

Original Research Article

Protective Effect of Ethanol Leaf Extract of *Carica papaya* Linn (Caricaceae) in Alloxan-induced Diabetic Rats

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Abstract

Purpose: To investigate the ameliorative effect of ethanolic extract of *Carica papaya* leaves in alloxan - induced diabetic rats.

Methods: Rats were randomly divided into five groups of eight animals each. Group A (control) comprised normal healthy animals which were orally administered 1.0 ml of distilled water daily for 21 days while groups B – E consisted of alloxan-induced diabetic rats. Group B comprised diabetic untreated rats, and groups C and D received 1.0 ml of 250 mg/kg and 500 mg/kg body weight of the extract, respectively. Group E received 300 mg/kg of metformin.

Results: Administration of the extract to the diabetic rats significantly reduced ($p < 0.05$) glucose level (123.50 mg/dl), total cholesterol (TC), triglyceride (1.24 mg/dl) and low density lipoprotein cholesterol (LDL-C), while significantly increasing ($p < 0.05$) high density lipoprotein cholesterol (HDL-C) and total protein level (66.51 g/dl) compared to the diabetic untreated rats. The extract also significantly decreased ($p < 0.05$) the concentration of serum urea (12.35 mg/dl), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of the diabetic rats.

Conclusion: The ethanol leaf extract of *Carica papaya* ameliorates metabolic disorder caused by diabetes in rats.

Keywords: Diabetes, *Carica papaya*, Glucose, Lipid profile, Alloxan monohydrate

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INTRODUCTION

Diabetes mellitus is a metabolic disorder associated with chronic hyperglycaemia and imbalance of carbohydrate, protein and fat metabolism. It is characterized by deficiency in insulin secretion and/or insulin action [1]. The diabetic state may result in the development of further metabolic disturbances among which are dyslipidemia, weight loss, atherosclerosis, gangrene, retinopathy, renal disease, neuropathy and coma [2].

Diabetes mellitus constitutes a major health and socioeconomic burden for diabetic patients and the healthcare providers. According to report by the World Health Organization (WHO), there were 150 million diabetic patients worldwide by the year 2000, with a projection of 221 million people in 2010 and 300 million in 2015 [3]. The International Diabetes Federation (IDF) also reported that diabetic population in Africa is 19.8 million and this is expected to increase to 41.5 million in 2035 [1].

Many synthetic oral anti-diabetic drugs are associated with drawbacks such as resistance

and side effects ranging from liver toxicity, increased cardiovascular risk, abdominal discomfort, flatulence and diarrhoea [4]. This has led to the usage of medicinal plants for the treatment of diabetes, but most of them lack scientific evidence to validate their usage and efficacy [5]. Some of the plants being used include *Ageratum conyzoides*, *Blighia sapida*, *Calotropis procera*, *Ficus exasperata* and *Carica papaya* [6].

Carica papaya is used traditionally for the treatment of many ailments including malaria and hypertension. Studies have reported the anti-diabetic potential of its seeds and aqueous extract of its leaves [7,8]. Despite the fact that ethanolic extract of *Carica papaya* leaf is used in the treatment of diabetes in Nigeria [6], there is dearth of information on its efficacy. Therefore, this study was aimed at evaluating the anti-diabetic effect of ethanolic extract of *Carica papaya* leaves in alloxan-induced diabetic rats.

EXPERIMENTAL

Plant material

Fresh leaves of *Carica papaya* were harvested from cultivated plantation in Ojo, Ojo Local Government area of Lagos State, Nigeria in the first week of December 2012. The plant was authenticated by Dr AB Kadirri of the Department of Botany, University of Lagos, Lagos, Nigeria and a voucher specimen (no. LUH 2151) was deposited in the University's herbarium.

Preparation of aqueous extract

Leaves of *Carica papaya* were dried under shade and crushed to powder by using Warring blender. The powdered sample was soaked in ethanol for 24 h after which it was filtered using Whatman no. 1 filter paper. The filtrate (extract) was concentrated under reduced pressure 40 °C using rotary evaporator (Cole Parmer SB 1100, Shanghai, China) and freeze-dried with the aid of Virtis Bench Top (SP Scientific Series, USA) freeze dryer.

