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Comparative study of aqueous, methanol and petroleum ether extracts of unripe *Carica papaya* seed on liver and kidney function in streptozotocin-induced diabetic rats

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Abstract

Background: Diabetes as chronic disease has serious complications which can affect multiple vital organ systems, thereby leading to more severe and irreversible pathological conditions. This study investigated the hepatoprotective and nephroprotective potentials of unripe *Carica papaya* (UCP) seed extract in diabetic rats using three different solvents.

Methods: Thirty adult male Wistar rats were used. Twenty-five out of thirty were induced with diabetes following an overnight fast, by a single intravenous injection of 60 mg/kg STZ. The rats were grouped into six groups (n=5):NC: normal control, DC: diabetic control, DSTD: diabetic and treated with glibenclaimide, aqueous (DAUCP), methanol (DMUCP) and petroleum ether (DPEUCP) rats were induced but treated with 200 mg of aqueous, methanol and petroleum ether extract of UCP seed extract respectively. The extracts were administered to the animals orally for 21 days.

Results: The animals administered with different extracts showed significant decrease ($P<0.05$) in blood sugar level, ALT, AST, ALP, γ-GT, urea, creatinine, BUN, total and direct bilirubin and an increase level in total protein, albumin, globulin and sodium, potassium, bicarbonate and chloride when compared to the diabetic control group.

Conclusion: It can be inferred from that data that the extracts have hepatoprotective and nephroprotective potentials on the liver and kidney. This implies that unripe *C. papaya* seed can be effectively used in the management of diabetes.

Keywords: Diabetes; *Carica papaya*; Hepatoprotective; Nephroprotective; Hyperglycaemia

1. Introduction

Diabetes mellitus (DM) is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. Concern regarding this chronic disease is the complications which can affect multiple vital organ systems, thereby leading to more severe and irreversible pathological conditions such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular diseases, as well as hepatopathy[1-3]. Research indicates that DM is associated with a number of liver abnormalities, such as abnormal glycogen deposition, non-alcoholic fatty liver disease (NAFLD), fibrosis, cirrhosis, hepatocellular carcinomas (HCCs),

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abnormal elevated hepatic enzymes, acute liver disease and viral hepatitis[4-6]. A fatty liver and hyperglycaemia can destroy the hepatocytes and contribute to increased morbidity and mortality among diabetic patients[4,7].

The burden of diabetes mellitus (DM) has increased globally. In 2019, approximately 463 million adults aged 20–79 years were living with diabetes worldwide[8], causing an estimated 1.5 million deaths[9]. This number is expected to rise to 700 million by 2045[8]. The burden of DM in terms of prevalence and number has risen dramatically, particularly in low-income and middle income countries[9-10].

During diabetes, the liver has been reported to be affected due to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin in the liver cells while the kidney has been reported to increase in weight due to glucose over-utilization and subsequent enhancement in glycogen synthesis[11], lipogenesis and protein synthesis. These changes may lead to serious microvascular renal complications, which involves a series of metabolic changes in the pathogenesis of diabetic nephropathy[12-14].

Plants and plant-based products have been employed to prevent various human diseases since ancient times. Papaya (*Carica papaya* Linn.) belongs to the family Caricaceae and is well known for its therapeutic and nutritional properties all over the world[15]. The papaya plant is perennial usually unbranched, smooth stem and long-stalked leaves are having 5–6 lobes and can grow up to 20 m in height[16]. Different parts of papaya plant viz. fruit, bark, roots, seeds, peel, pulp, and leaf have many known therapeutic uses around the world[17-18]. There is no data on the effect of unripe *C. papaya* seed on different organ functions and glycaemic control using solvents of different polarities. In this study, we investigated the nephroprotective and hepatoprotective potentials of aqueous, methanol and petroleum ether extracts of unripe *Carcia papaya* seed in streptozotocin-induced diabetic rats and comparison of these different extracts to ascertain the most potent in the management of diabetes.

2. Material and methods

2.1. Chemicals/Reagents

All chemicals and reagents used in this research were of analytical grade. Streptozotocin (STZ) was purchased from Sigma chemicals, (St. Louis, USA), others were obtained from Merck, United States while Kits for different enzyme assays were purchased from Biosystems S.A., Mexico.

