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Antioxidant-Mediated Hepatoprotective Effects of Crude Ethanolic Extract of *Carica papaya* Leaves in Streptozotocin-Induced Diabetic Wistar Rats

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ABSTRACT
This study investigates the antioxidant-mediated hepatoprotective effects of ethanolic extract of *Carica papaya* leaves (EECP) in streptozotocin (STZ)-induced diabetic Wistar rats. Forty male albino Wistar rats were randomized into five groups (n = 8): normal control, STZ-induced diabetic, STZ + metformin (500 mg/kg), STZ + EECP (200 mg/kg), and STZ + EECP (400 mg/kg). Diabetes was induced with a single intraperitoneal injection of STZ (50 mg/kg). EECP or metformin was administered daily for 21 days. Fasting blood glucose (FBG), body weight, liver function markers (ALT, AST, ALP, total protein, albumin, bilirubin), antioxidant biomarkers (SOD, CAT, GSH, MDA), and liver histopathology were assessed. EECP mitigated STZ-induced weight loss, hyperglycaemia, and hepatic dysfunction in a dose-dependent manner. The 200 and 400 mg/kg EECP treatments notably reduced FBG levels (to 7.04 ± 1.42 mmol/L and 8.61 ± 1.52 mmol/L, respectively) compared to diabetic controls (15.37 ± 0.44 mmol/L, $p < 0.05$). EECP also significantly restored liver enzyme activities—especially at 400 mg/kg—reducing AST (79.68 ± 4.78 U/L) and ALT (39.50 ± 7.12 U/L) towards normal levels. Hepatic antioxidant status was markedly improved, with elevated SOD (78.41 ± 2.74 u/mL), CAT (41.07 ± 1.47 u/mg Protein), and GSH (6.75 ± 0.58 mM), and reduced MDA levels (8.31 ± 0.74 uM) in treated groups. Histological evaluation confirmed preservation of liver architecture and reduced inflammatory infiltration in EECP-treated rats. Crude ethanolic extract of *Carica papaya* leaves confers hepatoprotective and antioxidant effects in diabetic rats, via mitigation of oxidative stress and restoration of hepatic function.

Keywords:
Antioxidant,
Carica papaya,
Liver enzymes,
Diabetes,
Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a long-term metabolic condition marked by sustained elevation of blood glucose levels, which may result from deficient insulin production, resistance to insulin activity, or a combination of both mechanisms (Alam *et al*., 2021). Type 1 diabetes mellitus (T1DM) primarily develops due to autoimmune destruction of pancreatic β-cells, while Type 2 diabetes mellitus (T2DM), the more common variant, is mainly associated with insulin resistance and progressive impairment of β-cell function (Kumar *et al*., 2020). Given its fundamental role in regulating carbohydrate and lipid metabolism, the liver is highly susceptible to diverse systemic complications linked to both forms of diabetes (Dilworth *et al*., 2021).

The 10th edition of the International Diabetes Federation (IDF) Diabetes Atlas reports that an estimated 537 million adults worldwide are currently living with diabetes, a number projected to increase to 643 million by 2030 and further to 784 million by 2045 (Saeedi *et al*., 2019). Notably, the majority of cases (81%) occur in low- and middle-income countries, where the prevalence of underdiagnosis, approximately 44%, intensifies the health burden, particularly in resource-constrained regions (Patel, 2023). In Nigeria alone, more than 3.6 million individuals were affected in 2021, with diabetes accounting for about 48,375 deaths during the same year (Orji *et al*., 2024).

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Oxidative stress, characterized by excessive generation of reactive oxygen species (ROS) that surpass the capacity of antioxidant defense mechanisms, is recognized as a key pathophysiological driver of diabetic complications, particularly within the liver. This has stimulated increasing research into therapeutic agents with antioxidant properties. Carica papaya (C. papaya), a tropical plant belonging to the family *Caricaceae*, has a long history of use in traditional medicine for the management of diabetes, liver disorders, and inflammatory conditions (Alara *et al*., 2022; Kong *et al*., 2021; Singh *et al*., 2020). Its leaves contain diverse bioactive phytochemicals, such as flavonoids, alkaloids, tannins, phenolic compounds, and saponins, many of which exhibit potent antioxidant and anti-inflammatory activities (Ogidi *et al*., 2022; Shoyshob *et al*., 2024; Singh *et al*., 2020). Evidence from preclinical studies further highlights the antidiabetic, hepatoprotective, and anti-inflammatory potential of C. papaya leaf extracts (Idu *et al*., 2024; Li *et al*., 2023). Nonetheless, the majority of existing research has concentrated on glycaemic regulation, with comparatively limited attention directed toward hepatic outcomes in diabetic conditions.

Despite these findings, the specific mechanisms by which *C. papaya* leaf extracts exert hepatoprotective effects, particularly through antioxidant-mediated pathways, remain insufficiently explored. This represents a significant gap, given that oxidative stress is a key driver of liver injury in diabetes. Addressing this gap could enhance our understanding of plant-based interventions in managing diabetic liver complications. Accordingly, the present study aims to evaluate the hepatoprotective potential of crude ethanolic extracts of Carica papaya leaves, with a particular focus on their antioxidant-mediated mechanisms, in streptozotocin (STZ)-induced diabetic Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Male albino Wistar rats, 10 to 12 weeks old and weighing 200 to 250 g, were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Ibadan, Nigeria. The animals were maintained in stainless steel cages (eight rats per cage) under standard laboratory conditions, including adequate ventilation, a controlled temperature of 25 to 28 °C, and a 12-hour light/dark cycle, at the Animal Care Unit, Faculty of Basic Medical Sciences, Bingham University, Karu, Nasarawa State, Nigeria. During the study period, the rats had ad libitum access to a standard rodent diet and clean water. A one-week acclimatization period was observed before the commencement of the experimental procedures. All animal handling and experimental protocols complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23) and received approval from the Institutional Animal Ethics Committee (Reg. No. NHREC/21/05/2005/01523).