Animals

Healthy albino rats weighing 180 – 200 g were obtained from the animal house of the College of Medicine, University of Lagos Teaching Hospital, Idi-araba, Lagos, Nigeria. The animals were housed in polypropylene cages and maintained under standard conditions. The animals were fed with standard rat pellet and water *ad libitum*. All experimental procedures were performed in compliance with institutional and international

policies governing the humane and ethical treatment of experimental animals as contained in the United States National Institutes of Health (NIH) guidelines [9] after ethical approval by the Research Committee of the Lagos State University (ref no. RC/BCH/0703).

Induction of experimental diabetes

Diabetes was induced in the wistar rats by intra-peritoneal administration of alloxan monohydrate (Sigma St Louis, USA) dissolved in phosphate buffer at a dose of 150 mg/kg body weight. After 72 h, blood samples were collected from the tail tip of the rats and diabetes mellitus was confirmed in the rats by testing for hyperglycemia using glucometer (Accu-chek ®, Johnson-Johnson California, USA). Animals with blood glucose level above 240 mg/dl were selected for the study.

Treatment of diabetic rats

Wistar rats were randomly divided into 5 groups comprising 8 rats each.

- Group 1: Normal control rats received distilled water.
- Group 2: Diabetic control rats received distilled water
- Group 3: Diabetic rats that were administered 250 mg/kg body weight extracts.
- Group 4: Diabetic rats that were administered 500 mg/kg body weight extracts.
- Group 5: Diabetic rats that were administered 300 mg/kg body weight of metformin.

The drug and extracts treatment was done for a period of 21 days using orogastric tube.

Collection of blood samples

Following 21 days of extract administration, the rats were humanely sacrificed by anaesthetization and the neck area was quickly cleared of fur before the jugular vein was sharply cut with sterile surgical blade. The blood was collected and centrifuged at 1282 xg for 5 min, and the serum was carefully aspirated with a Pasteur pipette into sample bottles for various biochemical assays.

Determination of biochemical parameters

The levels of total cholesterol (TC), triglyceride, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined in the serum of the animals using standard procedures [10]. Serum urea and creatinine were determined using the procedure

of Marsh *et al* [11]. Serum aspartate and alanine aminotransferase (AST and ALT) were determined using the method of Schmidt and Schmidt [12].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego MA, USA). The data were analysed by one way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as mean \pm SEM for eight rats in each group and were considered statistically significant at $p < 0.05$.

RESULTS

Table 1 showed the effect of oral administration of aqueous extract of *Carica papaya* leaves on the blood glucose level of alloxan-induced diabetic rats. At pre-induction of diabetes in rats, the glucose levels of the animals in all the groups were not significantly different ($p > 0.05$) from one another. After induction, there was significant difference ($p < 0.05$) between the control and other groups. However, at the end of

the treatment of the diabetic animals with the extract, there was significant reduction ($p < 0.05$) in the glucose level of the treated animals compared with diabetic untreated rats.

The effect of administration of *Carica papaya* leaf extract on the lipid profile of alloxan-induced diabetic rats is shown in Table 2. The significant increase ($p < 0.05$) witnessed in total cholesterol level of the diabetic untreated rats compared to the control was significantly reduced ($p < 0.05$) by the 250 mg/kg body weight of the extract. Administration of the extract also significantly increased ($p < 0.05$) the HDL-C concentration of the diabetic rats compared to the diabetic untreated ones. The significant elevation ($p < 0.05$) in the LDL-C and triglycerides of the diabetic untreated rats was significantly reduced ($p < 0.05$) by the administration of the *Carica papaya* extracts and this is comparable to the control and metformin-treated diabetic rats.

