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

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Abstract

Diabetes mellitus and liver diseases are regarded as the most causes of morbidity and mortality in human globally. In this study, antidiabetic effect and liver functions were evaluated in streptozotocin-induced diabetic rats treated with petroleum ether extract of unripe *Carica papaya* seed. Experimental diabetes was induced by a single intravenous injection of 60 mg/kg STZ freshly dissolved in 0.01 M citrate buffer, animals with fasting blood glucose level >200 mg/dL were considered diabetic. Glibenclamide (5 mg/kg) was used as a standard drug. Fasting blood glucose and body weight were used to assess the antidiabetic effect. The treatment lasted for twenty-one days. 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 ($P\geq0.05$) difference in total protein, albumin, bilirubin (total and direct), and globulin was observed in group V (NCPET) compared to normal control. Fasting blood glucose level significantly ($P>0.05$) decreased in group III (DPET) and IV (DSTD) compared with the initial blood glucose level at the beginning of the experiment. Body weight significantly ($P<0.05$) increase in all groups compared to the diabetic control with highest weight gain seen in group III (DPET). Conclusively, the use of unripe *Carica papaya* seed extract may be effective in the treatment of diabetes mellitus as it does not have any damaging effect on the liver as revealed in the study.

Keywords: *Carica papaya*; Diabetes mellitus; Fasting blood glucose; Total protein; Albumin; Globulin; Bilirubin

Abbreviations: STZ: Streptozotocin; CRUTECH: Cross River University of Technology; IREC: Institutional Research Ethical Committee; NIH: National Institute of Health; DC: Diabetic Control; DPET: Diabetic and 200mg Petroleum Ether Extract of unripe *Carica papaya* seed; DSTD: Diabetic and standard Drug; NPET: Normal Control and 200mg Petroleum Ether Extract of unripe *Carica papaya* seed; BCG: Bromocresol Green; ANOVA: One-Way Analysis of Variance; ROS: Reactive Oxygen Species; NC: Normal Control.

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, β-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 antidiabetic 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 1: Normal control (NC), Group 2: Diabetic control (DC), Group 3: Diabetic and 200 mg Petroleum Ether extract of unripe *Carica papaya* seed (DPET), Group 4: Diabetic and standard drug (glibenlamide) (DSTD), Group 5: 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 ≥ 200 mg/dL were selected for the study. Treatment began on the day the diabetic state was ascertained. Blood glucose level and body weight were determined weekly for three weeks throughout the period of the experiment.

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 Body Weight/Fasting Blood Glucose

Fasting blood glucose level was determined by using glucometer (Accu-Chek Sensor Comfort) and test strips by glucose oxidase method. Body weight was determined using a digital weighing balance (Denver, Model: IR-30). These were done weekly for three (3) weeks.

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 $P<0.05$.

Result

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 3

(DPET). This elevation was the highest, followed by group 4 (DSTD) compared to the diabetic control, and non-significant difference (P≤0.05) in group 5 compared to the normal control (Table 1). Also, the extract produced a significant increase (P<0.05) in the concentration of serum albumin in group 3 (DPET), this was followed by group 4 (DSTD) compared to the diabetic control but showed non-significant difference in group 5 (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 3 (DPET); this was followed by group 4 (DSTD) which received the standard drug compared to the diabetic control, and non-significant difference in group 5 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 3 (DPET), this was followed by group 4 (DSTD), which received the standard drug compared to the diabetic control, and non-significant difference in group 5 compared to the normal control. However, the extract produced a significant increase (P<0.05) in the concentration of globulin in group 3 (DPET); this was followed by group 4 (DSTD), which received the standard drug compared to the diabetic control, and non-significant difference in group 5 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^{e}	23.95±0.47^{e}	5.46±0.07^{a}	4.28±0.07^{ab}	20.75±0.45^{d}
DC	25.27±0.46^{a}	9.90±0.45^{a}	7.31±0.11^{c}	6.45±0.17^{f}	13.69±0.30^{a}
D + PET	35.18±0.60^{c}	19.38±0.78^{b}	6.25±0.23^{c}	5.41±0.25^{e}	16.05±0.42^{b}
D + STD	32.96±0.73^{b}	21.58±0.88^{c}	5.86±0.16^{b}	4.84±0.41^{d}	17.79±0.40^{c}
N + PET	43.49±0.52^{d}	22.91±0.70^{d}	5.47±0.07^{a}	4.45±0.22^{abc}	21.24±0.36^{d}

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.

The result in table 2 indicate the blood glucose level of streptozotocin-induced diabetic rats; it was revealed that the groups that received the extract (group 3) and standard drug (group 4) produced a significant decrease (P<0.05) in blood glucose level at the end of the experiment, compared to the diabetic control. However, non-significant difference (P≤0.05) was revealed in group 5 compared to the normal control (Table 2). The percentage difference of blood glucose level was evaluated in table 3. Group 3 (DPET) and group 4 (DSTD) progressive decrease in blood glucose level (-21%) compared to its high levels at the beginning of the experiment when determination of the percentage difference was carried out. The diabetic control (group 2) had drastic increase (-11%) in the blood glucose level. However, non-significant difference (1%) was revealed in group 5 compared to the normal control (Table 3).

Groups	D0	D7	D14	D21
NC	82.00±3.54^{a}	84.40±5.32^{a}	85.20±4.21^{a}	83.20±5.07^{a}
DC	254.60±9.17^{d}	264.00±13.56^{e}	275.60±19.19^{e}	284.80±20.95^{c}
D + PET	253.20±5.07^{bc}	233.40±12.34^{bc}	216.80±10.78^{b}	204.60±8.53^{b}
D + STD	262.20±5.07^{c}	242.80±6.69^{cd}	231.40±8.20^{cd}	210.80±6.72^{b}
N + PET	79.80±11.30^{a}	80.80±8.35^{a}	83.00±9.77^{a}	81.20±7.40^{a}

Table 2: Blood glucose level of 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; D0: Day zero; D7: Day seven; D14: Day fourteen; and D21: Day fourteen.