Collection and Extraction of Plant Material

Fresh leaves of Carica papaya were collected from the Forestry Research Institute of Nigeria (FRIN), Ibadan, and taxonomically authenticated by a plant taxonomist in the Department of Botany, Bingham University, Karu, Nasarawa State, Nigeria. A voucher specimen (FHI 1142134) was deposited in the departmental herbarium for reference purposes. The leaves were air-dried, pulverized into fine powder, and subjected to extraction using 96% ethanol (v/v) in a Soxhlet apparatus. The resulting extract was concentrated under reduced pressure at 40 °C with a rotary evaporator, yielding 10.5%. The crude ethanolic extract obtained was stored in an airtight container at 4 °C until further use in experimental procedures.

Induction of Diabetes Mellitus

The animals were divided into two groups: a non-diabetic control group and an experimental group designated for diabetes induction with streptozotocin (STZ). Diabetes induction was carried out according to the protocol described by Alhindi (2022). Prior to STZ administration, the experimental rats were pretreated with nicotinamide (Merck KGaA, Darmstadt, Germany). STZ was freshly dissolved in 0.1 M citrate buffer (pH 4.5) and administered as a single intraperitoneal injection at a dose of 50 mg/kg body weight. To prevent mortality from acute hypoglycemia, the STZ-injected rats were supplied with 5% glucose solution for 24 hours post-injection. After 72 hours, fasting blood glucose (FBG) levels were determined from blood samples collected via the retro-orbital plexus, using a glucometer (OneTouch Select Plus, LifeScan, USA). Rats with FBG values greater than 200 mg/dL were classified as diabetic and enrolled into the treatment protocol, which commenced four days after induction.

Experimental Design

Forty male rats were randomly allocated into two main groups. The first group (n = 8) served as the control rats, while the second group (n = 32) comprised the experimental cohort designated for induction of diabetes via STZ. The antidiabetic and hypolipidemic effects of the ethanolic extract of *C. papaya* (EECP) leaves were evaluated at doses of 200 mg/kg and 400 mg/kg in a diabetic rat model over a 21-day treatment period. The experimental group was further subdivided into four treatment subgroups. Group 2 (STZ), serving as the positive control, received a single intraperitoneal injection of STZ at a dose of 50 mg/kg to induce diabetes. Group 3 (STZ + MET), designated as the standard treatment group, received STZ (50 mg/kg, i.p.) followed by oral administration of metformin (MET) at a dose of 500 mg/kg body weight per day, in accordance with the

protocol described by Iwara *et al*. (2025), and calculated based on the Reagan-Shaw *et al*. (2008) dosage conversion formula. Group 4 (STZ + EECP200) received 200 mg/kg/day of *C. papaya* ethanolic extract orally, while Group 5 (STZ + EECP400) received 400 mg/kg/day of the same extract orally throughout the 21-day experimental period.

The selected doses of crude EECP leaf extract used in the current study were considered safe and required no further toxicity assessment, as they were well below the established recommended range of 2000 to 5000 mg/kg for rats (Adewuyi *et al*., 2024). Specifically, doses of 200 and 400 mg/kg were adopted, aligning with concentrations previously demonstrated to be effective in related studies (Kebebew & Shibeshi, 2013). For comparison, Naggayi *et al*. (2015) utilized a dose of 500 mg/kg and evaluated its effects relative to a higher dose of 700 mg/kg.

Weight measurements

Weekly body weight was measured for each rat, and cumulative weight gain was calculated as: Cumulative weight gain (g) = Final body weight (g) − Initial body weight (g)

Blood collection

At the end of the experimental period, rats were anesthetized, and blood was collected from the jugular vein. Samples were centrifuged at 4000 rpm for 30 minutes at 10 °C, and the separated serum was stored at –18 °C until analysis.

Determination of FBG

Fasting blood glucose (FBG) levels (mg/dL) were determined using an enzymatic colorimetric assay kit based on the glucose oxidase–peroxidase (GOD-PAP) method, following the procedure of Dominguez *et al*. (2017).

Determination of liver functions

Serum markers of hepatic function, including alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), alkaline phosphatase (ALP, U/L), total and direct bilirubin (mg/dL), total protein (g/dL), and albumin (g/dL), were measured using commercial diagnostic kits, following the manufacturer's instructions.

Oxidative stress biomarkers

Superoxide dismutase (SOD, U/L) activity was determined using a commercial assay kit according to the method of Giannopolitis and Ries (1977). Catalase (CAT, U/L) activity was evaluated with a CAT assay kit based on the protocol described by Aebi (1984). Reduced glutathione (GSH, g/dL) levels were quantified using a colorimetric assay kit following the procedure of Beutler *et al*. (1963). Lipid peroxidation, expressed as malondialdehyde (MDA, nmol/mL), was measured using a colorimetric assay kit in line with the method of Ohkawa *et al*. (1979). All oxidative stress parameters were assessed with an automated biochemical analyzer (Mindray BS-240, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

Histopathological Examination

Portions of liver tissue from each experimental group were excised and prepared for light microscopic evaluation. Samples were fixed in 10% neutral buffered formalin, dehydrated through graded concentrations of ethanol, cleared in benzene, and subsequently infiltrated and embedded in paraffin wax. The paraffin-embedded blocks were mounted on wooden holders and trimmed to an initial thickness of 20 µm. Serial sections of 7 µm were obtained using a rotary microtome and stained with hematoxylin and eosin (H&E) for histological assessment. Photomicrographs were captured with a 5-megapixel AmScope digital camera attached to an Olympus light microscope.

