



# Hepatoprotective Effect of Petroleum Ether Extract of Unripe *Carica papaya* Seed on Streptozotocin-Induced Diabetic Rats

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## Abstract

Liver plays a vital role in metabolism, detoxification and biotransformation process in the body; they are prone to xenobiotic induced injury due its central role in xenobiotic metabolism. Liver diseases are regarded as the most causes of morbidity and mortality in human globally. In this study, liver function biomarkers such as total protein, albumin, globulin and bilirubin (direct and total) were evaluated in streptozotocin (STZ) induced diabetic rats treated with petroleum ether extract of unripe *Carica papaya* seed. The treatment lasted for twenty-one (21) days. Four hundred grams (400 g) powder of the unripe *Carica papaya* (CP) seed was extracted using 1.5 L of petroleum ether for 72 hours at 60°C. The extract was evaporated to dryness at 40°C. Twenty-five (25) albino Wistar rats weighing 160 to 190 g were obtained and sub-divided into five (5) groups of five (5) rats each as follow: Group I (Normal control), Group II (Diabetic control), Group III (Diabetic and 200 mg Petroleum Ether extract), Group IV (Diabetic and standard drug), Group V (Normal Control and 200 mg Petroleum Ether extract). After the experimental period, the rats were sacrificed and blood was collected through cardiac puncture, then centrifuged and the sera were used for the evaluation of serum total protein, albumins, bilirubin (Direct and total) and globulin. The result revealed a significant ( $P < 0.05$ ) increase in total protein, albumin, and globulin in groups III (DPET) and IV (DSTD) compared to the diabetic control; however, non-significant ( $P > 0.05$ ) difference was revealed in bilirubin (direct and total) in groups III (DPET) and IV (DSTD). More so, non-significant ( $P \geq 0.05$ ) difference in total protein, albumin, bilirubin (total and direct), and globulin was observed in group V (NCPET) compared to normal control. Conclusively, it can be said that the unripe *Carica papaya* seed extract possess hepatoprotective effect as it does not have any damaging effect on the liver as revealed in the study.

**Keywords:** *Carica Papaya*; Liver Function; Diabetes Mellitus; Total Protein; Albumin; Globulin; Bilirubin

**Abbreviations:** STZ: Streptozotocin; CP: *Carica papaya*; CRUTECH: Cross River University of Technology; IREC: Institutional Research Ethical Committee; NIH: National

Institute of Health; NC: Normal Control; DC: Diabetic Control; DSTD: Diabetic and standard Drug; BCG: Bromocresol Green; ANOVA: One-Way Analysis of Variance; ROS: Reactive Oxygen

Species; IL-6: Interlukin-6; IL-1: Interlukin-1; DPET: Diabetic and 200 mg Petroleum Ether Extract of unripe *Carica papaya* seed; NPET: Normal Control and 200 mg Petroleum Ether Extract of unripe *Carica papaya* seed; TP: Total protein; ALB: Albumin; TB: Total bilirubin; DB: Direct bilirubin.

## Introduction

Liver diseases are momentous cause of morbidity and mortality in human globally [1], in spite of significant scientific advancement in the field of medicine. The principle of using medicinal plants or their preparations in the management of various diseases including liver diseases and diabetes mellitus is common in African countries and other developing countries and has been practiced for several decades and its extension in current dispensation is recognized [1]. Globally, more than 1000 species of medicinal plants have been used by different ethnic group in traditional medicine for their supposed antidiabetic activity [2], one of such medicinal plants is *Carica papaya*, commonly known as Pawpaw.

*Carica papaya* L. (*C. papaya*) belongs to the genus *Carica*. It is an herbaceous, soft-wooded tree-like plant in the *Caricaceae* family [3]. *Carica papaya* fruits consist mostly of water and carbohydrate, low in calories and rich in natural vitamins and minerals, particularly vitamins A and C, ascorbic acid and potassium [4]. The leaves, stem, fruits and seeds of *Carica papaya* contain different chemical constituents which includes carpain, pseudocarpain, dehydrocarpaine I and II, choline, carposide, vitamin C and E, myrosin, sinigrin, carpaine, benzylisothiocyanate, benzyl glucosinolate, glucotropacolin, benzylthiourea, hentriacontane,  $\beta$ -sitosterol, caricin, alkaloids, flavonoids, saponins, tannins, cardiac glycoside, anthraquinones and cardinolodes etc. [5] There are valuable natural compounds in the plant, which have anti-carcinogenic, anti-diabetic, anti-inflammatory, antipyretic, and analgesic activities [6]. It is commonly known for its food and nutritional values throughout the world. The leaves, fruits and latex obtained from the papaya plant are used medicinally and for various purposes [7]. The seeds are used as a potential post-testicular antifertility drug [8-10]. The latex and the seeds are used in the treatment of gastrointestinal nematode infections and they have shown anthelmintic activity [11]. The seeds and immature fruit have shown inhibitory activity against human enteric pathogens [7,12].

