isoenzymes have been employed (Panava *et al,* 1990) it has a very wide distribution and has been shown to be present in higher plants (Axelrod, 1947) animal tissues such as the prostate, breast, stomach, colon, thyroid, kidney and ovary. (Reiner *et al,* 1957, Atanka *et al,* 1975). It is also found in the placenta (Ahmed and King, 1959). Some of these acid phosphatases are organ specific (Albin *et al,* 1970). Its abundance in the kidney had been established by various workers (Perlmann and Ferry, 1942, Strauss, 1954, Shibko and Tappel, 1965, Avla and Convit 1973) it was shown by Davison and Conning, 1968 that acid phosphatase shows intense activity in the convoluted and straight parts of the proximal and distal convoluted tubules of rat kidney and moderate activitiy in all parts of the glomerulus.

Variations in serum acid phosphatase activity have been widely used in the diagnosis of many diseased states. For example, elevated serum enzyme levels have been reported in hyperthyroidism (Reuther and Webber, 1966) variety of non hematologic malignacies usually with metastateses (Deloroy *et al,* 1951, Gianfreda et al 1991) and liver diseases semen, a specific hereditary deficiency of lysosomal acid phosphatase

activity had also been shown to be very strong (William and Fishman, 1974). Acid phosphatase has also been observed in urine. Its activity in the urine is believed to originate from the kidney. The values in urine of normal male (human beings) are more than those in female due to secretion from prostate gland into the urine (Raab, 1968, Moss *et al,* 1995). Acid phosphates isoezymes produces a pattern typical of rheumatoid arthritis in synovial fluid. Kobayoshi *et al,* 1971, and Bull et al 2002 found elevated urinary acid phosphatase in patients suffering from chronic renal failure and inferred that the enzyme could be an important index of kidney disease.

Diabetes has long been a clinical model for general medicine. It is a catabolism disorder in which circulating insulin is virtually absent, plasma glucagon is elevated and the pancreatic B cell fail to respond to all insulinogenic stimuli. Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis, reduce hyperglucagonemia and bring the elevated blood glucose down (Volk and Arguilla, 1985) *Carica papaya* is cultivated for its fruits. Papain, the protedytic enzymes has a wealth of industrial uses. Fruit and seed extracts have pronounced bactericidal activity against staphyloccus aureus, Bacillus cereus, Esherischa acid etc. (Emeruwa, 1982). Its hypoglycemic effect have been reported by several workers, (Duke 1984b: Olagunju *et al,* 2005).

The aim of this study is to investigate the effect of unripe pulp on acid phosphatase level in alloxan induced diabetic rabbits.

MATERIAL AND METHOD

Plant material

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

Management of animals

Twenty four adult healthy rabbits of both sexes (local strain) weighing between 1.0 – 1.5kg obtained from the Animal breeding unit of the Department of Veterinary Physiology, University of Ibadan, Nigeria were used for the studies. The animals were kept in separate cages and were allowed free access to tap water and laboratory pellets. The cages were cleansed daily and washed every week. Animals were weighed weekly and their physical appearance examined.

Animal grouping

The rabbits were randomly divided into six groups of four animals each. The animals in group I to IV were normal and healthy (non – diabetic)

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

Group	Dose (mg/kg)	Serum	Small intestine	Stomach	Kidney	Liver
Normal untreated rabbits (control)	-	6.44±1.28^{a}	407.24±6.19^{a}	42.24±2.24^{a}	517.23±12.21^{a}	38.556±2.41^{a}
Normal	50	5.24±1.02^{a}	724.89±13.44^{b}	133.84±8.24^{b}	474.16±12.19^{b}	36.47±3.17^{a}
treated	100	7.54±1.11^{a}	507.41±11.29^{c}	85.27±5.64^{c}	1526.34±21.91^{c}	32.37±6.27^{a}
rabbits	200	1.58±0.34^{b}	347.68±5.19^{d}	78.37±7.39^{c}	2317.21±25.91^{d}	67.40±3.98^{b}

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

while the animals in group V – VI were made diabetic by the administration of alloxan monohydrate. Animals in group I served as control and they received distilled water only. The 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) respectively. Alloxan diabetic rabbits in group V were kept as diabetic control (Untreated) and were administered 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 injecting them intra-peritonially 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^{0}c 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.