Statistical Analysis

Data were analyzed using the SPSS, version 22.0 for Windows (IBM Corp., Houston, Texas, USA). Results were expressed as mean ± standard error of the mean (SEM). The Shapiro–Wilk test was applied to evaluate data normality, and Levene's test was used to confirm homogeneity of variances. Group comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the cumulative changes in body weight of STZ-induced diabetic Wistar rats treated with crude EECP leaves. Rats in the normal control group showed a progressive increase in body weight, reflecting normal growth, whereas the untreated diabetic group (STZ) exhibited significant ($p<0.05$) and persistent weight loss throughout the study duration. Treatment with metformin (STZ + MET) attenuated this weight loss, leading to a gradual recovery in body mass from -1.92 ± 1.92 g (Day 7) to a net gain of 1.74 ± 2.89 g (Day 21), with significant ($p < 0.05$) improvement compared to the STZ group. Likewise, administration of *C. papaya* at 200 mg/kg resulted in progressive weight gains while the 400 mg/kg dose produced slightly higher improvements, with both treatment groups showing statistically significant ($p < 0.05$) differences compared to the STZ group. Additionally, the 400 mg/kg group showed a significant ($p < 0.05$) increase between Day 7 and Day 21, suggesting a dose-dependent amelioration of STZ-induced catabolic weight loss.

Table 1: Effect of ethanolic extracts of *C. papaya* leaf on change in body weight

	Cumulative weight change (g)		
	Day 7	**Day 14**	**Day 21**
Control	5.62 ± 0.43	10.95 ± 2.10	18.74 ± 2.33^{+}
STZ	−7.14 ± 2.78*	−11.87 ± 3.45*	−13.95 ± 3.86*$^{+}$
STZ + MET	−1.92 ± 1.92*^{a}	0.62 ± 2.66*^{a}	1.74 ± 2.89*^{a}
STZ + EECP200	1.28 ± 1.44^{a}	3.74 ± 1.58*^{a}	5.93 ± 2.19*^{a}
STZ + EECP400	0.94 ± 1.76*^{a}	2.41 ± 2.63*^{a}	7.82 ± 4.76*$^{a+}$

Data are presented as mean ± SEM, with eight rats per group (n = 8). Statistical significance was considered at *$p < 0.05$ when compared with the normal control group. $^{a}p < 0.05$, compared to STZ group. $^{b}p < 0.05$, compared to normal control group. $^{+}p < 0.05$, compared to Day 7. STZ-Streptozotocin, MET - Metformin, EECP- ethanolic extract of *C. papaya*.

Table 2 summarizes the effects of EECP on fasting blood glucose levels in STZ-induced diabetic rats. All diabetic groups showed significantly elevated glucose levels at baseline compared to controls ($p < 0.05$), confirming hyperglycaemia. Glucose levels in the control group remained stable, while the STZ-only group maintained persistently high levels throughout. Metformin treatment significantly ($p < 0.05$) reduced glucose from Day 14 onward, reaching 10.33 ± 0.72 mmol/L by Day 21. EECP at 200 mg/kg produced a more pronounced reduction to 7.04 ± 1.42 mmol/L, while the 400 mg/kg dose lowered levels to 8.61 ± 1.52 mmol/L by Day 21. Both doses significantly ($p < 0.05$) improved glycaemic control in a time-dependent manner, with the 200 mg/kg dose showing a slightly stronger effect.

Table 2: Effect of ethanolic extracts of *C. papaya* leaf on fasting glucose level

	Fasting Glucose level (mmol/L)			
	Day 0	**Day 7**	**Day 14**	**Day 21**
Control	5.61 ± 0.18	4.77 ± 0.17	4.72 ± 0.23	4.68 ± 0.25
STZ	14.82 ± 0.51*	14.95 ± 0.39*	15.91 ± 0.53*	15.37 ± 0.44*
STZ + MET	14.63 ± 0.30*	13.05 ± 0.85*	12.10 ± 0.20*^{a}	10.33 ± 0.72*$^{+}$
STZ + EECP200	13.76 ± 0.24*	12.18 ± 0.31*	9.48 ± 0.33*$^{ab+}$	7.04 ± 1.42^{a+}
STZ + EECP400	13.29 ± 0.39*^{ab}	10.18 ± 0.27*^{ab}	9.37 ± 0.22*$^{ab+}$	8.61 ± 1.52^{a+}

Data are presented as mean ± SEM, with eight rats per group (n = 8). Statistical significance was considered at *$p < 0.05$, compared to normal control group. $^{a}p < 0.05$, compared to STZ group. $^{b}p < 0.05$, compared to normal control group. $^{+}p < 0.05$, compared to Day 0. STZ-Streptozotocin, MET - Metformin, EECP- ethanolic extract of *C. papaya*.

Table 3 summarizes the effects of EECP leaves on liver function enzymes such as AST, ALT, and ALP, in STZ-induced diabetic Wistar rats. In the normal control group, liver enzyme levels remained within physiological limits indicating normal hepatic function. In contrast, STZ-induced diabetic rats exhibited a significant elevation in all three liver enzymes compared to the control group ($p < 0.05$), with AST increasing to 166.18 ± 3.99 U/L, ALT to 93.10 ± 7.27 U/L, and ALP to 97.20 ± 3.80 U/L, reflecting hepatic injury and dysfunction associated with hyperglycaemia-induced oxidative stress. Treatment with metformin partially restored hepatic enzyme levels, though values remained elevated compared to controls. EECP administration produced a dose-dependent hepatoprotective effect. At 200 mg/kg, EECP significantly ($p < 0.05$) reduced AST and ALT, though ALP levels remained elevated. More notably, the 400 mg/kg EECP group showed near-normalization of enzyme activities, with AST and ALT significantly ($p < 0.05$) reduced, and ALP declining closer to the control values and significantly ($p < 0.05$) lower than the STZ group.

