

**Hypoglycemic, Hypolipidemic and Hepatoprotective Activities of Ripe and Unripe *Carica papaya* Methanol Extracts in Streptozotocin-Induced Diabetic Male Rats**Modupe O. Adetayo¹, Adekunle M. Adetayo², Oluwaseyi A. Adetunji^{1*}, Titilayo F. Coker-Osiwoga¹, Amaka C. Mordi¹¹Department of Biochemistry, Babcock University, Ogun State, Nigeria²Department of Surgery, Babcock University Teaching Hospital, Ogun State, Nigeria

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ABSTRACT

Diabetes mellitus is associated with elevated plasma glucose levels, hyperlipidemia and hepatic dysfunction. Hence, we investigated the effect of ripe and unripe *Carica papaya* methanol extract (CPME) on blood glucose levels, lipid profile, liver biomarker enzymes and plasma total protein in streptozotocin (STZ)-induced diabetic rats. Thirty rats were equally distributed into 6 groups: Group 1 (vehicle treated); group 2 received 500 mg/kg ripe CPME for 21 days; group 3 received 500 mg/kg unripe CPME for 21 days; group 4 received STZ (65 mg/kg once); group 5 received STZ (65 mg/kg once) and 500 mg/kg ripe CPME; group 6 received STZ (65 mg/kg once) and 500 mg/kg unripe CPME. Blood glucose was measured on days 0, 7, 14 and 21 after which animals were sacrificed and blood samples collected for estimation of biochemical parameters. Ripe and unripe CPME significantly ($p < 0.05$) reduced plasma glucose, total cholesterol (119.78 ± 2.67 mg/dL and 113.11 ± 2.51 mg/dL), triglyceride (164.79 ± 6.19 and 135.79 ± 4.44 mg/dL), low density lipoprotein (54.71 ± 3.24 and 44.70 ± 2.89 mg/dL), very low-density lipoprotein (30.80 ± 2.79 and 28.86 ± 2.28 mg/dL), AST (60.67 ± 3.79 and 58.33 ± 1.14 U/L), ALT (56.33 ± 9.57 and 56.00 ± 8.37 U/L) and TP (7.02 ± 0.23 and 6.85 ± 0.30 g/dL) levels and significantly ($p < 0.05$) increased high density lipoprotein (39.72 ± 4.26 and 31.44 ± 8.56 mg/dL) levels of the treated animals. Ripe and unripe *C. papaya* methanol extracts showed hypoglycemic, hypolipidemic and hepatoprotective activities in STZ-induced diabetic rats with the unripe fruit being more effective.

Keywords: Diabetes, *Carica papaya*, Hypoglycemic, Hypolipidemic, Hepatoprotective.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by the presence of hyperglycemia due to defect in insulin secretion, action or both. The World Health Organization estimates that about 422 million people worldwide have diabetes, with 1.6 million deaths being directly attributed to diabetes each year.¹ Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades with diabetes being one of the leading causes of death.² The chronic hyperglycemia of diabetes is associated with relatively specific long-term microvascular complications affecting the eyes, kidney and nerves, as well as increased risk of cardiovascular diseases.³ The abnormalities in carbohydrate, fat, and protein metabolism that are found in diabetes are due to the deficient action of insulin on target issues.⁴ Strategies involving the use of plant materials have shown a more promising result in not only the reduction of these complications but also the adverse effects.

Carica papaya, commonly known as Pawpaw, is a tropical fruit belonging to the plant family *Caricaceae* and the genus *Carica* native

to Mexico and Central America. It is the most economically important fruit in the *Caricaceae* family⁵ and is currently cultivated in tropical areas world-wide with Nigeria being the third largest producer globally. It is cultivated for its large, sweet melon-like fruits.⁵ *C. papaya* is well known for its exceptional nutritional and medicinal properties throughout the world. The whole *C. papaya* including its leaves, seeds, fruits and juice, is used as a traditional medicine.⁶ *C. papaya* has been considered as a nutraceutical fruit due to its versatile medicinal properties such as anti-fertility, diuretic, anti-hypertensive, hypolipidemic, anti-bacterial, anti-fungal, anti-diabetic, anti-tumor and free radical scavenging activities.⁷ Chloroform, aqueous and ethanol extracts of *C. papaya* leaf have been reported to exhibit hypoglycemic and hypolipidemic activities.^{8,9} Also, the unripe *C. papaya* fruit has been used ethnomedicinally in the treatment of hypertension, diabetes mellitus, hypercholesterolemia, malaria, helminthiasis in Nigeria.¹⁰ The phytochemical investigation suggested that *C. papaya* fruits contain alkaloids, saponins, tannins, flavonoids, glucosides, vitamins, minerals, proteins, polysaccharides, fat and oils and sterols; hence the therapeutic properties.¹¹

The *C. papaya* fruit is considered ripe when it feels soft and the skin has attained amber to orange hue, while the unripe fruit is green in colour and contain white latex in more amount. On ripening process, the fruit skin starts turning yellow colour to red, becomes aromatic, juicy and sweet.¹¹ Despite numerous uses of *C. papaya* in the traditional management of many diseases, research on the hypoglycemic and hypolipidemic activities of *C. papaya* fruit, there is insufficient information on the effect of ripening on these activities. The study examined the hypoglycemic, hypolipidemic and hepatoprotective activities of ripe and unripe *C. papaya* fruit methanol extract in streptozotocin-induced diabetic rats.