2.2. Plant material

Unripe fruits of *Carica papaya* were harvested from local farm at Okuku Yala Local Government Area of Cross River State, Nigeria. The plant was identified and authenticated by Dr. Michael Eko, a botanist in the Department of Biological Sciences, University of Calabar and a voucher specimens number 73 was deposited in the Herbarium, Department of Botany, University of Calabar, Nigeria. The fruits were cut into pieces and the seeds removed and thoroughly washed and dried at room temperature. Dried seeds were crushed and ground to powder using a domestic mixer grinder (binatone BLG-450).

2.3. Extraction using aqueous and organic solvents

The aqueous extraction was performed by soaking 400 g of powdered *C. papaya* seed in 1 L of distilled water over 48 hours. The extract was filtered with Whatman filter paper no 1 (24 cm) and dried with Water Bath (DWB20-P DLAB) at 40˚C. The extract was kept frozen at -20oC for use. It was reconstituted in distilled water for administration.

The methanol and petroleum ether extraction were performed each by wrapping 400 g powder sample of *C. papaya* seeds in a thimble and placed in a 1000 cm3 Soxhlet extractor (Mand G Scientific Co., England). The samples were Soxhlet extracted following standard analytical laboratory method at 60 °C for 72 h. The extract was evaporated to dryness at 40 ˚C. The extract was kept frozen at -20 °C for use. It was reconstituted in Tween 80 for administration.

2.4. Animals

Thirty male Wistar rats weighing 130 to 160 g were used. The animals were maintained under laboratory conditions of temperature (23 to 25 °C) and light 12 h light-dark cycle in the Animal House of Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus and allowed free access to grower's mash and water *ad libitum*. The animals were acclimatized for two weeks. The experiment which lasted for 21 days was carried out according to the guideline procedures of the Animal House. The rats were maintained in accordance with the principles of laboratory

animal care guidelines[19]. The experiment protocol was designed according to the Departmental Animal Ethics Committee guidelines.

2.5. Induction of Diabetes

Overnight-fasted rats were induced with diabetes by a single intraperitoneal injection of 60 mg/kgbody weight of streptozotocin (STZ) freshly dissolved in citrate buffer (0.01 M, pH 4.5). Control animals received 0.9% sterile saline. Hyperglycemia was confirmed 3 days after injection by measuring the tail vein blood glucose level with an Accu-Chek Active (Roche Diabetes Care GmbH, Mannheim, Germany. Animals with fasting blood glucose levels ≥ 200 mg/dL and ≤ 450 mg/dL were considered diabetic and used for the study.

2.6. Experimental Design

Thirty male Wistar rats were used but the animals were divided into six groups, each group containing five animals (n=5).

- NC: Normal Control
- DC: Diabetic Control
- DAUCP: Diabetic and 200 mg Aqueous Extract of unripe *C. papaya* seed
- DMUCP: Diabetic and 200 mg Methanol Extract of unripe *C. papaya* seed
- DPEUCP: Diabetic and 200 mg Petroleum Ether Extract of unripe *C. papaya* seed
- DSTD: Diabetic and standard Drug (0.1 mg glibenclamide)

2.7. Duration of Treatment

Treatment began on the day the diabetic state was ascertained. Blood glucose level was determined weekly for three weeks throughout the period of the experiment. On the 21stday treatment the animals were fasted overnight, anesthetized and sacrificed by humane decapitation.

2.8. Determination of Fasting Blood Glucose Level

Fasting blood glucose levels were determined by using glucometer (Accu-chek Active) and test strips by glucose oxidase method. This was done weekly for three weeks.

2.9. Collection of blood sample

Blood was collected directly through cardiac puncture. Five (5) mL of blood was collected from each rat, 3 mL was transferred into plain tube and were centrifuged at 3000 g for 10 min to obtain serum for some biochemical analysis while the remaining 2 mL was transferred into EDTA bottle for other analysis.

2.10. Determination of Biochemical Parameters

Serum was used for the evaluation of biochemical parameters, including urea, creatinine, blood urea nitrogen, total bilirubin, direct bilirubin, total protein, albumin, globulin, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferaseand alkaline phosphatase, using commercial kits from Randox Laboratories, UK, according to the manufacturer's protocol. Other biochemical parameters were assayed with the following methods; sodium and potassium[20], chloride ion[21] and bicarbonate (HCO_3) was determined using Forrester et al[22].

2.11. Statistical Analysis

Data obtained was analysed using the SPSS statistical package, version 23 with one-way analysis of variance (ANOVA) and statistical significance established at $p< 0.05$. Data is expressed as the mean ± SD.

3. Results

Results of the effect of daily treatment of streptozotocin-induced diabetic rats with various extracts of unripe *Carica papaya* seed and glibenclamide are presented below.