Table 3: Effect of ethanolic extracts of *C. papaya* leaf on liver function enzymes on rats

	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	72.89 ± 8.94	33.53 ± .32	62.84 ± 11.78
STZ	166.18 ± 3.99*	93.10 ± 7.27*	97.20 ± 3.80*
STZ + MET	107.19 ± 4.95*^{ab}	76.22 ± 7.90*	79.79 ± 9.78
STZ + EECP200	99.95 ± 3.08*^{a}	51.83 ± 3.19*^{ab}	80.28 ± 3.87
STZ + EECP400	79.68 ± 4.78^{a}	39.50 ± 7.12^{ab}	53.32 ± 7.09

Data are presented as mean ± SEM, with eight rats per group (n = 8). Statistical significance was considered at *p < 0.05, compared to normal control group. ^{a}p < 0.05, compared to STZ group. ^{b}p < 0.05, compared to normal control group. STZ- Streptozotocin, MET - Metformin, EECP- ethanolic extract of *C. papaya*. AST - Aspartate aminotransferase. ALT - Alanine aminotransferase. ALP – Alkaline phosphatase

Table 4 illustrates the effects of EECP leaves on liver function status in STZ-induced diabetic Wistar rats, based on markers such as total protein (TP), direct bilirubin (DB), total bilirubin (TB), and albumin levels. STZ-induced diabetic rats experienced significant ($p < 0.05$) liver dysfunction, with reduced TP and albumin levels, and elevated direct (DB) and total bilirubin (TB) compared to controls. Metformin treatment improved these markers but did not fully restore them. EECP at 200 mg/kg significantly increased TP (94.00 ± 2.32 g/L), reduced DB and TB, and improved albumin (3.76 ± 0.28 g/dL), surpassing control values in TP. The 400 mg/kg dose also restored liver function, normalizing DB and TB and improving TP and albumin levels ($p < 0.05$), confirming a dose-dependent hepatoprotective effect.

Table 4: Effect of ethanolic extract of *C. papaya* leaf on liver function status on rats

	TP (g/L)	DB (umol/L)	TB (umol/L)	Albumin (g/dL)
Control	71.29 ± 4.90	3.68 ± 0.74	12.78 ± 2.62	4.73 ± 0.51
STZ	38.10 ± 4.66*	8.79 ± 0.65*	30.32 ± 2.45*	2.43 ± 0.23*
STZ + MET	60.43 ± 0.94^{a}	1.62 ± 0.21*^{a}	13.98 ± 1.28*^{a}	4.08 ± 0.46^{a}
STZ + EECP200	94.00 ± 2.32*^{ab}	2.81 ± 0.34^{a}	11.44 ± 0.75*^{a}	3.76 ± 0.28^{a}
STZ + EECP400	79.09 ± 6.27^{a}	1.39 ± 0.55*^{a}	9.24 ± 1.14*^{ab}	4.44 ± 0.47^{a}

Data are presented as mean ± SEM, with eight rats per group (n = 8). Statistical significance was considered at *p < 0.05, compared to normal control group. ap < 0.05, compared to STZ group. bp < 0.05, compared to normal control group. STZ- Streptozotocin, MET - Metformin, EECP- ethanolic extract of *C. papaya*. TP- total protein, DB-direct bilirubin, TB-total bilirubin.

Table 5 presents the effects of EECP leaves on hepatic antioxidant enzyme activities and oxidative stress marker STZ-induced diabetic Wistar rats. STZ group showed significant ($p < 0.05$) oxidative damage, as evidenced by a sharp decline in SOD (52.16 ± 1.37 u/mL), CAT (33.04 ± 1.23 u/mg), and GSH (1.41 ± 0.16 mM), alongside a marked elevation in MDA (20.92 ± 0.74 µM) compared to control. Metformin treatment partially restored redox balance, significantly ($p<0.05$) improving the antioxidant activities of the liver while markedly reducing MDA when compared to STZ effect, however these changes were not commensurate to control values. Similarly, EECP at 200 mg/kg significantly enhanced antioxidant enzyme levels and decreased MDA when compared to STZ. The 400 mg/kg dose of EECP yielded even significant ($p < 0.05$) improvements, with SOD and CAT levels approaching control values, GSH rising to 6.75 ± 0.58 mM, and MDA moderately reduced.

Table 5: Effect of ethanolic extracts of *C. papaya* leaf on hepatic antioxidant activities and oxidative stress marker

	SOD (u/mL)	CAT (u/mg Protein)	GSH (mM)	MDA (uM)
Control	81.24 ± 0.42	44.15 ± 2.41	7.92 ± 1.36	4.88 ± 0.33
STZ	52.16 ± 1.37*	33.04 ± 1.23*	1.41 ± 0.16*	20.92 ± 0.74*
STZ + MET	64.33 ± 1.02*a	39.28 ± 0.79a	6.31 ± 0.61a	7.02 ± 0.29a
STZ + EECP200	69.87 ± 1.05*a	38.33 ± 0.95a	6.04 ± 0.45a	7.44 ± 0.69a
STZ + EECP400	78.41 ± 2.74ab	41.07 ± 1.47a	6.75 ± 0.58a	8.31 ± 0.74*a

Data are presented as mean ± SEM, with eight rats per group (n = 8). Statistical significance was considered at *p < 0.05, compared to normal control group. ap < 0.05, compared to STZ group. bp < 0.05, compared to normal

control group. STZ- Streptozotocin, MET - Metformin, EECP- ethanolic extract of *C. papaya*. SOD-superoxide dismutase, CAT-catalase, GSH-glutathione, MDA-malondialdehyde