Table 3 showed the effect of administration of the aqueous extract of *Carica papaya* leaf on some kidney function parameters of alloxan-induced diabetic rats. The urea and creatinine concentra-

Table 1: Effect of oral administration of ethanol extract of *Carica papaya* leaves on the blood glucose level (mg/dl) of alloxan-induced diabetic rats

Group	Period		
	Pre-induction	Post-induction	Day 21
Normal control	79.75 \pm 4.66 ^a	76.44 \pm 5.20 ^a	78.50 \pm 3.72 ^a
Diabetic untreated	71.00 \pm 4.14 ^a	240.20 \pm 9.00 ^b	318.50 \pm 15.72 ^b
Diabetic + Extract (250mg/kg)	72.65 \pm 3.11 ^a	244.75 \pm 10.78 ^b	137.50 \pm 7.10 ^c
Diabetic + Extract (500mg/kg)	76.80 \pm 6.67 ^a	252.75 \pm 6.21 ^b	123.50 \pm 8.76 ^c
Diabetic + Metformin	81.48 \pm 4.83 ^a	266.36 \pm 9.25 ^c	107.50 \pm 5.06 ^d

Values are mean \pm SEM (n= 8); values with different alphabetical superscripts are significantly different at $p < 0.05$

Table 2: Effect of administration of ethanol extract of *Carica papaya* leaves on the serum lipid profile (mg/dl) of alloxan-induced diabetic rats

Group	Lipid			
	TC	HDL-C	LDL-C	Triglyceride
Normal control	1.25 \pm 0.11 ^a	1.92 \pm 0.21 ^a	0.15 \pm 0.02 ^a	1.30 \pm 0.27 ^a
Diabetic untreated	1.89 \pm 0.35 ^b	0.67 \pm 0.03 ^b	0.89 \pm 0.05 ^b	3.90 \pm 0.90 ^b
Diabetic + Extract (250mg/kg)	1.63 \pm 0.95 ^c	1.73 \pm 0.21 ^a	0.28 \pm 0.31 ^a	1.54 \pm 0.03 ^c
Diabetic + Extract (500mg/kg)	1.83 \pm 0.27 ^b	1.53 \pm 0.32 ^c	0.27 \pm 0.03 ^a	1.24 \pm 0.04 ^a
Diabetic + Metformin	1.62 \pm 0.32 ^c	1.49 \pm 0.26 ^c	0.40 \pm 0.02 ^c	1.29 \pm 0.03 ^a

Values are mean \pm SEM (n= 8); values with different alphabetical superscripts are significantly different at $p < 0.05$ TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol

Table 3: Effect of administration of ethanol extract of *Carica papaya* leaves on some kidney function parameters of alloxan-induced diabetic rats

Group	Kidney function	
	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	14.82 ± 1.99 ^a	42.72 ± 0.26 ^a
Diabetic untreated	33.50 ± 1.05 ^b	124.12 ± 4.60 ^b
Diabetic + Extract (250mg/kg)	12.35 ± 1.82 ^a	50.09 ± 3.77 ^a
Diabetic + Extract (500mg/kg)	11.37 ± 0.79 ^a	46.88 ± 1.48 ^a
Diabetic + Metformin	10.67 ± 0.29 ^a	48.22 ± 2.49 ^a

Values are mean ± SEM (n= 8); values with different alphabetical superscripts are significantly different at $p < 0.05$

Table 4: Effect of administration of ethanolic extract of *Carica papaya* leaves on some liver function parameters of alloxan-induced diabetic rats

Group	Liver function		
	Total protein (g/dl)	ALT (U/L)	AST (U/L)
Normal control	78.18 ± 1.50 ^a	81.50 ± 4.62 ^a	123.30 ± 8.00 ^a
Diabetic untreated	53.23 ± 2.32 ^b	115.00 ± 7.08 ^b	376.10 ± 9.20 ^b
Diabetic + Extract (250mg/kg)	66.51 ± 1.58 ^c	91.50 ± 4.62 ^a	158.73 ± 9.76 ^c
Diabetic + Extract (500mg/kg)	62.16 ± 1.06 ^c	85.30 ± 6.68 ^a	143.30 ± 7.00 ^c
Diabetic + Metformin	74.75 ± 4.66 ^a	82.87 ± 9.89 ^a	127.30 ± 6.88 ^a

Values are mean ± SEM (n= 8); values with different alphabetical superscripts are significantly different at $p < 0.05$ ALT: alanine aminotransferase, AST: aspartate aminotransferase

tion of the diabetic untreated rats were significantly different ($p < 0.05$) from the control. However, administration of the extract to diabetic rats significantly reduced ($p < 0.05$) these parameters to a level similar to the control and metformin-treated diabetic animals.

