

Original Research Article

Anti-Diabetic and Toxicological Studies of *Carica papaya* Leaves ExtractHauwa M. Gujbama^{1*}, Muhammad Ibrahim Usman¹¹Department of Biochemistry, Faculty of science, Yobe State University, Damaturu

Article History

Received: 07.05.2022

Accepted: 03.07.2022

Published: 12.07.2022

Journal homepage:

<https://www.easpublisher.com>

Quick Response Code



Abstract: Diabetes mellitus, a chronic non-communicable disease is ranked 7th killer disease in the world. With the current prevalence rate in the country, the number of people living with diabetes mellitus in Nigeria will increase to 4.8 million by the year 2030. The research aimed at evaluating LD₅₀ and antidiabetic of solvents (Aqueous, Ethyl acetate, Chloroform and Hexane) leaves extracts of *Carica papaya* in alloxan induced diabetic rats as well as characterization of most active extract. Twenty rats were used for acute toxicity studies, while thirty-five rats were used for the second phase of the study in which solvents extract of the leave were screened for hypoglycaemic activities. Forty rats were used for the third phase of the research to screen the column chromatography fractions obtained from the most active solvent extract. Acute toxicity studies showed all extracts to be practically non-toxic with oral LD₅₀ greater than 5000mg/kg. Second phase of the research observed that chloroform extract possesses the highest activity among the four extracts, while fraction II was shown to possess the highest activity from the column chromatography fractions. The fraction was also found to ameliorate liver damage as a result of diabetes. Conclusively, leaves extract of *Carica papaya* was found to lower fasting blood glucose of alloxan induced diabetes rat, the plant was also found to be non-toxic up to a dose of 5000mg/kg.

Keywords: Anti-diabetic; Acute toxicity; *Carica papaya*; Solvents and Extract.

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1.0 INTRODUCTION

Diabetes mellitus is an endocrine disease characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both, and is typically associated with failure of pancreatic β cells. There are two major types of diabetes mellitus—type 1 and type 2. In type 1 diabetes, or insulin dependent diabetes mellitus, the body has little or no insulin secretory capacity and depends on exogenous insulin to prevent metabolic disorders and death. In type 2 diabetes, a non-insulin dependent diabetes mellitus, the body retains some endogenous insulin secretory capability; however, its insulin level is low relative to its blood glucose level and/or there is a measure of insulin resistance (Maria-Rotella *et al.*, 2013).

Type 2 diabetes is the most common form of diabetes that goes undiagnosed at the early stage because there are no physical clinical manifestations. However, when hyperglycemia is severe, it usually presents with classic symptoms of untreated diabetes such as weight loss, polyuria (frequent urination),

polydipsia (increased thirst), and polyphagia (increased hunger) or a complication related to diabetes (retinopathy, nephropathy) or as a chance finding in an asymptomatic individual. Other symptoms include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin (Picot *et al.*, 2009). The underlying causes of diabetic complications have been attributed to hyperglycemia which results in oxidative stress, alterations in enzyme activities, protein glycosylation and several structural changes (Muhammad *et al.*, 2015).

Medicinal plants have been used extensively as a source for numerous active constituents for treating human diseases and they, as well, have high contain of therapeutic value. *Carica papaya* is a genus of eight or nine species of an unbranched tree with large leaves and fruits. It originated in south and Central America, particularly in Mexico (Ikeyi *et al.*, 2013) The name itself is said to be Caribbean in origin. Its wild ancestor is unknown, but it has been cultivated since ancient times in tropical America. It spread very rapidly at the time of great 16th –century explorers and has become

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cosmopolitan in all tropical and sub –tropical regions the world over (WHO, 2015).

Carica papaya is commonly called paw-paw and it belongs to the family Caricaceae and possesses excellent medicinal properties for treatment of different ailments (Sudhakar *et al.*, 2014). Paw-paw is locally called ‘Ibepe’ (Yoruba), ‘Okworo beke’, ‘ojo’ or ‘Okworo’ (Igbo) and ‘Gwanda’ (Hausa) in Nigeria (Dike *et al.*, 2012). In Africa *Carica papaya* may have arrived on the coast and in Madagascar as early as pre-colonial era by the specific route. In the 17th century it was observed in East African countries where the Arabs certainly played a role in its distribution. According to some documents, it appeared on the west coast as early as 15th century, brought into Guinea by Portuguese navigators. More certainly, it was carried to, and spread in Atlantic coast countries by slave traders and merchants in the 17th –century. In the interior of the continents, its expansion followed the routes of early explorers (Isela *et al.*, 2014).