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Materials and Methods

Chemicals

All chemicals used in the study were of analytical grade and procured from standard chemical dealers. Chemicals and reagents were stored based on the storage instructions indicated for each.

Identification of plant material and preparation of extract

Fresh ripe and unripe *C. Papaya* Linn fruit were collected directly from the *C. papaya* tree Ilishan-Remo, Nigeria, in January 2019. The authentication of the fruit was done at the Forest Herbarium, Ibadan, Oyo State, and it was given a voucher specimen number of FHI. 112487.

The fruits were peeled, and the seeds were removed, the fruit pulps were then washed with distilled water. The fruits were diced and soaked separately in 80% methanol (BDH Chemicals, Bois-Franc, Canada) to enable the dissolution of wide range of compounds with different polarity, at room temperature, for 24 h. This was filtered with filter paper (Whatman No. 1, 125mm, England), and the filtrate was collected and concentrated to dryness using a rotary evaporator (RE-1050, Shanghai Yuhua Instrument Equipment CO., China) to obtain the ripe and unripe *Carica papaya* methanol extract (CPME).

Experimental animals

Thirty (30) male rats aged 4 weeks and weighing between 80-100 g were purchased from the animal facility, University of Ibadan, Ibadan, Oyo State. The rats were housed in clean cages and were fed commercial rat chow and water *ad libitum*. The rats were acclimatized for two weeks. The dose 500 mg/kg administered to rats has no toxic effect.¹² The rats were divided into six groups of five rats each, as follows.

Group 1: Normal rats with no treatment (normal control)

Group 2: Normal rats treated with 500 mg/kg body weight ripe CPME

Group 3: Normal rats treated with 500 mg/kg body weight unripe CPME

Group 4: Type 1 Diabetes Mellitus (DM)-induced rats with no treatment (diabetic control)

Group 5: Type 1 DM-induced rats treated with 500 mg/kg body weight ripe CPME

Group 6: Type 1 DM-induced rats treated with 500 mg/kg body weight unripe CPME

Ethical consideration

Ethical approval (Number: BUHREC 308/19) was obtained from the Babcock University Research and Ethics Committee in September 2019 prior to commencement of the study.

Induction of diabetes

Diabetes was induced, in groups 4, 5 and 6, by intraperitoneal injection of streptozotocin (Sigma, USA) 65 mg/kg body weight in 0.1 M cold citrate buffer pH 4.5.¹³ Animals in the control group received the vehicle alone. Blood was extracted from the tail vein, after 48 h, for glucose analysis using the Accucheck™ Glucometer, model 35NZ (Roche Diabetes Care, Mannheim, Germany). Rats with fasting blood glucose of 200 mg/dL and above were considered diabetic.

Sacrificing of experimental Animals

At the end of 21 days treatment, the rats were allowed to fast overnight for 10-12 hours. Blood samples were collected from the experimental animals through the ocular puncture into lithium heparin bottles using a syringe after which the rats were sacrificed through pelvic dislocation.

Measurement of blood glucose level

Blood samples were collected from the tip of the tail to determine the fasting blood glucose level which was measured using Accucheck™ Glucometer based on the glucose oxidase method.¹⁴ This was done on days 0, 7, 14 and 21.

Biochemical parameters

Plasma total cholesterol, triglyceride, low density lipoprotein, high density lipoprotein, aspartate aminotransferase (AST), alanine amino

transferase (ALT) and total protein were determined by the kit method with the use of Randox Kit (Randox Laboratory Limited, United Kingdom). The liver function tests were carried out spectrophotometrically for AST and ALT.¹⁵

Statistical Analysis

Data obtained from this study were expressed as Mean \pm standard error of mean (SEM). Statistical analysis of the data was carried out using a one-way analysis of variance (ANOVA). Post-hoc test was done using Duncan Multiple-Range Test (DMRT). Graphs were constructed using GraphPad Prism 6. $P < 0.05$ was considered statistically significant.