Groups	Initial blood glucose	Final blood glucose	Average blood glucose	Blood glucose difference	Percentage difference
NC	82.00±3.54[a]	83.20±5.07[a]	82.6±4.30[a]	1.2	1%
DC	254.60±9.17[d]	284.80±20.95[c]	269.7±15.33[c]	30.2	11%
D + PET	253.20±5.07[bc]	204.60±8.53[b]	228.9±6.80[b]	-48.6	-21%
D + STD	262.20±5.07[c]	210.80±6.72[b]	236.5±5.89[b]	-51.4	-21%
N + PET	79.80±11.30[a]	81.20±7.40[a]	80.5±9.35[a]	1.4	1%

Table 3: Result showing percentage difference of the blood glucose level of 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.

The result in table 4 indicate the body weight of streptozotocin-induced diabetic rats; it was revealed that the body weight of the rats in all groups significantly increases (P<0.05) except that of the diabetic control (group 2). Group 3 (received the extract) produced the highest body weight. Non-significant difference (P≤0.05) was revealed in group 5 compared to the normal control (Table 4). The percentage difference was evaluated in table 5. Group 3 (DPET) progressive increase in body weight (15%) more than the other groups compared to their initial weights at the beginning of the experiment. The diabetic control (group 2) had drastic decrease (-24%) in the body weight. Group 1 (NC) was used as the reference for the body weight gain, in which all the groups except group 2 (diabetic control) showed great improvement (Table 5).

GROUPS	D0	D7	D14	D21
NC	164.60±6.34[a]	173.20±8.90[b]	181.40±4.21[a]	191.20±3.11[bc]
DC	168.20±9.12[a]	150.20±6.69[a]	142.00±7.11[a]	131.60±2.88[a]
D + PET	168.60±11.59[a]	174.60±10.26[b]	183.80±8.44[b]	196.80±12.52[c]
D + STD	168.60±4.39[a]	176.60±2.51[b]	184.80±2.77[b]	193.40±4.39[c]
N + PET	170.60±3.85[a]	180.40±6.02[b]	190.60±5.59[b]	196.40±7.23[c]

Table 4: Body weight of 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; D0: Day zero; D7: Day seven; D14: Day fourteen; and D21: Day fourteen.

GROUPS	Initial body weight	Final body weight	Average body weight	Body weight difference	Percentage difference
NC	164.60±6.34[a]	191.20±3.11[bc]	177.9±4.72[b]	26.6	14%
DC	168.20±9.12[a]	131.60±2.88[a]	149.9±6.00[a]	-36.6	-24%
D + PET	168.60±11.59[a]	196.80±12.52[c]	182.7±12.05[c]	28.2	15%
D + STD	168.60±4.39[a]	193.40±4.39[c]	181.0±4.39[b]	24	13%
N + PET	170.60±3.85[a]	196.40±7.23[c]	183.5±5.54[c]	25	13%

Table 5: Result showing percentage difference of the body weight of 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.

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]. Streptozotocin (STZ) induced diabetes mellitus is characterized by loss of body weight and increase in blood glucose level. Likewise, in the present study, streptozotocin (STZ) caused an enormous loss of body weight and increase of blood glucose in the diabetic induced control groups. However, the unripe *Carica papaya* seed extract administered to STZ-induced rats significantly ameliorated hyperglycemia-mediated damages by diminishing the blood glucose level and elevating body weight compared to the diabetic control group, indicating that the unripe *Carica papaya* seed extract possess antidiabetic effect. This antidiabetic effect of unripe *Carica papaya* seed extract maybe possibly due to the similar mechanism of action as glibenclamide that boost the release of insulin from the pancreatic beta cells. Few researchers have confirmed this similar mode of action using different plant extracts [15,30-33], thereby validating our study.

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 [34,35]. The liver plays an important role in metabolism, detoxification and biotransformation. The liver is one of the major organs usually affected by xenobiotics [36]. 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 [37]. 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 [38]. 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 [39]. 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 [40,41]. 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 interlukin-6 (IL-6), interlukin-1 (IL-1) and tumor necrosis factor [42]. Globulins are a heterogeneous group of large serum proteins other than albumins, these include clotting proteins, complement, many acute phase proteins, immunoglobulins, and lipoproteins [43]. 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 management 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 [44]. These findings appear to validate previous studies [37, 45-48]. Although there are reports of decreases in serum total proteins, albumin and globulin in STZ-diabetic rats [49-51]. 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 [37].

Bilirubin occurs in the normal catabolic pathway when the body breaks down hemoglobin, which is the protein in red blood cells that carries oxygen. This process is a necessary in the body for the clearance of waste products that arise from the destruction of aged or abnormal red blood cells [52]. It is excreted in bile and urine, and elevated levels may indicate certain diseases [53]. Bilirubin (direct and total) levels were significantly ($p>0.05$) decrease in groups treated with the plant extract and standard drug (glibenclamide) compared with the diabetic control. These finding accords to the previous studies by Oze et al. [54] and Omonkhua et al. [55] 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%) [56]. Total bilirubin measures both conjugated bilirubin and unconjugated bilirubin [57]. Total and direct bilirubin levels can be measured from the blood [58]. 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. The plant extract is effective in the management of diabetes mellitus as it does not have any damaging effect on the liver.

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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