3.1. Weekly blood glucose levels of streptozotocin-induced diabetic rats treated with various extracts of Unripe *Carica papaya* seed

Figure 1 shows the mean fasting blood glucose of experimental rats treated with the aqueous, methanol, petroleum ether extracts and glibenclamide. On day 0 there was a significant difference (p<0.05) between the normal control and other groups. Other groups except normal control exhibited hyperglycemia showing that induction of diabetics was successful. After the experimental period (3-week), STZ-diabetic rats exhibited significant (p<0.05) hyperglycemia compared with the control rats (Figure 1). The extracts and glibenclamide decreased blood glucose level in the diabetic rats compared to the untreated diabetic rats (P <0.05). On day 7 the glucose level of DC group increase when compared to the day 0 while in the treated groups it reduced. On day 21 the reduction in glucose level of DMUCP and DSTD were significant (p<0.05) when compared to DAUCP and DPEUCP. The reduction in glucose level of DMUCP and DSTD were statistically similar. Among the extracts, DMUCP seems to be more potent in the reduction of glucose.

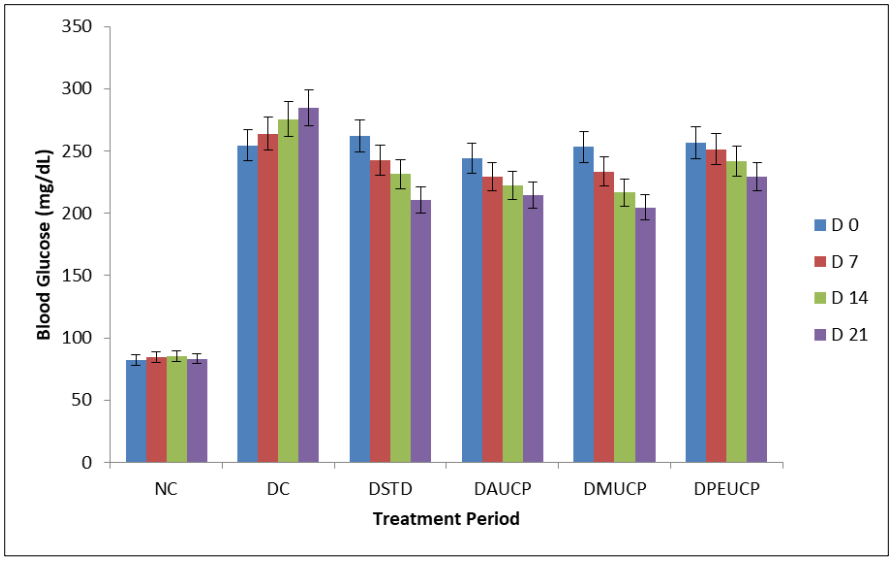

Figure 1 Weekly Blood Glucose Level of Rats treated with Aqueous, Methanol, Petroleum Ether extracts and Glibenclamide

3.2. Liver enzymes activities of streptozotocin-induced diabetic rats treated with various extracts of unripe *Carica papaya* seed.

In Table 1 there was a significant (p> 0.05) increase in AST, ALT, ALP and GGT in the diabetic control (DC) group when compared to other groups. AST and ALP of DMUCP were statistically similar when compared to the normal control. The extract reversed the alteration in the liver enzymes to normal. While DAUCP and DPEUCP reduced these liver enzymes but not statistically similar to the normal control. Furthermore, the levels GGT were statistically similar in DMUCP and DSTD. All these data indicate that methanol extract seems to be the most potent in protecting the hepatocytes. All the treated groups significantly (p< 0.05) reduced the elevated levels of the liver enzymes (AST, ALT, ALP and γ-GT) compared to the diabetic control.

3.3. Total Protein (TP), Albumin (ALB), Total Bilirubin (TB), Direct Bilirubin (DB) and Globulin (GLOB) Concentrations of Rats treated with Aqueous, Methanol, Petroleum Ether extracts and Glibenclamide

In Table 2 there was significant (p> 0.05) increase in TP, ALB and GLOB when compared to the diabetic control (DC). The levels of TP in DMUCP and DSTD were statistically similar. The increase in the level of TP was highest in DPEUCP which significantly differ when compared to other treated groups. The levels of ALB and GLOB were highest in DMUCP and differs significantly (p> 0.05) when compared to other treated groups. The levels of TB and DB were appreciably reduced in the treated groups as compared to the diabetic control group. The levels were statistically similar in DMUCP and DSTD. The liver function seems to be most improved in DMUCP group.

