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Biochemical effect of Aqueous Carica papaya Seed and Leaf Extracts on Serum Biochemistry of Alloxan Induced Diabetic Rats

*Ojo Rotimi Johnson[1] *Seriki Samuel[2] *Wang Davou Elnathan[1]
*****Mhya Hyelni John[1]
[1]*Department of Biochemistry, Faculty of Science and Technology, Bingham University Karu Nasarawa state, Nigeria*
[2]*Department of Physiology, College of Medicine Bingham University Karu Nasarawa state, Nigeria*

Abstract: *The cost of administering modern anti diabetic drugs is usually beyond the reach of most people in the low income group especially those in the developing world where the disease is on the increase. This has led to the current shift to the use of preparations from various parts of plants because of the current increase in the knowledge of their toxicity, side effects, active constituents and doses. This study was conducted to compare the effects of the aqueous Carica papaya seed and leaf extracts on serum biochemistry of alloxan induced diabetic rats especially glucose level. Male Wister rats weighing 150-200g were induced with single freshly prepared alloxan monohydrate (150 mg/kg body weight).Diabetes was confirmed after seven days in alloxan-induced rats showing fasting blood glucose levels ≥ 200mg/dl. The diabetic rats were randomly allocated into three experimental groups which received Seed extract, leaf extract or normal saline depending on the group. The extracts were administered orally for twenty-eight days after which the animals were sacrificed and blood samples were collected for Biochemical analyses. The results showed that both extracts have significant hypoglycaemic, hepatoprotective and nephroprotective effects although extract of the seed proved to be more potent than that of the leaf.*

Keywords*: alloxan, diabetics, Carica papaya, kidney, liver; rats.*

I. Introduction

Development of drugs for the treatment of Diabetes mellitus, one of the major health problems in the world requires experimental studies using diabetic and anti-diabetic agents[1,2,3,4,5,6].One of such potent diabetic agents that has being used for this purpose in experimental animals is alloxan[2].

Alloxan is a chemical substance that exerts its diabetogenic action when administered intravenously, intraperitoneally or subcutaneously. When administered, alloxan selectively destroys the insulin-producing beta-cells found in the pancreas leaving the non-beta cells and other endocrine and non-endocrine islet cell types intact[7,8].The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphydryl (-SH) groups, inhibition of glucokinase and generation of free radicals [9,10].Alloxan injection induce an insulin-dependent type I like diabetes syndrome and all the morphological features of beta cell destruction that are characteristics of necrotic cell death[7,11,12].The dose required for inducing diabetes depends on the animal species, route of administration and nutritional status[13].Alloxan is non-toxic to the human beta-cells, even in very high doses, this may be due to the differences in glucose uptake mechanisms in humans and rodents[2,10,14,15,16].

The hypoglycemic effect of Carica papaya leaf and seed extracts has been widely reported [17, 18]. Carica papaya (pawpaw) belongs to the family of Caricaceae. It is a large perennial herb with a rapid growth rate[17,18,19,20].It contains broad spectrum of chemical compounds including, polysaccharides, vitamins, minerals, enzymes, proteins, alkaloids, glycosides, fats and oils, lectins, saponins, flavonoids and steroids[21] , the active chemical components of Carica papaya include; alkaloid, α-carpaine, β-D-glucosides, β-sitosterol, papain, choline, carotene, riboflavin, vitamin C, phenyethyl- β- D- glucosides,[21]. The present study was therefore carried out to evaluate the effect of the aqueous extracts of Carica papaya leaf and seed in alloxan-induced diabetic rats.

II. Materials And Methods

2.1 Collection of Plant Samples

Plant samples were obtained from Masaka market in Nasarawa State and identified in Biological Sciences Department, Bingham University karu, Nasarawa State Nigeria.

2.2 Preparation of Carica papaya Leaf and seed extracts

The fresh leaves collected were sterilized using 30% alcohol and dried under a shade. The dried leaves were then ground and passed through a sieve. The powdered sample of a known weight was then boiled in hot water for 30 minutes to mimic the traditional procedure used by local people after which it was filtered using a piece of white cotton gauze. The filtrate was evaporated to dryness at 40^{o}C producing brown colour solid residue. The residue was weighed and stored in air and water proof container, kept in a refrigerator at 4^{o}C, from which fresh stock was made when required.

To Prepare Carica papaya Seed extract, mature, unripe Carica papaya fruits were cut into pieces and the wet seeds were removed and rinsed in tap water two times. The seeds were air-dried at room temperature for 4 weeks and pulverized into fine powder using a grinder, 80 g of the powdered Carica papaya seeds was boiled in 1000cm^{3} of distilled water for 30 minutes after which it was filtered using a piece of clean white cotton gauze. The filtrate was evaporated to complete dryness at 40^{o}C, producing a fine sweet smelling and chocolate colour solid residue [yield: 24.0% (w/w)]. The extraction process was repeated 4 times and the solid residue was weighed after extraction and stored in an air and water proof container which was kept in a refrigerator at 4^{o}C. From which fresh preparations were made whenever required.