Diabetes mellitus is a chronic disorder that affect the metabolism of proteins, fats and carbohydrates [13,14]. Diabetes mellitus has become one of the leading causes of morbidity and mortality globally, and it is associated with acute and chronic complications, which are accountable for the majority of the diabetes mellitus-related morbidity

and mortality [15]. Globally, the socio-economic and health burden of diabetes is rising at alarming rate with its devastating complications [16]. The number of patients is predicted to grow to 642 million in the year 2040, with the greatest increase expected in low and middle countries [17]. The high rate of diabetes mellitus in developed and even developing country is disastrous, so there is a need for studies that will foster remedies as soon as possible by restoring the function of pancreatic tissue, by causing an increase in insulin output or by inhibiting the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes [18]. Thus, this study seeks to investigate the possible effect of petroleum ether extract of unripe *Carica papaya* seed on liver function in streptozotocin (STZ) induced diabetic rats.

## Materials and Methods

### Plant Material

Unripe fruits of *Carica papaya* were harvested from a local farm at Okuku, Yala Local Government Area of Cross River State, Nigeria. The fruits were cut into pieces; seeds were removed and thoroughly washed. They were dried at room temperature. The plant was identified and authenticated at the Department of Plant Science, Cross River University of Technology (CRUTECH), Calabar, Cross River State, and the voucher number: CRUTECH/PSB/0024 deposited in the herbarium.

### Preparation of Petroleum Ether Extract of Unripe *Carica papaya* Seed

Dried seeds were crushed and grinded in a domestic mixer grinder and coarse powder was prepared. 400 g of the powder was used for the extraction with petroleum ether (1.5 L) in a soxhlet extractor for 72 hours at 60°C. The extract was evaporated to dryness at 40°C. The obtained extract was in chocolate colour with aromatic odour [19]. It was reconstituted in 0.1 % of Tween 80 for administration.

### Chemicals/Reagents

Streptozotocin (STZ) was purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). Glibenclamide was purchased from AdvacarePharma (Cheyenne, USA). All other chemicals of analytical grade were obtained from E. Merck (Darmstadt, Germany). Kits for different assays were purchased from BioSystems S.A. (Barcelona, Spain).

### Experimental Animals

Twenty-five (25) male albino Wistar rats weighing 160 to 190 g were used. The animals were allowed to acclimatize

for a period of two (2) weeks in a well-ventilated room. The animals were maintained under standard laboratory conditions (12 h light/12 h dark cycle, temperature 21-22 °C, air humidity 55-60 %). The animals were bred at the Animal House of the Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH), Okuku Campus. The animal had free access to water and animal feed *ad libitum*. The feed was of dietary formulation of commercial pelletized feed (Vital feeds, Jos). The research study was carried out according to the guidelines approved by CRUTECH Institutional Research Ethical Committee (IREC) following the principle laid down in the Declaration of Helsinki (1964), as revised in 2013 and National Institute of Health (NIH) Principles of Laboratory Animal Care. No human participants were involved in the study.

### Experimental Design

Twenty-five (25) Wistar rats were used, the animals were divided into five (5) groups, each group containing five animals (n=5). Group I: Normal Control (NC), Group II: Diabetic Control (DC), Group III: Diabetic and 200 mg Petroleum Ether Extract of unripe *Carica papaya* seed (DPET), Group IV: Diabetic and standard Drug (glibenlamide) (DSTD), Group V: Normal Control and 200 mg Petroleum Ether Extract of unripe *Carica papaya* seed (NPET).

### Induction of Diabetes

Experimental diabetes was induced following an overnight fast, by a single intravenous injection of 60 mg/kg STZ freshly dissolved in 0.01 M citrate buffer (pH 4.5). Control animals received 0.9 % sterile saline. Hyperglycemia was confirmed three (3) days after injection by measuring the tail vein blood glucose level with an Accu-Chek Sensor Comfort glucometer (Roche, Mexico City). Only the animals with fasting blood glucose levels  $\geq 200$  mg/dL were selected for the study. Treatment began on the day the diabetic state was ascertained.

### Termination of Experiment and Sample Collection

After the experimental period, the rats were sacrificed and blood was collected through cardiac puncture, then centrifuged and the sera were used for the evaluation of serum total protein, albumins, bilirubin (Direct and total) and globulin.

### Determination of Biochemical Parameters

**Estimation of Total Protein:** The most widely used method for measuring serum protein is the biuret reaction [20],

which was adopted for this estimation. The principle of this reaction is that serum proteins react with copper sulphate in sodium hydroxide to form a violet biuret complex. The intensity of the violet colour was measured using a DRE 3000 HACH spectrophotometer and is proportional to the concentration of protein [21].