3.4. Kidney function indices of streptozotocin-induced diabetic rats treated with various extracts of unripe *Carica papaya* seed

From Table 3, urea, creatinine and blood urea nitrogen of diabetic control were significantly increased (p<0.05) when was compared to normal control. The treatment significantly reduced the levels of urea, creatinine and blood urea nitrogen. The level of decrease was highest in DMUCP when compared to other extract treated groups. The reduction in the level of creatinine was statistically similar among NC, DMUCP and DSTD. The reduction in blood urea nitrogen is statistically in similar among DMUCP and DSTD. Indicating that methanol extract seems to most potent in protecting the kidney.

Data on electrolytes indicates that bicarbonate, sodium, potassium and chloride levels of DC were significantly reduced when compared to the normal control. When treated groups were compared to the diabetic control, the treatment significantly (p> 0.05) increased the levels of these electrolytes except in the bicarbonate level of DAUCP. The increase in the level of bicarbonate was statistically similar in NC, DMUCP, DPEUCP and DSTD. Conversely the decrease observed in DC and DAUCP was statistically similar. This means that the aqueous extract does not have any significant effect on the bicarbonate level when compared to the non-treated diabetic group. The increase in the level of potassium was statistically similar in DMUCP, DPEUCP and DSTD. Furthermore, the increase was statistically similar in NC and DAUCP. The increase in the level of chloride was statistically similar in all the treated groups and normal control.

Table 1 Liver enzymes activities of streptozotocin-induced diabetic rats treated with various extracts of unripe *Carica papaya* seed

Group	AST (U/I)	ALT (U/I)	ALP (U/I)	γ-GT (U/I)
NC	27.38±0.80^{a}	24.04±1.27^{a}	35.27±0.71^{a}	10.63±0.39^{c}
DC	66.59±1.09^{d}	61.40±1.26^{f}	95.25±1.29^{e}	14.87±0.54^{d}
DSTD	33.02±0.72^{b}	28.47±0.36^{c}	48.56±0.46^{b}	8.69±0.47^{a}
DAUCP	32.94±0.45^{b}	44.83±0.96^{e}	53.99±0.78^{c}	9.31±0.47^{b}
DMUCP	26.65±0.49^{a}	25.64±0.52^{b}	34.65±0.49^{a}	8.48±0.26^{a}
DPEUCP	42.48±0.69^{c}	37.32±0.80^{d}	57.01±0.64^{d}	10.23±0.27^{c}

Values are mean ±SD (n=5). Values with different superscript on the same row are statistically different (P<0.05).NC: Normal Control; DC: Diabetic Control; DSTD: Diabetic + 0.1 mg glibenclamide; DAUCP: Diabetic + 200 mg Aqueous Extract of unripe C. papaya seed; DMUCP: Diabetic + 200 mg Methanol Extract of unripe C. papaya seed; DPEUCP: Diabetic + 200 mg Petroleum Ether Extract of unripe C. papaya seed.

Table 2 Total Protein, Albumin, Total Bilirubin, Direct Bilirubin and Globulin Concentrations of Rats treated with Aqueous, Methanol, Petroleum Ether extracts and Glibenclamide

Group	TP (g/d)	ALB (g/d)	TB (g/d)	DB (g/d)	GLOB (g/d)
NC	44.45±0.28^{e}	23.95±0.47^{e}	5.46±0.07^{a}	4.28±0.07^{a}	20.75±0.45^{d}
DC	25.27±0.46^{a}	9.90±0.45^{a}	7.31±0.12^{d}	6.45±0.17^{d}	13.69±0.30^{a}
DAUCP	34.29±0.39^{c}	20.63±0.29^{c}	6.23±0.08^{c}	5.40±0.07^{c}	16.74±0.90^{b}
DMUCP	33.19±0.54^{b}	21.64±0.41^{d}	5.70±0.27^{b}	4.67±0.33^{b}	17.64±0.54^{c}
DPEUCP	35.18±0.60^{d}	19.38±0.78^{b}	6.25±0.23^{c}	5.41±0.25^{c}	16.05±0.42^{b}
DSTD	32.96±0.73^{b}	21.58±0.88^{d}	5.86±0.16^{b}	4.84±0.41^{b}	17.79±0.40^{c}

Values are means ±SD of five replicate determinations. Values with different superscript on the same row are statistically different (P<0.05).NC: Normal Control; DC: Diabetic Control; DAUCP: Diabetic + 200 mg Aqueous Extract of unripe C. papaya seed; DMUCP: Diabetic + 200 mg Methanol Extract of unripe C. papaya seed; DPEUCP: Diabetic + 200 mg Petroleum Ether Extract of unripe C. papaya seed; DSTD: Diabetic + 0.1 mg glibenclamide.