Histological analysis of liver tissues from the control group (Figure 1A) revealed moderate portal triaditis without evidence of periportal inflammatory cell infiltration. The central venule appeared normal, hepatocytes exhibited typical morphology, and hepatic sinusoids were devoid of inflammatory infiltration. In contrast, liver sections from the STZ-induced diabetic control group (Figure 1B) demonstrated severe portal triaditis characterized by marked infiltration of inflammatory cells within the central venule, as well as extensive inflammatory infiltration across the liver parenchyma and sinusoids, with only a limited number of hepatocytes retaining normal architecture. The STZ + metformin group (Figure 1C) showed perivascular inflammatory cell infiltration, with the majority of hepatocytes maintaining normal morphology, although isolated hepatocellular necrosis was noted; the sinusoids, however, remained unaffected by inflammatory cells, as illustrated in Figure 1. Liver tissues from diabetic rats treated with 200 mg/kg of crude ethanolic extract of *C. papaya* (Figure 1D) exhibited mild portal triaditis. Hepatocytes within most regions of the parenchyma retained their morphology, interspersed with areas displaying spongy and foamy cytoplasm, while the sinusoids showed only mild inflammatory infiltration. Notably, the group treated with 400 mg/kg EECP (Figure 1E) demonstrated largely preserved liver architecture, with intact central venules and portal triads, uniformly normal hepatocyte morphology, and sinusoids exhibiting minimal inflammatory cell infiltration.

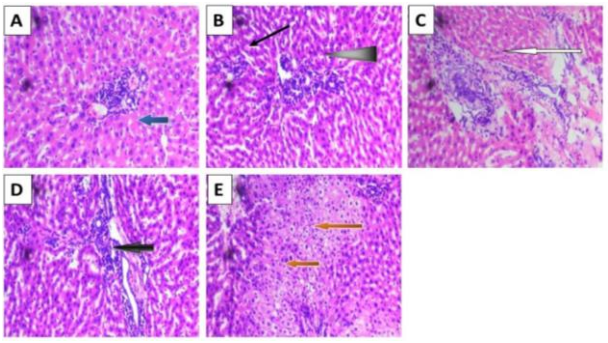

Figure 1: Photomicrograph of liver section stained by H & E (x100 magnification). A: Control rat B: STZ-induced diabetic rat C: Diabetic rats treated with Metformin D: Diabetic rats treated with 200mg/Kg *C. papaya* E: Diabetic rats treated with 400mg/Kg *C. papaya*. White arrow shows normal central venules, and normal portal triads (black arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids show very mild infiltrates.

This study investigated the antioxidant-mediated hepatoprotective effects of EECP leaves in STZ-induced diabetic Wistar rats. The results demonstrate that EECP significantly improves glycaemic control, restores liver function markers, preserves hepatic architecture, and mitigates oxidative stress in a dose-dependent manner. At 400 mg/kg, these effects were comparable to or exceeded those of metformin, the standard antidiabetic agent.

Progressive weight loss is a hallmark of STZ-induced diabetes, attributed to insulin deficiency, which triggers protein catabolism and lipid mobilization (Bauer *et al*., 2023). In this study, EECP, especially at 400 mg/kg, significantly attenuated weight loss, reflecting improved metabolic regulation. This change could be as a result of the phytochemical catechins present in *C. papaya* (Sharma *et al*., 2022) which according to Liu *et al*. (2023) improved weight of rats in their study. These results align with those of Ajibade *et al*. (2019), who observed weight recovery in diabetic rodents treated with *C. papaya*. However, they differ from Roy *et al*. (2022), who found non-significant changes in weight despite improved glycaemia. The disparity likely reflects variations in extraction methods, dosage, or treatment duration, emphasizing the role of phytochemical composition in therapeutic efficacy.

EECP also produced significant reductions in fasting blood glucose, with the 200 mg/kg dose showing superior effects by Day 21. This non-linear response suggests a hormetic effect, where moderate doses confer greater benefit. Loureiro & Martel (2019) reported similar findings with plant polyphenols, highlighting optimal efficacy at intermediate concentrations due to improved bioavailability and reduced metabolic saturation. While previous studies have attributed *C. papaya*'s antidiabetic action to enhanced insulin sensitivity and glucose uptake, our findings suggest that moderate doses may also offer pancreatic β-cell protection via flavonoids (Nyakundi & Yang, 2023). Nonetheless, Juárez-Rojop *et al*. (2012) noted that papaya extracts may be insufficient as monotherapy for long-term glycaemic control, indicating the need for adjunctive strategies.

In addition to its hypoglycaemic effects, EECP demonstrated hepatoprotective properties through significant reductions in hepatic enzymes (AST, ALT, and ALP) which are elevated during liver injury. The 400 mg/kg dose restored enzyme levels toward baseline, supporting hepatocellular integrity. This effect may be attributed to the extract's antioxidant constituents, which reduce lipid peroxidation, stabilize hepatocyte membranes, and enhance endogenous detoxification pathways. These findings are consistent with Shaban *et al*. (2021), who reported similar enzyme normalization with *C. papaya* in paracetamol-induced hepatotoxicity. Comparable outcomes have been documented with other

plant-based antioxidants, such as Moringa oleifera and Curcuma longa (Ibrahim Salih *et al*., 2022; Uchio *et al*., 2017). However, Idehen *et al*. (2024) observed limited effects with aqueous papaya extracts, suggesting that solvent polarity influences the concentration and activity of bioactive constituents—ethanolic extraction likely yields higher flavonoid and phenolic content.