**Estimation of Albumin:** Albumin is generally measured by a dye-binding technique that utilizes the ability of albumin to form a stable complex with bromocresol green dye [20]. The absorbance of the samples and of the standard was measured against reagent blank at 546 nm, and temperature of 37°C. These tubes and their contents were mixed and incubated for 90 minutes at 37°C. Estimation of albumin level (g/dL) was obtained using a DRE 3000 HACH spectrophotometer.

**Estimation of Globulin:** Since, bromocresol green (BCG)-albumin complex absorbs light at a different wavelength from the unbound dye, the method may overestimate albumin by binding to other proteins [20]. Hence, the total globulin fraction is generally determined by subtracting the albumin fraction from the total protein fraction.

### Estimation of Bilirubin (Total and Direct)

Serum bilirubin was estimated by the method described by Jendrassik and Grof [22].

### Statistical Analysis

Statistical differences between the mean was analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows, version 19.0. Resulting data were represented as mean and standard deviation. Differences in mean were considered significant at  $P < 0.05$ .

### Results

The result in table 1 indicates the effect of petroleum ether extract of unripe *C. papaya* on liver functions in streptozotocin-induced diabetic rats, it was observed that the extract produced a significant increase ( $P < 0.05$ ) in the concentration of serum total protein as seen in group III (DPET). This elevation was the highest, followed by group IV (DSTD) when compared to the diabetic controls, and non-significant difference ( $P \leq 0.05$ ) in group V compared to the normal control. Also, the extract produced a significant increase ( $P < 0.05$ ) in the concentration of serum albumin in group III (DPET), this was followed by group IV (DSTD) compared to the diabetic control but showed non-significant difference in group V (NPET) compared to the normal control.

More so, the extract produced a significant decrease ( $P > 0.05$ ) in the concentration of total bilirubin in group III (DPET); this was followed by group IV (DSTD) which received the standard drug when compared to the diabetic control, and non-significant difference in group V when compared to the normal control (Table 1). Likewise, the extract produced

a significant decrease ( $P>0.05$ ) in the concentration of direct bilirubin in group III (DPET), this was followed by group IV (DSTD), which received the standard drug when compared to the diabetic control, and non-significant difference in group V when compared to the normal control. However, the extract

produced a significant increase ( $P<0.05$ ) in the concentration of globulin in group III (DPET); this was followed by group IV (DSTD), which received the standard drug when compared to the diabetic control, and non-significant difference in group V when compared to the normal control (Table 1).

GROUPS	TP (g/dL)	ALB (g/dL)	TB (g/dL)	DB (g/dL)	GLOBULIN (g/dL)
NC	44.45±0.28 <sup>e</sup>	23.95±0.47 <sup>e</sup>	5.46±0.07 <sup>a</sup>	4.28±0.07 <sup>ab</sup>	20.75±0.45 <sup>d</sup>
DC	25.27±0.46 <sup>a</sup>	9.90±0.45 <sup>a</sup>	7.31±0.11 <sup>c</sup>	6.45±0.17 <sup>f</sup>	13.69±0.30 <sup>a</sup>
D + PET	35.18±0.60 <sup>c</sup>	19.38±0.78 <sup>b</sup>	6.25±0.23 <sup>c</sup>	5.41±0.25 <sup>e</sup>	16.05±0.42 <sup>b</sup>
D + STD	32.96±0.73 <sup>b</sup>	21.58±0.88 <sup>c</sup>	5.86±0.16 <sup>b</sup>	4.84±0.41 <sup>d</sup>	17.79±0.40 <sup>c</sup>
N + PET	43.49±0.52 <sup>d</sup>	22.91±0.70 <sup>d</sup>	5.47±0.07 <sup>a</sup>	4.45±0.22 <sup>abc</sup>	21.24±0.36 <sup>d</sup>

**Table 1:** Result showing effect of petroleum ether extract of unripe *Carica papaya* seed on liver functions in streptozotocin (STZ) induced diabetic rats.

Values are expressed as Mean ± SD. Identical superscript (i.e. a) means there is no significant difference between the comparing group  $P>0.05$ . Non-identical superscripts (i.e. a,b,c,d,e) means there is significance between the comparing groups at  $P<0.05$ .

**Legend:** NC: Normal control; DC: Diabetic control; D+PET: Diabetic and 200 mg Petroleum Ether extract; D+STD: Diabetic and standard drug and N+PET: Normal Control and 200 mg Petroleum Ether extract; TP: Total protein; ALB: Albumin; TB: Total bilirubin; and DB: Direct bilirubin.