Table 3 Kidney function indices of streptozotocin-induced diabetic rats treated with various extracts of Unripe *Carica papaya* seed

Group	Urea (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)	HCO_3^- (mmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
NC	27.21±1.01^a	1.85±0.48^a	20.39±0.53a	19.24±0.36^b	87.86±0.48^e	7.62±0.05^c	27.14±0.65^b
DC	42.71±0.42^e	2.44±0.23^b	60.77±0.48d	17.54±0.15^a	77.32±0.42^a	6.27±0.06^a	24.59±0.42^a
DSTD	31.10±0.44^c	2.00±0.16^a	36.95±0.93b	19.05±0.58^b	84.15±0.39^d	7.43±0.06^b	27.28±0.31^b
DAUCP	32.33±0.63^d	2.12±0.15^{ab}	38.77±0.55c	17.99±0.40^a	81.76±1.10^b	7.54±0.08^c	27.15±0.37^b
DMUCP	29.04±0.50^b	1.96±0.36^a	37.27±0.27b	19.10±0.47^b	83.19±0.63^c	7.41±0.12^b	27.29±0.28^b
DPEUCP	30.79±0.61^c	2.07±0.21^{ab}	38.43±0.55c	19.30±0.29^b	81.13±0.65^b	7.39±0.06^b	27.40±0.33^b

Values are mean ±SD (n=5). Values with different superscript (a, b, c, d, e) on the same row are statistically different (P<0.05).NC: Normal Control; DC: Diabetic Control; DSTD: Diabetic + 0.1 mg glibenclamide; DAUCP: Diabetic + 200 mg Aqueous Extract of unripe C. papaya seed; DMUCP: Diabetic + 200 mg Methanol Extract of unripe C. papaya seed; DPEUCP: Diabetic + 200 mg Petroleum Ether Extract of unripe C. papaya seed.

4. Discussion

Diabetes affects many organs, including the liver, which plays a key role in the regulation of carbohydrate, lipid, and protein metabolism[4,6,23,24]. Elevated serum aminotransferases level; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltransferase (γ-GT) which was observed in this study due to induction of diabetes was in line with other precious findings on diabetes[25-27]. Several studies have shown the elevation of these enzymes in diabetic patients[26,28,29,30]. Alanine aminotransferase and aspartate aminotransferase are the most specific markers of hepatic injury, which is located in the hepatocellular cytosol and mitochondria, respectively[31-33]. The administration of the extracts showed a significant declined in activities of AST, ALT, ALP and γ-GT. Though an increase in γ-GT concentration has been regarded as a marker of alcohol-associated liver disease than diabetes associated hepatic injury[34]. But elevated level of γ-GT can account for liver injury when considered along other biomarkers. Glycation is the most common complication of diabetes that results from oxidative stress in tissue[35]. This oxidative stress and cytokine production in the liver cause alterations of liver enzymes due to the hepatocellular damage[36,37]. Thus, further results dysregulation of blood glucose maintenance, since it plays a key role in such maintenance[38]. This condition results in the abnormal introduction of liver enzymes into the circulation and become elevated.

The administration of the extracts showed significant increases in values of total protein, albumin and globulin while significant declined were observed in AST, ALT, ALP and γ-GT. The improvement in the function of the liver might be because of the ability of the extracts to significantly reduce the high blood glucose levels which eventually led to reverse in the alteration of hepatocytes. It also observed that methanol extract proved most potent in this amelioration.

The kidneys remove metabolic wastes such as urea, uric acid, creatinine and ions and thus optimum chemical composition of body fluids is maintained. The concentrations of these metabolites increase in blood during renal diseases or renal damage associated with uncontrolled diabetes mellitus. Blood urea and creatinine are considered as significant markers of renal dysfunction[39,40,41]. Observed increase in urea and creatinine level in the diabetic control were reduced following the administration of the extracts of UCP when compared to the diabetic control. Due to continuous catabolism of amino acid during diabetic state, high quantity of urea will be formed from urea cycle.