The effect of administration of aqueous extract of *Carica papaya* leaves on some liver function parameters was presented in table 4. There was significant reduction ($p < 0.05$) in the level of total protein in the diabetic rats compared to the control. Administration of the *Carica papaya* leaf extract significantly increased ($p < 0.05$) total protein level of diabetic rats though not to the level of the control. The significant increase ($p < 0.05$) in the activities of ALT in the diabetic rats was significantly reduced ($p < 0.05$) to a level similar to the control rats. Though, the significant elevation ($p < 0.05$) witnessed in the AST of diabetic untreated rats was reduced by the administration of the extract but not comparable to the control rats.

DISCUSSION

Diabetes mellitus is the commonest disorder of carbohydrate, protein and fat metabolism. Its features include chronic and persistent hyperglycemia, neuropathy and degenerative vascular changes which are due to deficiency in insulin secretion or insulin resistance [2]. The management approaches of diabetes mellitus

include nutritional adjustment, insulin injection, and administration of various classes of oral hypoglycaemic drugs [4]. However, occurrence of various side effects such as hypoglycaemia, abdominal discomfort and liver diseases associated with oral antidiabetic drugs necessitated the search for alternatives from medicinal plants.

Alloxan monohydrate induces diabetes by destroying the insulin producing beta-cells of the pancreas causing cell necrosis [13]. The increased levels of glucose in alloxan-induced diabetic rats were lowered by the administration of aqueous extract of *Carica papaya* leaves. The reduced glucose levels suggested that *Carica papaya* leaves might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism [14], or by absorption of glucose into the muscle and adipose tissues through the stimulation of a regeneration process and revitalisation of the remaining beta cells [15]. In this study, elevated levels of serum lipids such as cholesterol and triglycerides were noticed in diabetic rats. This is because under normal circumstances, insulin activates lipoprotein lipase and hydrolyzes triglycerides [16] as well as inhibits lipolysis. However in diabetes, there is increased lipolysis which finally leads to hyperlipidemia. Administration of the *Carica papaya* leaves extract eventually suppressed these lipids in the diabetic rats.

The level of HDL-cholesterol, which increased after *Carica papaya* leaf extracts administration, might be due to the increase in the activity of lecithin cholesterol acyl transferase (LCAT), which may contribute to the regulation of blood lipids [17]. Diabetic untreated rats also experienced elevation in the level of LDL-cholesterol which may be due to inhibitory action of insulin on β -hydroxyl- β -methyl glutaryl CoA reductase (HMG-CoA reductase), a key rate-limiting enzyme responsible for the metabolism of cholesterol rich LDL particles. Since aqueous extract of *Carica papaya* leaves restored all these alterations, it suggests that this plant prevents cardiovascular complications.

The kidneys remove metabolic wastes such as urea, creatinine and ions and thus optimum chemical composition of body fluids is maintained. But the increase in the concentrations of these metabolites (urea and creatinine) in diabetic untreated rats may suggest renal damage associated with uncontrolled diabetes mellitus. Blood urea and creatinine are considered as significant markers of renal dysfunction [18]. However, administration of the *Carica papaya* leaf extract normalizes these metabolic changes in diabetic rats.

Aspartate aminotransferase, alanine aminotransferase and total protein are considered as part of liver toxicity markers [19]. The increase in the activities of these plasma enzymes indicated that diabetes might be induced due to liver dysfunction. Therefore an increase in the activities of AST and ALT in serum might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which gives an indication on the hepatotoxic effect of alloxan monohydrate. On the other hand, treatment of the diabetic rats with aqueous extracts of *Carica papaya* caused reduction in the activities of these enzymes when compared to the diabetic group and consequently alleviated liver damage [20] caused by alloxan-induced diabetes.

CONCLUSION

The findings of this study show that the ethanol leaf extract of *Carica papaya* at the doses used in this study ameliorates metabolic derangement caused by alloxan-induced diabetes in rats.

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