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection Preparation and Administration of Plant Extract

Carica papaya leaves were collected from a Botanical garden of Yobe state University, Damaturu and identified at the Department of Plant Biology of the same institution. The samples were shade dried ground and sieved to powdered form. Two hundred gram (200g) of the powder was weighed and soaked in 500ml of respective solvents (aqueous, ethyl acetate, chloroform, and n-hexane). The resulting solution was vigorously shaken and left to stand at room temperature for 24 hours with intermittent shaking. After the 24 hours of percolation, the mixture was filtered using Whatman’s filter paper No. 1. The filtrate was concentrated by complete evaporation of the solvent using rotary evaporator to yield aqueous, ethyl acetate, chloroform, and n-hexane extract; labelled as **E1**, **E2**, **E3** and **E4** respectively. The dried sample will then be reconstituted with distilled water or DMSO and be administered to the animals according to appropriate dosage using the relation (Muhammad *et al.*, 2016)

Volume to be administered (ml) = weight of rats (kg) × dose (mg/kg)

Concentration of extract (mg/ml)

2.1.2 Experimental Animals

Healthy albino rats weighing 120g-150g were purchased from Department of Biological Science, Bayero University Kano. The rats were housed in metal cages in a well-ventilated room and allowed to acclimatize for 14 days before the commencement of research. They will be allowed free access to standard palletised growers’ feed and clean drinking water.

2.2 Methods

2.2.1 Lethal Mean Dose (LD₅₀) Determination

The limit test procedure described by organization for economic cooperation and development (OECD) guidelines was adopted (OECD, 2001). A total of twenty (20) rats were grouped into five (5) rats of four (4) rats representing the extracts (aqueous, ethyl acetate, chloroform, and n-hexane extract). The animals were fasted after which the plant extracts were administered orally at a dose level of 5000mg/kg body weight to each rat. The rats were observed for the first 4 hour and within the period of the 48 hour. Behavioral changes (weakness, difficulty in movement, sedation, hyperactivity, hair loss, reduced response to sound, loss of appetite, grooming e.t.c), body weight, and mortality was observed for a period of 14 days

2.2.2 Induction of Diabetes

Diabetes mellitus was induced by injecting alloxan hydrate intraperitoneal at a dose of 150 mg/kg using. The volume of the solution containing 150 mg/kg to be given to each experimental rat will be determined by the following relationship.

Volume (ml) = Dose (mg/kg) × weight of rat (kg)
Concentration of alloxan (mg/ml)

After 48 hours window period, rats with fasting glucose level of 11.1 mmol/L were considered diabetic.

2.2.3 Grouping and Treatment of Experimental Rats

A total of Thirty-five (35) rats were placed into seven groups of five rats each:

Group I: normal control

Group II: diabetic control

Group III: standard drug (Chlorpropamide, 100 mg/kg body weight)

Group IV: diabetic, administered with aqueous extract 200mg/kg

Group V: diabetic, administered with ethyl acetate extract at 200mg/kg

Group VI: diabetic, administered with chloroform extract at 200mg/kg

Group VII: diabetic, administered with n-hexane extract at 200mg/kg

Fasting blood glucose concentrations of rats will be monitored at the end of first and second week of extracts administration. The most potent extract from the four will then be subjected to further fractionation using column chromatography.

2.2.4 Fractionation of the Most Active Extract Using Column Chromatography

The most active chloroform extract from was subjected to column chromatography to further separate the extract into its component fractions. The packing,

elusion and collection of fractions were done as reported by (Jerry *et al.*, 2010).

2.2.5 Screening of the Fractions for Hypoglycaemic Activities

Forty albino rats were grouped into eight groups of five rats each. Diabetes will be induced using the method described previously for the solvents extract. The grouping of the rats was as follows:

Group I: Normal control: non-diabetic, no extract will administered

Group II: diabetic control: diabetic, no extract will be administered

Group III: standard drug (Metformin, 100mg/kg bodyweight)

Group IV: diabetic, administered with 100 mg/kg body weight of fraction I

Group V: diabetic, administered with 100 mg/kg body weight of fraction II

Group VI: diabetic, administered with 100 mg/kg body weight of fraction III

Group VII: diabetic, administered with 100 mg/kg body weight of fraction IV

Group VIII: diabetic, administered with 100 mg/kg body weight of fraction V

Fasting blood glucose concentrations of rats were monitored at an interval of three days for a period of two weeks. The rats were euthanized and blood samples collected for biochemical analysis of liver and kidney functions, as well as tissues antioxidant levels.