2.3 Experimental Animal

Male Wister rats weighing 150-200g were purchased from Bingham University animal house Nasarawa state. The rats were approved for the experiment by the local committee and were housed in individual plastic cages with stainless steel covers kept at room temperature (25$\pm$3^{o}C) under 12 hr dark-light cycles. All the rats were allowed free access to their respective diets and water and acclimatized for 14 days before the treatment.

2.4 Preparation of alloxan

Two grams of crystalline alloxan monohydrates were dissolved in 50mls of normal saline (0.9% NaCl solution) to yield a concentration of 40mg/ml.

2.5 Induction of diabetics

The rats were divided into 2 groups before the induction of diabetes: non-diabetic control group and experimental group (to be induced with alloxan).

Diabetes was induced in the experimental rats after overnight fasting (12hrs) by intraperitoneal administration of 150mg of alloxan per kg body weight of rat (150mg/kg body weight).After the induction, all the rats were allowed free access to the same feed and water and the alloxan-induced rat were carefully examined for the next 24hrs for evidence of allergic reactions, behavioural changes and convulsion. After one week blood was collected from the tail vein and diabetes was confirmed in alloxan treated rat with fasting blood glucose levels greater than 200mg/dl.

2.6 Experimental design

The control and experimental animals were divided into different groups and treated accordingly.
Group 1 (non-diabetic group): normal control
Group 2 (Diabetic control): receive 1ml normal saline through direct stomach intubation every day.
Group 3 (Carica papaya seed group): Diabetic rats receiving 400mg Carica papaya seed extract per kg body weight daily.
Group 4 (Carica papaya leaf group): Diabetic rats receiving 400mg Carica papaya leaves extract per kg body weight daily.

2.7 Preparation of samples for biochemical analysis

After four weeks of treatment with the different extracts, the body weights of all the rats were taken again and were allowed to fast for 12 hours before they were sacrifice under sodium pentobarbitone anaesthesia. Whole blood was collected via cardiac puncture using sterile syringes and needles and emptied into plain bottles; this was allowed to clot for about two hours. The clotted blood was centrifuged at 3,500rpm for 30mins to recover the serum from clotted blood. Serum was separated with sterile syringes and needles and stored frozen until it is used for biochemical analysis.

2.8Assays

The blood glucose in a protein free serum was determined as described by Sood [22]. Urea, creatinine and total bilirubin concentrations were determined by the methods of Patton and Crouch [23]; Henry et al. [24] and Pearlman and Lee [25] respectively. Total cholesterol was measured by the procedure described by Allain et al.,[26]. Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method described by

Reitman and Frankel [27]. Protein content was determined by the method of Lowry et al. [28]. All the assays were carried out at the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria

III. Result and Discussion

This study showed that oral administration of Carica papaya seed and leaf extracts to alloxan- induced diabetic rats significantly reduced the blood glucose levels (TABLE 1). This hypoglycemic effect is similar to the findings of previous researchers on the antidiabetic effects of Carica papaya seed and leaf extracts[17,18],the action of the extracts might be ascribed to the ability of the extract to decrease the rate of intestinal glucose absorption [17],increase peripheral glucose utilization[29,30];stimulates the few surviving β-cells to produce insulin or regenerates β-cells of the islets, since β-cells have been shown to have a remarkable potential for regeneration[17,31,32].All the above proposed mechanisms of action of these extracts may be due to the presence of alkaloids, flavonoids, saponin, tannin, anthraquinones, and anthacyanosides in the extract [18]. Also, these active components of the extracts might have acted as a chain-breaking antioxidant and scavenge the free radicals genenerated by the alloxan which has been suggested to be responsible for eventual destruction of β-cells and other vital organs leading to the hyperglycemic effects observed after alloxan induction[33,34,35,36]

Table 1: Effect of Different Treatment on Blood Glucose Level.

TREATMENT	DAY 1 (mg/dl)	DAY 28 (mg/dl)	CHANGE IN GLUCOSE LEVEL (%)
NORMAL CONTROL	83 ± 8.90	85.50 ± 8.99	+ 3.01
DIABETIC CONTROL	208 ± 5.77	410 ± 9.93	+ 97.12
CAPRICA PAPAYA SEED(400mg/kg)	426.5 ± 13.08	100.50 ± 7.84	-76.43
CAPRICA PAPAYA LEAF(400mg/kg)	255.50 ± 21.36	130 ± 6.93	-49.02

All Values Are Expressed As Mean ± Standard Deviation Of Four Observations.

