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Effects of *Carica papaya* on lactate and glutamate dehydrogenase activities in selected tissues of alloxan induced diabetic rabbits

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ABSTRACT

The activities of lactate and glutamate dehydrogenase in tissues like liver, kidney, stomach, small intestine and blood were investigated. Administration of aqueous extract of unripe pulp from *Carica papaya* resulted in a dose dependent inhibition of the activities of Lactate dehydrogenase. Significant reduction in Lactate dehydrogenase activity was observed ($p<0.05$) in the small intestine and kidney when compared with control values. However diabetic rabbits treated with aqueous extract (100mg/kg) showed increase in Lactate dehydrogenase activity when compared with diabetic untreated rabbits. In diabetic rabbits, Glutamate dehydrogenase activities reduced significantly ($p<0.05$) in serum, small intestine, stomach of animals treated with 100mg/kg body weight of the aqueous extract as opposed to increase in activity observed in the liver and kidney. The decreased level of Lactate dehydrogenase (LDH) and glutamate dehydrogenase (GDH) in the tissues of normal and diabetic rabbits could be related to enzymes in activation at both cellular and molecular levels. Since there is no corresponding increase in the serum. These results suggest the safe use and validity of the aqueous extract of unripe pulp from *Carica papaya* in the management of diabetes mellitus.

Key words: Lactate dehydrogenase, glutamate dehydrogenase, Alloxan and Diabetes.

INTRODUCTION

Lactate dehydrogenase is an anaerobic glycolytic enzyme which catalyses the reversible conversion of lactic acid to pyruvate in the presence of nicotinamide adenine dinucleotide. (NAD) (Rockwell, 1985). Lactate dehydrogenase is a tetramer of molecular weight 140, 000. It is a zinc metalloenzyme found in the soluble portion of the cell and it has a very wide distribution in animal tissue including skeletal muscle, kidney, liver and heart and it has long been shown to be located in the extractable fraction in these tissue (Johnson, 1960: Babson and Babson 1973). The human enzyme is inhibited by mercuric ions and p-chlomecuribenzoate, the effect being reversible by cysteine and glutathione. Dilute iodine solution, oxalic and oxanic acids also inhibit the enzyme (Neilands, 1954). Lactate dehydrogenase is a cytoplasmic enzyme and is present in the kidney (Rosalki and Wilkinson, 1959) and its distribution in various parts of this organ has been described for rats (Bonting *et al*, 1960; Mattenheiner, 1968: Ponc *et al,* 2001).

Human sera contain several lactate dehydrogenase isoenzymes and their relative proportions changed significantly in certain pathology conditions (Rodwell, 1985). An elevated level of LDH activity has been observed in pathological conditions in serum and other biological fluid, examples are myocardial infarction, leukaemia, anaemia and liver disease (Bodansky, 1961). In myocardial infarction, the concentration of serum

lactate dehydrogenase rises within 24 hours after the infarction and returns to normal range within 5-6 days. (Rodwell, 1985). In liver diseases, release of lactate dehydrogenase activity can vary, but very high levels have been reported in serum during infective hepatitis, infectious mononucleosis and toxic jaundice (Hsieh and Blumenthal, 1956, Wroblewski *et al*, 1956).

The presence of lactate dehydrogenase activity has previously been shown in human urine (Klaus, 1958; Rosalki and Wilkinson 1959). Urinary lactate dehydrogenase activity has been found to be elevated in infective hepatitis, infectious mononucleosis and toxic jaundice (Wrobleski et al, 1956). The elevated enzyme activity in the urine as reported by Wright and Plummer (1974) resulted from toxic renal damage which is accompanied by an impairment of tubular reabsoption capacity.

Glutamate dehydrogenase is a mitochondrial matrix enzyme that catalyses the reversible oxidative deamination of glutamate to ketoglutarate plus free ammonia using either NAD^+ or $NADP^+$ as a cofactor. The enzyme is expressed at high levels in liver, brain, pancreas and kidney, but not in muscle (Jie *et al*, 2002). Smaller amounts are found in adipose tissue, lungs, lymph nodes, gastric mucosa and myocardium (Zimmerman and Seeff 1970). Glutamate dehydrogenase is a useful biochemical indicator of injury to michondria since it is confined to it (Hanley *et al,* 1966.)

Glutamate dehydrogenase has about six isoenzymes. It is inhibited by metal ions such as $Zn^{2+,}$ Ag^+ and Hg^+ and several chelating agents as well as L-thyroxine (Aldelstein and Vallee, 1958). It is activated by Adenosine diphosphate (ADP), which reserves the inhibition caused by thyroxine and diethylstilbestrol (Midrand and Gireville, 1962; Katchmer, 1970). Allosteric control of mammalian GDH activity by positive effectors (e.g. ADP and leucine) and negative effectors (e.g. GTP) has been studied extensively (Jie *et al*, 2002). There is virtually no glutamate dehydrogenase activity in normal serum. Moderate elevations of serum levels are found in most cases of acute hepatitis in some forms of hepatic necrosis, and in 70 to 90% of patients with cirrhosis (Zimmerman and Seef, 1970). The enzyme activity is also elevated in the latter stage of chlorpromazine jaundice (Zimmerman and Seef, 1970), and in extraheptic obstruction (Schmidt *et al*, 1963).