2.2.6 STATISTICAL ANALYSIS

The data were expressed as mean ± standard deviation. One-way ANOVA was used to determine the differences between extract administered groups and diabetic control groups with p value <0.05 considered significant. GraphPad Instat3 Software was used for statistical analyses.

3.0 RESULTS AND DISCUSSIONS

3.1 Results

Table 1 presents the result for oral LD₅₀ determination of the extracts, although some rats exhibited symptoms of weakness within 48 hours of extract administration, no mortality was observed

Table 1: Oral LD₅₀ of Solvents Extracts of *Carica Papaya*

	Aqueous extract	ethyl acetate extract	Chloroform extract	n-hexane extract
Initial (5000 mg/kg)	0/5	0/5	0/5	0/5
After 4 hrs	0/5	0/5	0/5	0/5
After 48 hrs	0/5	0/5	0/5	0/5

The effect of solvents (aqueous, ethyl acetate, chloroform, and n-hexane) extract on fasting blood glucose concentrations in diabetic rats was presented in Table 2. Fourty eight (48) hours after alloxan administration, the blood glucose concentration of diabetic control group (group II) and all test groups (groups III –VII) increase significantly (p<0.05)

compared to normal control (group I). After administration of the extracts, a significant (p<0.05) fall in fasting blood glucose was observed in standard drug, aqueous, ethyl acetate, chloroform extract, with chloroform extract exhibiting the highest anti hyperglycemic activity compared to the remaining extract.

Table 2: Fasting Blood Glucose of Rats Administered with Solvents Extracts of *Carica Papaya* for Two Weeks

Groups	Before Alloxan (mmolL⁻¹)	48 hours after Alloxan (mmolL⁻¹)	Week 1 (mmolL⁻¹)	Week 2 (mmolL⁻¹)
Group I	5.15±0.99	5.18±0.52	5.85±1.45	4.70±0.55
Group II	5.25±0.58 ^{a,b,c}	20.10±2.04 ^a	28.12±2.93 ^b	30.80±5.64 ^c
Group III	5.80±0.68 ^a	22.45±2.02 ^{a,b}	16.24±2.32	7.30±1.56 ^b
Group IV	4.35±0.45 ^a	25.92±3.33 ^{a,b}	21.25±3.20	17.22±4.15 ^b
Group V	5.00±0.31 ^a	26.55±2.72 ^a	22.42±4.77	18.40±3.54 ^b
Group VI	4.85±0.21 ^a	25.65±3.46 ^{a,b}	18.73±2.05	10.50±4.53 ^b
Group VII	4.20±1.02 ^a	26.56±2.80 ^b	22.50±2.92	21.83±3.62

The most active chloroform extract was subjected to further fractionation using column chromatography. A total of 58 fractions were obtained, these fractions were then pooled into five fractions according to their R_f values. The fractions were

screened for anti-hyperglycemic activity, the result shows significant reduction in blood glucose level by fraction II and fraction V administered groups with fraction II been the most active.

Table 3: Fasting Blood Glucose of Rats Administered with Column Chromatography Fractions from Chloroform Extract of *Carica Papaya* for Two Weeks

Groups	Before Alloxan (mmolL ⁻¹)	After Alloxan (mmolL ⁻¹)	Day 3 (mmolL ⁻¹)	Day 6 (mmolL ⁻¹)	Day 9 (mmolL ⁻¹)	Day 12 (mmolL ⁻¹)	Day 15 (mmolL ⁻¹)
G I	6.05±1.12	5.96±1.25	4.98±1.64	6.70±1.85	5.20±1.14	6.85±2.05	5.95±1.25
G II	5.35±1.88 ^a	25.10±3.64 ^a	27.43±2.92	28.83±3.64	29.9±4.21	30.92±3.24	29.12±3.09
G III	6.22±0.98 ^a	23.68±4.02 ^{a,b,c,d}	19.33±2.41	17.32±4.34 ^b	12.5±2.85 ^c	10.93±2.34 ^d	8.12±0.54 ^e
G IV	6.32±1.07 ^a	22.90±3.11 ^a	23.37±3.20	22.00±2.14	23.39±5.99	23.5±4.59	21.65±3.09
G V	6.88±1.11 ^a	24.50±2.67 ^{a,b,c,d}	20.45±1.77 ^b	18.40±3.54	13.70±2.85 ^b	12.07±2.42 ^c	11.43±1.52 ^d
G VI	5.55±0.81 ^a	24.94±3.56 ^a	25.75±2.05	24.50±4.53	20.8±5.66	19.45±4.21	19.01±3.01
G VII	6.20±1.02 ^a	24.55±2.88 ^a	21.50±2.97	21.38±2.62	23.34±3.91	20.28±4.46	19.65±2.86
G VIII	4.98±0.56 ^a	22.90±3.49 ^{a,b,c}	22.3±3.64	19.3±3.85	18.65±3.27	16.9±3.99 ^b	14.54±2.78 ^c

The effect of the fractions on Liver function indices (AST, ALT, ALP, DB, TB, TP and ALB) was presented by Figure 1 and 2. A significant (p<0.05) increase in serum liver enzymes was recorded in diabetic control group and ethyl acetate extract administered groups compared to the normal control.