Further evidence of hepatoprotection was provided by improvements in serum total protein, albumin, and bilirubin. EECP reversed hypoalbuminemia and hyperbilirubinemia typically associated with diabetic liver dysfunction, indicating restored synthetic and excretory liver function. These findings are comparable to those of Kyei-Barffour *et al*. (2021), who observed enhanced protein synthesis and bilirubin clearance with botanical treatments in diabetic models. The notable increase in total protein at 200 mg/kg, exceeding control levels, may reflect enhanced hepatic biosynthesis or an adaptive response to oxidative stress. However, this observation requires further investigation to rule out overcompensation or stress-induced hepatic remodeling. In contrast, Ayeni *et al*. (2017) found no significant bilirubin changes with papaya extract, highlighting the influence of extract composition and experimental design.

EECP also restored antioxidant enzyme levels and reduced oxidative stress markers. Elevated levels of SOD, CAT, and GSH in treated groups, particularly at 400 mg/kg, indicate restoration of redox balance. Simultaneously, EECP significantly reduced MDA, a key lipid peroxidation marker, suggesting protection against oxidative liver damage. These findings align with those of Kumar *et al*. (2024), who reported that *C. papaya* leaves are rich in flavonoids, tannins, and phenolics capable of neutralizing reactive oxygen species. Studies by Sharma *et al*. (2022) and Khor *et al*. (2021) similarly support the role of plant-based antioxidants in modulating oxidative and inflammatory mediators. Interestingly, while Nxumalo *et al*. (2024) found increased antioxidant enzyme activity with *C. papaya*, they reported modest reductions in MDA levels. In contrast, our study achieved a substantial reduction, though not full normalization, implying that EECP reduces but may not fully reverse oxidative damage within short treatment durations.

Histological analysis corroborated the biochemical findings. EECP-treated liver tissues, especially in the 400 mg/kg group, exhibited near-normal hepatic architecture with minimal inflammatory infiltration and necrosis. These morphological improvements support those of Ibrahim *et al*. (2022), who demonstrated hepatocyte regeneration following polyphenol-rich treatments. The presence of foamy hepatocytes in the 200 mg/kg group suggests partial recovery or mild steatosis, possibly indicative of early reparative changes (Cichoż-Lach & Michalak, 2014). Notably, histological outcomes in the EECP group were superior to those in the metformin-treated group, which exhibited residual necrosis. This suggests that beyond glycaemic regulation, *C. papaya* exerts broader protective effects, potentially due to its concurrent anti-inflammatory and antioxidant properties (Zhang *et al*., 2010).

Overall, the findings highlight the multifaceted therapeutic potential of EECP in diabetic hepatopathy. While metformin primarily targets glycaemic pathways, EECP acts on multiple fronts—modulating glucose metabolism, preserving liver integrity, and restoring antioxidant balance. However, several limitations should be acknowledged. The crude extract lacks phytochemical standardization, which limits reproducibility and precision in dosing. Additionally, the specific molecular pathways involved—such as Nrf2, NF-κB, or MAPK signaling—were not evaluated, representing a critical gap that should be addressed in future mechanistic studies. The relatively short treatment duration also restricts conclusions about long-term safety and efficacy, warranting extended studies.

CONCLUSION

This study demonstrates that ethanolic extract of *C. papaya* leaves exerts significant antioxidant-mediated hepatoprotective and antidiabetic effects in streptozotocin-induced diabetic rats. The leaf extract improved glycaemic control, normalized liver enzyme levels, enhanced antioxidant defense, and preserved hepatic architecture. These findings suggest that *C. papaya* extract may serve as a complementary agent in managing diabetic liver complications through its integrated effects on metabolism, oxidative stress, and tissue repair. However, further research is needed to elucidate its molecular mechanisms of action, and evaluate long-term efficacy and safety in both preclinical and clinical settings.

REFERENCE

Adewuyi, H. A., Kabiru, A. Y., Muhammad, H. L., Lukman, H. Y., Owolabi, M. S., Jonathan, I., El-Gazzar, A. M., Mahmoud, M. H., Batiha, G. E.-S., & Lawal, B. (2024). Pre-clinical protective potentials of Carica papaya constituents in experimentally induced anemia. *American Journal of Translational Research*, *16*(7), 3259–3272. https://doi.org/10.62347/ZQDC9694

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, *105*, 121–126. https://doi.org/10.1016/s0076-6879(84)05016-3

Ajibade, A. J., Fakunle, P. B., Adetunji, T. J., & Kehinde, B. D. (2019). Protective Effects of Aqueous Extract of Carica papaya Leaf on the Liver of Streptozotocin (STZ)-

Induced Diabetic Adult Wistar Rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, *7*(4 SE-Original Research Article), 1–12. https://doi.org/10.9734/ajrimps/2019/v7i430127

Alam, S., Hasan, M. K., Neaz, S., Hussain, N., Hossain, M. F., & Rahman, T. (2021). Diabetes Mellitus: insights from epidemiology, biochemistry, risk factors, diagnosis, complications and comprehensive management. *Diabetology*, *2*(2), 36–50. https://doi.org/10.3390/diabetology2020004

Alara, O. R., Abdurahman, N. H., & Alara, J. A. (2022). Carica papaya: comprehensive overview of the nutritional values, phytochemicals and pharmacological activities. *Advances in Traditional Medicine*, *22*(1), 17–47.

Alhindi, Y. Z. (2022). *Anti-Diabetic and Antihyperlipidemic Potential of Combined Melatonin and Garlic in Nicotinamide-Streptozotocin Induced Diabetic Mice*.