Results and Discussion

Effect of ripe and unripe *C. papaya* methanol extract on blood glucose levels of streptozotocin-induced diabetic rats

Figure 1 shows the effect of ripe and unripe CPME on changes in blood glucose levels of experimental rats. The ripe and unripe CPME significantly ($p < 0.05$) reduced the blood glucose levels of animals in the treated groups, with the unripe CPME being more effective on days 14 and 21. The non-diabetic treated animals also had lowered blood glucose levels but was not significantly ($p > 0.05$) different from the untreated animals, with values falling within the normoglycemic range. The elevated blood glucose levels of diabetic animals on day 0 showed that they were hyperglycemic and after treatment with the ripe and unripe CPME, there was a reduction in the blood glucose levels, to the normal range. This shows the potential of ripe and unripe CPME in bringing about hypoglycemic effect however, the unripe CPME was observed to be more effective than the ripe CPME in reducing blood glucose levels of the animals. This is similar to findings from previous studies which reported that aqueous extract of unripe *C. papaya* fruit helped to reduce blood glucose levels of diabetic rats.^{12,14,16} Thus, the unripe pulp of *Carica papaya* may probably contain active substances that possess blood glucose lowering activities.

Group 1: Normal rats with no treatment (normal control); Group 2: Normal rats treated with 500mg/kg body weight ripe CPME; Group 3: Normal rats treated with 500mg/kg body weight unripe CPME; Group 4: Type 1 DM-induced rats with no treatment (diabetic control), Group 5: Type 1 DM-induced rats treated with 500mg/kg body weight ripe CPME; Group 6: Type 1 DM-induced rats treated with 500mg/kg body weight unripe CPME

Effect of ripe and unripe *C. papaya* methanol extract on lipid profile of streptozotocin-induced diabetic rats

Table 1 shows the effect of ripe and unripe CPME on the lipid profile of experimental animals. The ripe and unripe CPME significantly ($p < 0.05$) reduced the plasma total cholesterol, triglyceride, low density lipoprotein, very low-density lipoprotein and significantly increased ($p < 0.05$) the high density lipoprotein levels of animals in the treated groups. Unripe CPME was more effective than the ripe CPME in regulating the lipid profile parameters of the animals within a healthy range. The groups treated with unripe CPME were found to have better lipid profile levels compared to the groups treated with ripe CPME. This suggests that methanol extract of unripe *C. papaya* has a more effective hypolipidemic action than the methanol extract of ripe *C. papaya* extract. All lipid profile parameters were within the normal range in the groups treated with unripe CPME. This suggests that unripe *C. papaya* fruit may be an excellent nutraceutical for the prevention of cardiovascular diseases which may result from improper metabolism of fat in diabetic conditions.

This finding is in congruence with that of a previous research on the effect of feeding ripe and unripe papaya fruit pulps on blood lipid profile of normal male adult rabbits. It was revealed that both ripe and fruit pulps resulted in a consistent lowering of serum cholesterol, low density lipoprotein cholesterol and triglycerides and elevation in phospholipids and high-density lipoprotein cholesterol levels at 1,3 and 6 weeks of fruit pulp feeding.¹⁷

Table 1: Effect of ripe and unripe *C. papaya* methanol extract on lipid profile of streptozotocin-induced diabetic rats

Group	Parameter	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)
1		102.22 ± 3.02 ^b	108.54 ± 2.43 ^b	29.80 ± 4.49 ^a	24.17 ± 3.16 ^a	57.85 ± 4.01 ^e
2		104.22 ± 2.32 ^b	116.70 ± 1.14 ^c	25.99 ± 0.13 ^a	22.48 ± 4.34 ^a	51.24 ± 4.56 ^d
3		84.44 ± 3.10 ^a	101.63 ± 2.08 ^a	32.03 ± 4.22 ^a	20.10 ± 5.19 ^a	58.65 ± 3.26 ^c
4		120.22 ± 1.6 ^d	237.92 ± 5.62 ^f	75.97 ± 0.06 ^d	47.37 ± 2.59 ^c	20.04 ± 5.03 ^a
5		119.78 ± 2.67 ^d	164.79 ± 6.19 ^e	54.71 ± 3.24 ^c	30.80 ± 2.79 ^b	39.72 ± 4.26 ^c
6		113.11 ± 2.51 ^c	135.79 ± 4.44 ^d	44.70 ± 2.89 ^b	28.86 ± 2.28 ^b	31.44 ± 8.56 ^b

Data were expressed as mean ± SEM. Values in the same column having the same superscript alphabets have no statistically significant difference at $p < 0.05$. **Group 1:** Normal rats with no treatment (normal control); **Group 2:** Normal rats treated with 500 mg/kg body weight ripe CPME; **Group 3:** Normal rats treated with 500 mg/kg body weight unripe CPME; **Group 4:** Type 1 DM-induced rats with no treatment (diabetic control); **Group 5:** Type 1 DM-induced rats treated with 500mg/kg body weight ripe CPME; **Group 6:** Type 1 DM-induced rats treated with 500 mg/kg body weight unripe CPME. TC – Total cholesterol; TG – Triglyceride; LDL – Low density lipoprotein cholesterol; VLDL – Very low density lipoprotein cholesterol; HDL – High density lipoprotein cholesterol