Upon administration of the fraction, a fall in the level of serum AST, ALT, ALP level was observed in standard drug and chloroform administered groups compared with diabetic control group. No significant difference was observed in serum bilirubin, total protein and albumin.



Figure 1: Liver Enzymes Levels of Diabetic Rats Administered with Column Chromatography Fractions of *C. Papaya*

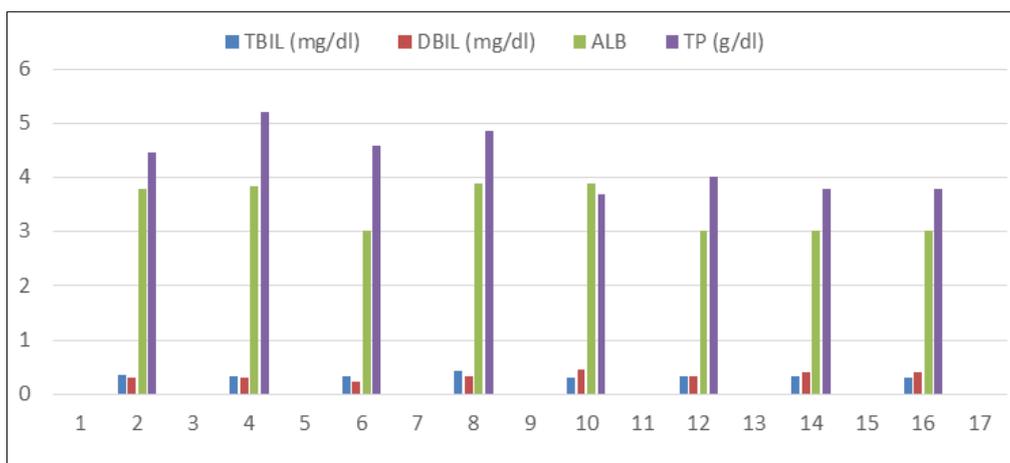


Figure 2: T.Bil, D.Bil, ALB and TP Levels of Diabetic Rats Administered with Column Chromatography Fractions of *C. Papaya*

Figure 3 below shows the effects of the fractions on serum electrolytes, while figure 2 present the urea and creatinine concentration of fractions

administered groups. No significant variation was observed within the groups in all parameters

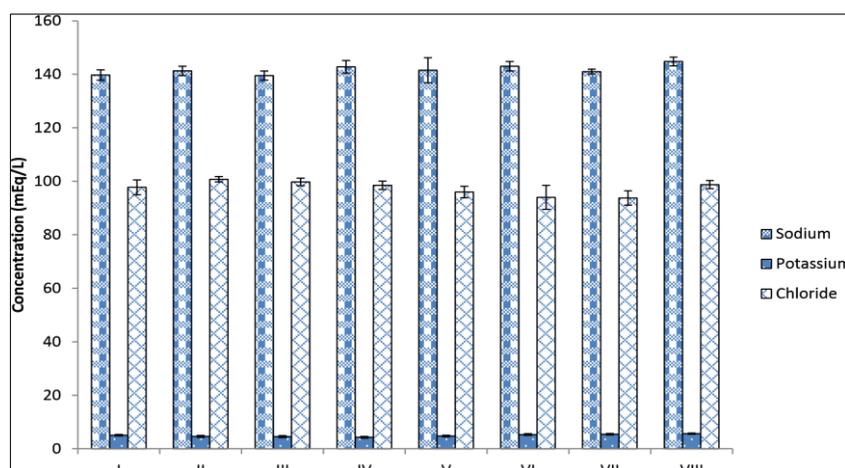


Figure 3: Serum Electrolytes of Diabetic Rats Administered with Column Chromatography Fractions of Active Chloroform Extract. Values are expressed as Mean \pm SEM