Diabetic nephropathy is a major long-term complication affecting approximately 30% of patients with type 1 diabetes (T1D) and 40% of those with type 2 diabetes (T2D)[42,43]. In this present study, kidney damage was assessed via kidney function parameters, including urea, creatinine, blood urea nitrogen (BUN) and some selected electrolytes. Creatinine is a breakdown product of creatine phosphate in muscle and its clearance rate from blood to urine correlates with glomerular filtration rate[44]. Therefore, creatinine clearance rate can be used as an indicator for kidney function[45]. In this study the urea, creatinine and blood urea nitrogen level increased significantly while sodium, potassium, bicarbonate and chloride significantly reduced in diabetic control group. Treatment with the extracts ameliorated the kidney dysfunctions by the reduction in the level of urea, creatinine and BUN, indicating the protective effects of the extracts in rats. Methanol extract was most effective among three extracts in reversing the alteration observed in the kidney.

Electrolytes in the serum are crucial in metabolic activities, systematic operation of cells and enzymes, and concentration gradient[46,47]. Serum concentration of electrolytes have been shown to change with plasma glucose levels.

Alterations in their concentrations designate development of various diseases. Diabetes is characterized by increased volume and metabolites excretion via the kidney, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes. Diabetic patients may experience disorganization of water, and electrolytes balance evolved from insulin inadequacy, hyperglycemia, and hyperketonemia[48,49]. Diabetes mellitus which causes hyperglycemia, finally resulted in cell dehydration and movement of K^+ ions into extracellular fluid (ECF). This process intensified the activity of parietal cells of distal and cortical collecting tubules, resulting in increased renal excretion of K^+. However, glycosuria discovered in diabetes leads to excretion of abundant water, Na^+, and K^+ in urine. Hence, it is evidenced that electrolytes and water loss associated with diabetes would result in loss of ECF, resulting in loss of Na^+ and K^+ concentration[50,51]. Hyponatremia is a decrease in serum Na^+ concentration which is often observed in uncontrolled hyperglycemia and it may result in cognitive impairment, osteoporosis and fractures[52]. Since, glucose is an osmotically active substance, the hyperglycemia which occur in diabetes increases serum osmolality, resulting in movement of water out of the cells and subsequently causing a reduction of serum sodium concentration (Na^+) by dilution[53].

Studies have shown that the incidence of hypokalemia in diabetes mellitus is higher than in general population[54]. This hypokalemia may be due to the redistribution of K^+ from the extracellular to intracellular fluid compartment, gastrointestinal loss due to malabsorption syndromes or K^+ loss through osmotic diuresis[55]. From this study, administration of the extracts increased the K^+ concentration in diabetes mellitus and this implies that the extracts may be acting through the pathways that ameliorate hypokalemia.

The function of Na^+/K^+-ATPase, Ca^{2+}/ Mg^{2+}-ATPase, Na^+/Ca^{2+} exchanger, and Ca^{2+} pump established in cell membrane, mitochondria, and endoplasmic reticulum has been diminished in hyperglycemia. It was also observed that diabetic ketoacidosis initiated promotion in the level of Cl^- due to serum glucose generation via gluconeogenesis, glycogenolysis, ketogenesis, and ketoacidosis, resulting in blood acidification causing acid–base imbalance in body. Therefore, to balance it, Cl^- level is increased in the body[56,57,58]. There was a significant change in serum chloride and bicarbonateion concentrations in all the treated groups when compared to the diabetic control. However, in the present study, it was discovered that extracts revised the electrolyte imbalance significantly by increasing the diminished levels of Na^+, K^+ in treated rats.

The significant increase in Na^+ concentration observed in treated groups from our study may be due to its ability to stimulate glucose excretion by increase the rate of filtration and preventing the reabsorption of glucose in the renal tubular cells of the kidneys[47,59,60]. Glucose being an osmotically active substance may therefore cause more water to be excreted through the kidney therefore, elevating the concentration of Na^+ in the blood serum[53]. The extracts administration reversed the status of the electrolytes and exhibited properties capable of boosting the buffering function of the body system.

5. Conclusion

Findings from the present work indicate that the extracts of unripe *Carica papaya* have hepatoprotective and nephroprotective potentials because the integrity of the liver and kidney tissues altered by induction of diabetes were revised by the extracts.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Ugwu Melvin Nnaemeka, Ijeomah Ann Ukamaka, Usin Saviour God'swealth and George Regina Resame declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

Statement of ethical approval

The ethical approval is obtained from Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku, Cross River State, Nigeria Animal Researches Ethic Committee in the session held on 24.01.2022 (decision number 2022.08.12).

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