From the results (TABLE 2), alloxan induction resulted in the elevation of liver biomarkers in the serum which gives an indication of the hepatotoxic effect of alloxan[34] , that is, alloxan is able to distort hepatocyte membrane leading to the leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of the serum marker enzymes like ALT and AST [37] and other biomarkers. The administration of Carica papaya extracts to the diabetic rats resulted in reduction in the activities of these enzymes in the serum (Table 2) compared to the diabetic group. This reduction in the levels of ALT and AST agreed with the general observation that serum levels of transaminases tend to return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes[34,38]. This observation implies that the extract used contained some active compounds that can heal the liver damage caused by alloxan. This assumption is further confirmed by decrease in serum bilirubin of diabetic rats treated with the extract compared to the untreated group (Table 2). The increase in serum bilirubin may result from the decrease in liver uptake, conjugation or increase bilirubin production from haemolysis[39,40]. Considering the elevated level of ALT which is the most reliable marker of liver damage, it may be said that alloxan is hepatotoxic and administration of the extract leads to reduction in transaminase level and subsequently healing of the liver damage caused by alloxan. This is in agreement with previous finding that flavonoids, alkaloids, saponin, tannin, anthraquinones, and anthacyanosides in medicinal plants extract like Carica papaya posses hepatoprotective actions which is mediated via antioxidant and free radicals scavenging activities of the compounds [36, 41, and 42].

Table 2: Effect of Different Treatment on Liver Biomarkers

BIOCHEMICAL PARAMETER	AST (U/I)	ALT(U/I)	Bilirubin(mg/dl)
NORMAL CONTROL	19.25^{c} ± 0.29	15.25^{d} ± 1.49	2.76^{c} ± 0.39
DIABETIC CONTROL	58.00^{b} ± 5.77	31.50^{b} ± 1.73	40.08^{b} ± 3.47
CAPRICA PAPAYA SEED(400mg/kg)	23.62^{a} ± 0.47	11.50^{c} ± 1.23	3.00^{c} ± 0.24
CAPRICA PAPAYA LEAF(400mg/kg)	24.50^{a} ± 0.58	13.62^{a} ± 0.53	3.96^{a} ± 0.43

All Values are expressed as Mean ± Standard Deviation of four (4) Observations. Values with different superscript in the same column are significantly different at $p< 0.05$

Increase in serum cholesterol levels during diabetes have been reported in animal models [43,44] and this present study is not an exception to this observation (TABLE 3).The abnormal high concentration of serum cholesterol in the diabetic rats may be due to the increase in the mobilization of free fatty acids from the peripheral fat deposits due to the absence of insulin[34] while the observed significant reduction in the serum concentrations of cholesterol of treated rats may be due to depressed hepatic gluconeogenesis by Carica papaya extract since there is a positive correlation between gluconeogenesis and lipogenesis that is, any substance that interferes with gluconeogenesis also interferes with lipogenesis[45]. Carica papaya extract has also been shown to reduce total cholesterol by interfering with their biosynthesis[46]. The results of the phytochemical analysis

of Carica papaya extract showed the presence of saponin which is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids. In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia.

The results showed an increase in the level of serum urea and creatinine in the diabetic rats compare to the control (Table 3),since kidney removes these metabolic wastes (urea and creatinine).Increase in concentration of these metabolites in blood is associated with renal diseases[47]. Thus, diabetes could lead to renal dysfunction while treatment of alloxan induced diabetic rats with Carica Papaya seed and leaf extract, significantly reduced serum urea and creatinine compared to the mean value of diabetic group (Table3).Thus the active components of Carica papaya extract could heal renal dysfunction resulting from diabetes.

Table 3: Serum Urea, Creatinine, Cholesterol And Protein Levels After Treatment.

TREATMENT	UREA (mg/dl)	CREATININE (mg/dl)	CHOLESTEROL (mg/dl)
NORMAL CONTROL	34.78^{a}+0.40	0.38^{c} + 0.01	47.25^{d} + 6.33
DIABETIC CONTROL	59.78^{c}+5.75	2.13^{b} + 1.52	180.00^{c} + 13.34
CAPRICA PAPAYA SEED(400mg/kg)	38.82^{b}+1.30	0.70^{a} + 0.30	68.50^{b} + 2.23
CAPRICA PAPAYA LEAF(400mg/kg)	33.82^{a}+ 1.15	0.73^{a} + 0.05	61.50^{a} + 1.29

All Values Are Expressed As Mean ± Standard Deviation Of Four Observations.

IV. Conclusion

The results showed that both seed and leaf extracts have significant hypoglycaemic effect on the diabetic rats, although the extract of the seed seems to be more potent than that of the leaf in reducing the blood glucose.Also, elevations in the liver biomarkers examined were significantly attenuated compare to the diabetic group which showed that the extracts are capable of protecting the liver and the kidney in alloxan-induced diabetic rats.

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