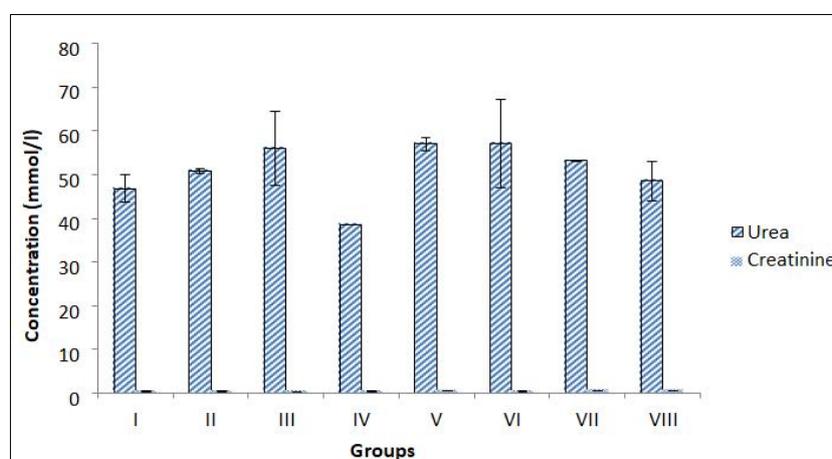


Figure 2: Urea And Creatinine Level of Diabetic Rats Administered with Column Chromatography Fractions of Active Chloroform Extract. Values are expressed As Mean \pm SEM

The effect of administration of the fractions liver function indices was presented in figure 3. A significant increase ($p < 0.05$) in serum AST, ALT, ALP was observed in diabetic control groups compared to the normal control. Administration of fraction II and V lead to decrease ($p < 0.05$) in serum AST, ALT, ALP level. Although there is slight variation in the level of total protein, albumin, total and direct bilirubin, no significant difference was recorded between the groups.

3.2 DISCUSSION

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually within 24-48 hours). To be described as *acute* toxicity, the adverse effects should occur within 14 days following the administration of the substance (IUPAC, 1997). The study established the plant to be practically non-toxic with oral LD₅₀ value greater than 5000mg/kg body weight. The result is in line with the finding various studies (Kanadi *et al.*, 2019, Zakiah *et al.*, 2014).

Diabetes Mellitus is a major health challenge globally with no permanent cure, over the years the

prevalence of this disease shows no retardation. This leads to enormous researches on plant based therapy that will support the currently used drug. *Carica papaya* leaves were reported to be used traditionally effective in the management of diabetes, hence the study seeks to establish the scientific basis of this claim.

Successful induction of the disease was achieved intraperitoneal administration of 150 mg/kg of alloxan. Which selectively destroys insulin producing β - cells. This causes an insulin dependent diabetes mellitus to the animals, which is characteristically similar to Type 1 diabetes in humans. These finding is in accordance with several studies that reported successful induction of diabetes with alloxan (Kolawole *et al.*, 2012), (Szudelski, 2001).

Extraction of bioactive compounds was done using different solvents of varying polarity and screening for anti-hyperglycemic activity shows chloroform extract to be to possess highest activity. This may suggest that the bio compounds from the leaves to be non-polar or hydrophobic in nature. This is however contrary to the work of (Augustine *et al.*,

2019) who reported ethanolic extract of *Carica papaya* leaves to possess anti-diabetic properties.

The hypoglycemic ability exhibited by the extract may be due to the presence of secondary metabolites extracted from by the solvent. In order to identify the bioactive compound(s) the extract was subjected to further fractionation using column chromatography. The fractions obtained shows significant reduction in groups administered with fraction II and V with fraction II having the highest activity. The fractions were also observed to ameliorate liver damage as a result of diabetes.

The exact structure of the bioactive compound(s) and its mode of action(s) is yet to be understood. One possible mechanism may be through antioxidant potential of the plant. The plant was reported to be rich in flavonoids and phenolics which may slow down/reverse the action of alloxan (Muhammad *et al.*, 2017). I may also act by inhibiting α -glucosidase and α -amylase activities and/or stimulating insulin production and release from the destroyed beta cells (Kaboré *et al.*, 2011).

CONCLUSION

From the findings of the study, it can be concluded that *Carica papaya* leaves extract is practically non-toxic with an oral LD₅₀ greater than 5000 mg/kg body weight. Secondly, the plant was established to possess hypoglycemic activity against alloxan induced diabetes mellitus.

ACKNOWLEDGEMENT

The authors wish to thank Tertiary Education Trust Fund (TETFund) for their immense contribution in financing this research under the Institutional based Research scheme.

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Cite This Article: Hauwa M. Gujbama & Muhammad Ibrahim Usman (2022). Anti-Diabetic and Toxicological Studies of *Carica papaya* Leaves Extract. *East African Scholars J Med Sci*, 5(7), 192-197.