Preparation of Serum

The animals were anaesthetised in a jar containing cotton wool soaked in chloroform. As soon as the rabbit become unconscious, they were sacrificed by cutting the jugular veins swiftly using sterile blade. The blood was collected into clean dry glass beaker and allowed to coagulate for 1hour. Pasteur pipette was used to remove the liquid (Serum) from the clot and collected into centrifuge tubes. Clear serum was then obtained by centrifugation at 3000rpm for 15 minutes. The samples were then frozen until required for analysis (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$^{w}/_{v}$ (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).

Table 2:Effect of oral administration of aqueous extract of Carica papaya Lactate dehydrogenase activities (nM/mg protein/min) in some diabetic Rabbit tissues*

Group	Dose (mg/kg)	Serum	Small intestine	Stomach	Kidney	Liver
Diabetic untreated rabbit	-	9.98±0.47c	821.20±2.43e	154.21±4.74d	371.37±11.7d	23.48±2.76c
Diabetic treated rabbits	100	2.67±0.79b	217.38±14.27f	188.81±11.91e	2764.24±19.23e	10.47±1.79d

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

Protein concentration and measurement of acid phosphatase

Enzyme and protein assays were carried out at conditions optimum for the present studies. All measurement were carried out using Spectronic 21 Spectrophotometer. Glass cuvetters of 1cm light path were used throughout. The protein contents of serum and homogenates were determined using Biuret Method (Plummer, 1978). Method of Wright *et al,* (1972) was employed to determine activity of Acid phosphatase. Acid phosphatese activities were determined by monitoring the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol and phosphoric acid at pH 10.1. The colour intensity was measured Spectrophometrically at 400nm.

Tissue dilution

The homogenates were appropriately diluted with ice – cold 0.25M, sucose solution before being used for protein and enzyme assays.

RESULTS AND DISCUSSION

Acid phosphatase was chosen and assayed based on its specific location in the cell such that any change in their activities is likely to give a strong indication of cellular impairment. It was previously reported that the site of injury to the cell could be correlated and determined by assaying the level of activities of "marker" enzymes in such tissues. The activity of acid phosphatise in selected tissues of rabbits following administration of different dose of aqueous extract of unripe pulp from Carica Papaya are as shown in Table 1 & 2. Significant reduction ($p<0.05$) in enzyme activity was observed in the liver of normal rabbits administered 100mg/kg body weight. While other tissues revealed, significant increase in activity when compared with control values. (Table 1) .

For diabetic rabbits administered 100mg/kg body weight of the aqueous extract, all the tissues studied demonstrated significant changes ($p<0.05$) in acid phosphatase activity when compared with diabetic untreated rabbit. (Table 2).

The reduction in acid phosphatase activity as recorded in kidney of normal animals treated with aqueous extract from unripe pulp (Table 1) may be due to leakage of enzymes through altered lysosomal membrane and the release of its components (Dean and Barret, 1976; Akanji, 1984). It was previously shown that compounds that labilize lysosomal membrane invariably lead to the escape of lysosomal enzymes into the extracellular environment and consequently loss of the enzymes from such tissues (Ngaha, 1982; Akanji, 1984). Since the activity of Acid phosphatase increased in normal and treated diabetic tissues, with corresponding low level of the enzyme in serum, it shows that their is no cellular leakage into extracellular environment. This results indicate the protective effect of *Carica papaya* fruit extract (i.e. not injurious to the body system).

It is expected that chemical changes that caused changes in membrane structure and function could also affect membrane bound or membrane associated enzymes. However, damage to lysosomal membrane have been reported to cause leakage of enzymes (Wills, 1985). Increased acid phosphatase activity observed in the small intestine, stomach and liver of normal rabbits administered aqueous extract of *Carica papaya* and the kidney of diabetic rabbits treated with aqueous extract (Table 2) might be due to increased *de novo* synthesis of enzymes molecules in these organs in response to assault by chemical agent (aqueous extract from unripe pulp). Indiscriminate increase in acid phosphatase activity may bring about a degree of autolytic damage to the cells, because of its hydrolytic nature, leading in some cases to cell death and necrosis (de Duve *et al,* 1962.)

Acid phosphatase was also found to be significantly low ($p<0.05$) in the serum of all the animals. This indicates that there was no leakage of the enzyme into the blood. Variation in serum acid phosphastase activity has been widely used in the diagnosis of many diseased states. For example, serum acid destruction (Ryman, 1978). In renal failure, the enzymes could also be an important index of kidney disease (Hoeder and Wilkinson, 1979). Generally this result indicates no cellular leakage into extracellular environment which shows that *Carica Papaya* fruit is not injurious to the body system

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