Ayeni, M., Akanmu, M., Bolaji, O., Osasan, S., Olayiwola, G., Afolabi, M., & Morohunfola, A. (2017). Bilirubin Lowering Potential of Aqueous Carica Papaya Extract in Induced Jaundice in Rats. *Journal of Pharmacy and Pharmacology*, *5*. https://doi.org/10.17265/2328-2150/2017.07.011

Bauer, B. M., Bhattacharya, S., Bloom-Saldana, E., Irimia-Dominguez, J. M., & Fueger, P. T. (2023). Dose-dependent progression of multiple low-dose streptozotocin-induced diabetes in mice. *Physiological Genomics*, *55*(9), 381–391. https://doi.org/10.1101/2023.04.08.536122

Beutler, E., Duron, O., & Kelly, B. M. (1963). Improved method for the determination of blood glutathione. *The Journal of Laboratory and Clinical Medicine*, *61*, 882–888.

Dilworth, L., Facey, A., & Omoruyi, F. (2021). Diabetes mellitus and its metabolic complications: the role of adipose tissues. *International Journal of Molecular Sciences*, *22*(14), 7644. https://doi.org/10.3390/ijms22147644

Dominguez, R. B., Orozco, M. A., Chávez, G., & Márquez-Lucero, A. (2017). The Evaluation of a Low-Cost Colorimeter for Glucose Detection in Salivary Samples. In *Sensors* (Vol. 17, Issue 11). https://doi.org/10.3390/s17112495

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, *59*(2), 309–314. https://doi.org/10.1104/pp.59.2.309

Ibrahim Salih, A., Mohammed Saleh, H., Sulaiman Khalaf, A., & Sabbar Ayed, H. (2022). Effect of Moringa oleifera Leaves against Hepatotoxicity Induced by Bisphenol A. *Archives of Razi Institute*, *77*(3), 1083–1089. https://doi.org/10.22092/ARI.2022.357266.2005

Idehen, I. C., Yibala, O., Yakubu, B. S., Onyinyechi, B., Dic-ijiewere, E. O., & Chelimo, J. J. (2024). Histopathological and biochemical impacts of aqueous Carica papaya leaf extract on the liver of streptozotocin-induced diabetic Albino Rats. *Journal of Survey in Fisheries Sciences*, *11*(4), 27–31.

Idu, S. I., Bokpe, G. F., Toryila, J. E., & Mallo, M. J. (2024). Hepatoprotective effect of ethanolic leaves extract of Carica papaya (Pawpaw) on Alloxan-Induced diabetic wistar rats. *Journal of Basics and Applied Sciences Research*, *2*(4), 55–60.

Iwara, I. A., Ibor, O. R., Mboso, E., Eyong, E. U., & Arukwe, A. (2025). Molecular and biochemical responses in Wistar rats treated with extracts from a hyperglycemia and diabetes-associated therapeutic Hensia crinita. *Phytomedicine Plus*, *5*(3), 100849. https://doi.org/https://doi.org/10.1016/j.phyplu.2025.100849

Juárez-Rojop, I. E., Díaz-Zagoya, J. C., Ble-Castillo, J. L., Miranda-Osorio, P. H., Castell-Rodríguez, A. E., Tovilla-Zárate, C. A., Rodríguez-Hernández, A., Aguilar-Mariscal, H., Ramón-Frías, T., & Bermúdez-Ocaña, D. Y. (2012). Hypoglycemic effect of Carica papaya leaves in streptozotocin-induced diabetic rats. *BMC Complementary and Alternative Medicine*, *12*, 236. https://doi.org/10.1186/1472-6882-12-236

Kebebew, Z., & Shibeshi, W. (2013). Evaluation of anxiolytic and sedative effects of 80% ethanolic Carica papaya L. (Caricaceae) pulp extract in mice. *Journal of Ethnopharmacology*, *150*(2), 665–671. https://doi.org/https://doi.org/10.1016/j.jep.2013.09.023

Khor, B.-K., Chear, N. J.-Y., Azizi, J., & Khaw, K.-Y. (2021). Chemical Composition, Antioxidant and Cytoprotective Potentials of Carica papaya Leaf Extracts: A Comparison of Supercritical Fluid and Conventional Extraction Methods. *Molecules (Basel, Switzerland)*, *26*(5). https://doi.org/10.3390/molecules26051489

Kong, Y. R., Jong, Y. X., Balakrishnan, M., Bok, Z. K., Weng, J. K. K., Tay, K. C., Goh, B. H., Ong, Y. S., Chan, K. G., & Lee, L. H. (2021). Beneficial role of Carica

papaya extracts and phytochemicals on oxidative stress and related diseases: a mini review. *Biology*, *10*(4), 287. https://doi.org/10.3390/biology10040287

Kumar, R., Saha, P., Kumar, Y., Sahana, S., Dubey, A., & Prakash, O. (2020). A review on diabetes mellitus: type1 & Type2. *World Journal of Pharmacy and Pharmaceutical Sciences*, *9*(10), 838–850.

Kumar, S. S., V, G. T., K, K., & John, M. (2024). Antioxidant potential and mineral elemental profiling of young and mature fruit and leaf of Carica papaya L. cultivar "Red Lady." *Journal of Trace Elements and Minerals*, *9*, 100166. https://doi.org/https://doi.org/10.1016/j.jtemin.2024.100166

Kyei-Barffour, I., Kwarkoh, R. K. B., Acheampong, D. O., Brah, A. S., Akwetey, S. A., & Aboagye, B. (2021). Alkaloidal extract from Carica papaya seeds ameliorates CCl4-induced hepatocellular carcinoma in rats. *Heliyon*, *7*(8), e07849. https://doi.org/https://doi.org/10.1016/j.heliyon.2021.e07849

Li, H., Beg, O. U., Rafie, A. R., Kanwal, S., Ovalle-Cisneros, A., Faison, M. O., & Siddiqui, R. A. (2023). Characterization of green and yellow papaya (carica papaya) for anti-diabetic activity in liver and myoblast cells and wound-healing activity in fibroblast cells. *Nutrients*, *15*(8), 1929. https://doi.org/10.3390/nu15081929

Liu, J., Ding, H., Yan, C., He, Z., Zhu, H., & Ma, K. Y. (2023). Effect of tea catechins on gut microbiota in high fat diet-induced obese mice. *Journal of the Science of Food and Agriculture*, *103*(5), 2436-2445. https://doi.org/10.1002/jsfa.12476

Loureiro, G., & Martel, F. (2019). The effect of dietary polyphenols on intestinal absorption of glucose and fructose: Relation with obesity and type 2 diabetes. *Food Reviews International*, *35*(4), 390–406.