## Discussion

Diabetes mellitus is age-long disease and medicinal plant extracts have remained rich source of therapeutically useful phytoconstituents and viable alternative in combating various diseases plaguing the mankind [23]. In cooperation, human and experimental diabetes have been shown to exert certain level of liver dysfunction [24-26]. Regardless of the availability of approved antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continue to be a major medical problem [27]. Streptozotocin (STZ) is the most common well established chemical model used for induction of experimental diabetes. It has proven to be a better diabetogenic agent than alloxan as it is linked with wider species effectiveness and reproducibility. Its diabetogenic action is mainly due to the DNA alkylating activity of its methylnitrosourea moiety, release of nitric oxide from the nitroso group in its further course of action and generation of reactive oxygen species (ROS) [28,29].

It is important to note that most compounds absorbed by the intestine pass through the liver, making the liver to function as a control center that integrates various metabolic processes and regulating the traffic of biological fuel molecules such as the carbohydrates [30,31]. The liver plays an important role in metabolism, detoxification and biotransformation. The liver is one of the major organs usually affected by xenobiotics [32]. An alteration in the biomarkers of the liver function indices might be used to monitor the level of injury or damage by the plant extract

before biopsy [33]. In this study, the functional integrity of the liver was assessed by investigating the levels of liver biomarkers such as total protein, albumin, bilirubin (total and direct) and globulin.

Total protein is a biochemical investigation for assessing the total amount of protein in serum [34]. Low total protein concentrations may be a sign of immunodeficiency, whereas those above the reference range are found in paraproteinaemia, Hodgkin's lymphoma, leukaemia or any condition causing an increase in immunoglobulins [35]. Albumin is the protein with the highest concentration in the plasma. It transports many molecules in the blood. It prevents the fluid in the blood from leaking out the tissue into the extravascular spaces [36,37]. Serum albumins concentration reflects the synthetic capacity of the liver. Factors that stimulate albumin synthesis include the action of hormones such as insulin and growth hormone, but it can be inhibited by pro-inflammatory mediators such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor [38]. Globulins are a heterogeneous group of large serum proteins other than albumins, these include clotting proteins, complement, many acute phase proteins, immunoglobulins, and lipoproteins [39]. Some globulins are produced in the liver, while others are made by the immune system. In this study, elevated levels of total protein, albumin and globulin concentrations were observed in groups treated with the plant extract and the standard drug (glibenclamide), thereby suggesting that the treatment of diabetes mellitus with petroleum ether extract of unripe *Carica papaya* seed did not alters the liver ability to mobilize proteins rather produced an increase in protein

synthesis.

However, the observed elevation in serum total protein maybe due to marked change in circulating amino acid level, hepatic amino acid uptake and muscle output of amino acid concentrations [40]. These findings appear to validate previous studies [33,41-44]. Although there are reports of decreases in serum total proteins, albumin and globulin in STZ-diabetic rats [45-47]. Also, the observed elevation in serum albumin is an indication that the extract may promote good functioning of the liver thereby possess a hepatoprotective property and may also help calcium in the blood stream to regulate the movement of water into body tissue. The observed elevation in globulin level may indicate the efficiency of the plant extract to produce antibody or due to the phytoconstituent of the plant extract [33].

Bilirubin occurs in the normal catabolic pathway when the body breaks down haemoglobin, which is the protein in red blood cells that carries oxygen. This process is necessary in the body for the clearance of waste products that arise from the destruction of aged or abnormal red blood cells [48]. It is excreted in bile and urine, and elevated levels may indicate certain diseases [49]. Bilirubin (direct and total) levels were significantly ( $p > 0.05$ ) decrease in groups treated with the plant extracts and standard drug (glibenclamide) compared with the diabetic control. These finding accords to the previous studies by Oze et al. [50] and Omonkhua et al. [51] using different plant extracts. Direct bilirubin is any form of bilirubin which is water-soluble; it is often made up largely of conjugated bilirubin, but some unconjugated bilirubin (up to 25 %) [52]. Total bilirubin measures both conjugated bilirubin and unconjugated bilirubin [53]. Total and direct bilirubin levels can be measured from the blood [54]. The reduction in bilirubin (total and direct) levels is an indication that the extract might not induce injury to the hepatic tissue or caused conjugated hepatobiliary injury on the Wistar rats.

## Conclusion

This present study suggests that the plant extract exhibit the potential to prevent hepatic dysfunction by increasing the concentration of serum protein, albumin and globulin which is an indication of enhanced liver functionality.

## Disclosure of Conflict of Interest

The authors declare that there is no conflict of interests.

## Ethical Approval

Ethical approval was secured from CRUTECH Institutional Research Ethical Committee (IREC) in accordance with

principle laid down in the Declaration of Helsinki (1964), as revised in 2013 and National Institute of Health (NIH) Principles of Laboratory Animal Care. No human participants were involved in the study.

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