It has previously been shown that the site of injury to a cell could be determined by assessing the level of activities of 'marker' enzymes in such cell (Ngaha, 1981).These enzymes and their isoenzymes also have a high organ specificity, which increases their diagnostic importance. Moreover, the morphological changes in cell injury are known to become apparent only after some critical biochemical systems within the cell have been deranged different parts of the cell possess qualitative differences in their enzyme elements. Thus elevation of marker enzymes in the serum or urine can lead to the identification of the site of injury to the cell (Ngaha, 1981).

The aim of this study is to investigate the toxic effect of aqueous extract of unripe pulp from *Carica papaya.*

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, 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 10^0c.

RESULTS

The activities of lactate dehydrogenase in the various tissues of normal as well as diabetic rabbits following administration of aqueous extract of unripe pulp from *Carica papaya* are shown in Table below. Significant reduction in enzyme activity was observe ($p<0.05$) in the small intestine and kidney at the various dose levels used, when compared with control values (Table 1). However, no significant change was noticed in serum of animals treated with 200mg/kg body weight. For diabetic rabbits administered 100mg/kg body weight of the aqueous extract, all the tissues studied demonstrated significant increase in lactate dehydrogenase activity

when compared with diabetic untreated rabbits (Table 2).

The activity of glutamate dehydrogenase in selected tissues of rabbit following administration of different doses of aqueous extract of unripe pulp from *Carica papaya* are as shown in (Table 3). Significant decrease ($p<0.05$) in enzyme activity was observed in the serum of animals treated with 200mg/kg body weight. However, significant reduction ($p<0.05$) was noticed in all other tissues. In diabetic rabbits, there was significant difference in enzyme activity when compared with diabetic untreated animals. The enzyme activity reduced

Table 1: 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
Normal untreated rabbits (control)	_	29.53±4.87^a	199.91±21.91^a	123.33±14.49^a	1009.66±29.69^a	53.08±3.58^a
Normal	50	37.00±1.04^b	154.21±4.11^b	177.40±1.27^b	525.00±4.26^b	29.07±2.17^b
treated	100	23.64.±2.24^a	134.71±3.91^c	124.41±5.21^a	179.34±13.90^c	66.79±3.49^c
rabbits	200	24.79±2.19^a	137.02±1.24^c	196.24±9.41^c	274±3.95^d	116.98±1.44^d

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

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	_	0.33±0.01^c	10.93±1.24^d	7.42±1.45^d	7.66±2.96^e	3.24±0.76^e
Diabetic treated rabbits	100	28.37±3.84^a	175.23±5.77^e	117.33±3.23^a	421±5.28^f	15.24±1.17^f

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

Table 3:Effect of oral administration of aqueous extract of *Carica papaya* Glutamate dehydrogenase activities (nM/mg protein/min) in some diabetic Rabbit tissues*

Group	Dose (mg/kg)	Serum	Small intestine	Stomach	Kidney	Liver
Normal untreated rabbits (control)	_	0.50±0.05^a	6.17±1.93^a	14.43±1.28^a	71.15±15.00a	11.77±1.57a
Normal	50	0.13±0.08^b	0.85±0.07^b	24.87±4.87^b	25.57±4.01^b	9.82±0.37b
treated	100	0.36±0.04^c	1.27±0.34^c	18.89±2.23^{bd}	71.11±13.60^a	12.57±0.43a
rabbits	200	0.09±0.005^d	1.16±0.31^c	3.94±1.04^c	47.23±7.30^c	5.44±1.00c

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

significantly in serum, small intestine and stomach of animals treated with 100mg/kg body weight of the aqueous extract as opposed to increase in glutamate dehydrogenase activity observed in the liver and kidney (Table 4).

Table 4: Effect of oral administration of aqueous extract of *Carica papaya* Glutamate dehydrogenase activities (nM/mg protein/min) in some diabetic Rabbit tissues*

Group	Dose (mg/kg)	Serum	Small intestine	Stomach	Kidney	Liver
Diabetic untreated rabbit	_	0.70±0.07e	18.14±5.24d	16.32±0.91d	15.11±0.52d	4.72±0.32c
Diabetic treated rabbits	100	0.38±0.09f	2.10±0.06e	8.56±1.41e	41.28±3.39c	10.47±0.04d

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

DISCUSSION

The activity of lactate dehydrogenase was found to increase in liver, kidney and small intestine of diabetic animal treated with aqueous extract. This may be due to increased *de novo* synthesis of the enzyme molecules. Also the increase in lactate dehydrogenase activity implies that the part of the cell (cytosol) for which the enzymes serves as 'marker enzyme' have been affected by the extract. Significant reduction observed in the small intestine and kidney of normal animals administered the extract (Table 1) may be attributed to the destruction of the plasma membrane and hence efflux of cytoplasmic content into the extra cellular space.

Reduction of glutamate dehydrogenase (GDH) activity observed in liver and kidney of normal rabbits treated with aqueous extract from unripe pulp (Table 3) gave an indication to the level of destruction of the cellular organelles which signifies damage to the mitochondria. Glutamate dehydrogenase (GHD) being a mitochondria enzyme is a useful biochemical indicator of injury of the organelle (Hanley et al, 1966). Elevation of glutamate dehydrogenase activities observed in the liver and kidney of animals treated with aqueous extract from unripe pulp (Table 1) when compared to control values may indicate *de novo* synthesis of the enzyme molecules. The decease in serum glutamate dehydrogenase therefore imply that the extract have no effect on the liver, rather they may be inactivated at the cellular level or as a result of the enzyme being inhibited by the extract. It is possible that the low serum values may be due to released enzymes not getting into the serum due to inhibition of enzyme molecules *in situ.*

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