Effect of ripe and unripe *C. papaya* methanol extract on liver function biomarker enzymes and total protein of streptozotocin-induced diabetic rats

Table 2 shows the effect of ripe and unripe CPME on liver function biomarker enzymes and total protein concentration of experimental animals. The ripe and unripe CPME significantly ($p < 0.05$) reduced the plasma AST and ALT levels of treated animals with the unripe CPME being most effective among the treated groups while the total protein levels of all groups had no significant ($p < 0.05$) difference and were within the normal range except for the diabetic control (untreated) group which was lower than normal. There was a decrease in the levels of AST and ALT in the groups treated with both the ripe and unripe methanol extract of *C. papaya* for the diabetic groups. However, the unripe *C. papaya* methanol extract was more effective in decreasing the AST levels of the treated animals compared to the ripe *C. papaya* methanol extract. These results showed that the extracts may have hepatoprotective properties. This reduction of AST and ALT to their normal levels indicate that the extracts had a protective effect on the liver as high levels indicate damage to the liver.

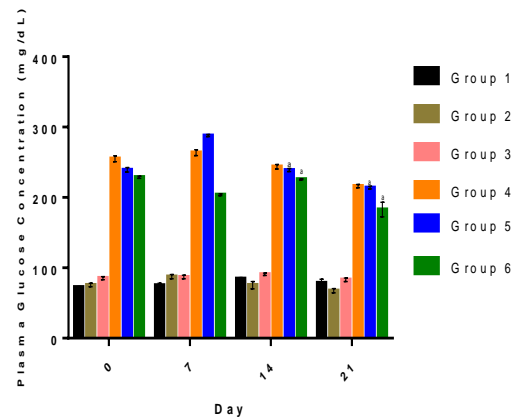
Table 2: Effect of ripe and unripe *C. papaya* methanol extract on liver function biomarker enzymes and total protein of streptozotocin-induced diabetic rats

Group	AST(U/L)	ALT (U/L)	TP (g/dL)
1	31.00 ± 1.08 ^a	32.67 ± 0.62 ^a	7.06 ± 0.02 ^b
2	44.67 ± 5.57 ^b	51.00 ± 4.14 ^b	7.52 ± 0.32 ^d
3	41.33 ± 5.15 ^b	45.00 ± 6.14 ^b	7.22 ± 0.19 ^c
4	84.67 ± 2.39 ^d	69.00 ± 4.55 ^d	4.56 ± 0.04 ^a
5	60.67 ± 3.79 ^c	56.33 ± 9.57 ^c	7.02 ± 0.23 ^b
6	58.33 ± 1.14 ^c	56.00 ± 8.37 ^c	6.85 ± 0.30 ^b

Values are expressed in mean ± SEM. Values in the same column having the same superscript alphabets have no statistically significant difference at $p < 0.05$.

Group 1: Normal rats with no treatment (normal control); **Group 2:** Normal rats treated with 500 mg/kg body weight ripe CPME; **Group 3:** Normal rats treated with 500 mg/kg body weight unripe CPME; **Group 4:** Type 1 DM-induced rats with no treatment (diabetic control); **Group 5:** Type 1 DM-induced rats treated with 500 mg/kg body weight ripe CPME; **Group 6:** Type 1 DM-induced rats treated with 500 mg/kg body weight unripe CPME.

AST – Aspartate transaminase; ALT – Alanine transaminase; TP – Total protein

**Figure 1:** Effect of ripe and unripe *C. papaya* methanol extract on blood glucose levels of streptozotocin-induced diabetic rats. Values were expressed as the mean ± SEM (n = 5). The difference is statistically significant compared to control using one-way ANOVA test; ^a $p < 0.05$ vs control

This finding is similar to previous report which showed that chloroform extracts of unripe *C. papaya* fruit caused a decrease in AST and ALT levels of experimental animals compared to the diabetic control.^{16,18-19}

The total protein concentration of all study groups was found to have no significant difference. This shows that ripe and unripe CPME helped to maintain the total protein levels of plasma within the normal range. The diabetic untreated group had lower levels of total protein, below the normal range, confirming the uncontrolled breakdown of protein associated with the disease condition when it is not properly managed.²⁰

Conclusion

It can be concluded from the findings of the study that methanol extract of ripe and unripe *C. papaya* possesses hypoglycemic, hypolipidemic and hepatoprotective properties with the unripe CPME being more effective. *C. papaya* may therefore be a promising fruit containing potential antidiabetic nutraceuticals for the management of diabetes and its complications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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