Naggayi, M., Mukiibi, N., & Iliya, E. (2015). The protective effects of aqueous extract of carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats. *African Health Sciences*, *15*(2), 598–605. https://doi.org/10.4314/ahs.v15i2.37

Nxumalo, M. B., Ntanzi, N., Kumalo, H. M., & Khan, R. B. (2024). Mitigating hyperglycaemic oxidative stress in HepG2 cells: the role of carica papaya leaf and root extracts in promoting glucose uptake and antioxidant defence. *Nutrients*, *16*(20), 3496. https://doi.org/10.3390/nu16203496

Nyakundi, B. B., & Yang, J. (2023). Uses of Papaya Leaf and Seaweed Supplementations for Controlling Glucose Homeostasis in Diabetes. *International Journal of Molecular Sciences*, *24*(7), 6846. https://doi.org/10.3390/ijms24076846

Ogidi, O. I., Tobia, P. S., Ijere, D. N., Akpan, U. M., Omu, O., Carbom, H. E., & Iyosayi, A. R. (2022). Investigation of bioactive compounds and antimicrobial sensitivity of pawpaw (Carica papaya) leave extracts against morbific micro-organisms. *Journal of Applied Pharmaceutical Research*, *10*(1), 21–28. https://doi.org/10.18231/J.JOAPR.2020.21.28

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Orji, I. A., Baldridge, A. S., Ikechukwu-Orji, M. U., Banigbe, B., Eze, N. C., Chopra, A., Omitiran, K., Iyer, G., Odoh, D., Alex-Okoh, M., Reng, R., Hirschhorn, L. R., Huffman, M. D., & Ojji, D. B. (2024). Evaluating diabetes care in primary healthcare centers in Abuja, Nigeria: a cross-sectional formative assessment. *BMC Primary Care*, *25*(1), 243. https://doi.org/10.1186/s12875-024-02487-1

Patel, J. (2023). Diabetes, A global epidemic. *SACAD: John Heinrichs Scholarly and Creative Activity Days*, *2023*(2023), 103.

Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2008). Dose translation from animal to human studies revisited. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *22*(3), 659–661. https://doi.org/10.1096/fj.07-9574LSF

Roy, J. R., Janaki, C. S., Jayaraman, S., Periyasamy, V., Balaji, T., Vijayamalathi, M., & Veeraraghavan, V. P. (2022). Carica papaya reduces muscle insulin resistance via IR/GLUT4 mediated signaling mechanisms in high fat diet and streptozotocin-induced type-2 diabetic rats. *Antioxidants*, *11*(10), 2081. https://doi.org/10.3390/antiox11102081

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., & Williams, R. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *157*, 107843. https://doi.org/10.1016/j.diabres.2019.107843

Shaban, N. Z., El-Kot, S. M., Awad, O. M., Hafez, A. M., & Fouad, G. M. (2021). The antioxidant and anti-inflammatory effects of Carica Papaya Linn. seeds extract on CCl(4)-induced liver injury in male rats. *BMC Complementary Medicine and Therapies*, *21*(1), 302. https://doi.org/10.1186/s12906-021-03479-9

Sharma, A., Sharma, R., Sharma, M., Kumar, M., Barbhai, M. D., Lorenzo, J. M., Sharma, S., Samota, M. K., Atanassova, M., Caruso, G., Naushad, M., Radha, Chandran, D., Prakash, P., Hasan, M., Rais, N., Dey, A., Mahato, D. K., Dhumal, S., … Mekhemar, M. (2022). Carica papaya L. Leaves: Deciphering Its Antioxidant Bioactives, Biological Activities, Innovative Products, and Safety Aspects. *Oxidative Medicine and Cellular Longevity*, *2022*, 2451733. https://doi.org/10.1155/2022/2451733

Shoyshob, T. Z., Heya, I. A., Afrin, N., Enni, M. A., Asha, I. J., Moni, A., Hannan, M. A., & Uddin, M. J. (2024). Protective Mechanisms of Carica papaya Leaf Extract and Its Bioactive Compounds Against Dengue: Insights and Prospects. *Immuno*, *4*(4), 629–645. https://doi.org/10.3390/immuno4040037

Singh, S. P., Kumar, S., Mathan, S. V, Tomar, M. S., Singh, R. K., Verma, P. K., Kumar, A., Kumar, S., Singh, R. P., & Acharya, A. (2020). Therapeutic application of Carica papaya leaf extract in the management of human diseases. *Daru : Journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, *28*(2), 735–744. https://doi.org/10.1007/s40199-020-00348-7

Uchio, R., Higashi, Y., Kohama, Y., Kawasaki, K., Hirao, T., Muroyama, K., & Murosaki, S. (2017). A hot water extract of turmeric (Curcuma longa) suppresses acute ethanol-induced liver injury in mice by inhibiting hepatic oxidative stress and inflammatory cytokine production. *Journal of Nutritional Science*, *6*, e3. https://doi.org/10.1017/jns.2016.43