
RESEARCH ARTICLE

Hypoglycemic and Hepatoprotective Potentials of Dichloromethane (DCM) Fraction of *Gongronema Latifolium* Extract in Streptozotocin-Induced Diabetic Wistar Rats

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ABSTRACT

Diabetes is a chronic health issue with devastating but preventable consequences. The increasing proportion of the aging population, intake of calorie-dense diets, weight problems and sedentary lifestyles have been directly implicated in the increased number of diabetics globally. Furthermore, diabetes confers a huge economic burden due to its management costs, especially in low income countries. This study investigated the hypoglycemic and hepatoprotective potentials of the dichloromethane (DCM) fraction of *G. latifolium* stem bark extract on some biochemical parameters in streptozotocin-induced hyperglycemic rats. A total of thirty (30) Wistar rats were randomly assigned into five (5) groups with six (06) animals per group. Type 2 diabetes was induced with intraperitoneal administration of streptozotocin in Groups 2 – 4, with animals in Groups 1 and 2 acting as normal and untreated diabetic controls, respectively. Group 3 rats were treated with the standard drug, metformin, while groups 4 and 5 were orally administered 200 and 400 mg/kg body weight of DCM fraction of *G. latifolium* stem bark extract. Changes in the body weight, biochemical assays (ALT, AST, total protein, albumin and urea), and expression levels of selected genetic markers, as well as the changes in liver histology, were determined and compared. Data obtained from this study show that the fasting blood glucose levels in hyperglycemic rats were significantly ($p < 0.05$) reduced, and the body weight steadily increased in rats treated with both doses of the DCM fraction of *G. latifolium* stem bark extract compared to the untreated diabetic rats. Total protein, AST and ALT levels, but not albumin levels, were significantly ($p < 0.05$) increased in rats treated with both doses of the extract. In addition, administration of the extracts significantly ($p < 0.05$) reversed the streptozotocin-induced repression of NRF2 and CAT expression in diabetic rats. Furthermore, histology results show an improvement in the liver architecture in the rats treated with the extract. The results from this study show that the DCM fraction of *G. Latifolium* stem bark extract possesses hypoglycemic, hepatoprotective and anti-diabetic activities and could be used in the management of diabetes.

KEYWORDS

Gongronema latifolium, DCM fraction, Diabetes, Genetic markers.

ARTICLE INFORMATION

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1. Introduction

Diabetes is a chronic health issue with devastating but preventable consequences. It is characterized by excessive blood glucose levels as a consequence of defects in insulin production, insulin action, or both. The increasing proportion of the aging population, intake of calorie-dense diets, weight problems and sedentary lifestyles have been directly implicated in the increased number of diabetics globally [Fiskin, 2021]. The global diabetes prevalence in 20–79 year olds in 2021 was estimated to be 10.5 % (536.6 million people), and this number is projected to rise to 12.2 % (783.2 million) in 2045 [Sun, 2023]. Furthermore, diabetes confers a huge economic burden due to its management costs as well as its complications are skyrocketing [Tan, 2019]. The current diabetes treatment regimen that focuses on insulin secretion and insulin sensitization results in undesired side effects in patients. In addition, the use of gene therapy as well as induced β -cells regeneration are not widely used in Nigeria as most patients cannot afford the cost associated with such treatment. This has led to a sizeable number of households in Nigeria exclusively depending on

medicinal herbs for the management of diabetic disease conditions. *Gongronema latifolium* is a tropical rainforest plant that belongs to the family Asclepiadaceae and genus *Gongronema*. It is mostly grown in West Africa [Okafor, 1996]. The plant is edible, with green leaves, yellow flowers and a stem that produces milky latex when broken or cut. The leaf *Gongronema latifolium* is high in fats, proteins, vitamins, minerals and essential amino acids [Eleyinmi, 2007]. It is then taken as a purgative for colic and stomach pains as well as to treat symptoms of worm infection [Okafor, 1975; Onike, 2010]. Apart from its dietary values, *G. latifolium* is believed to have strong medicinal qualities due to its composition of different active constituents. An infusion or decoction of the whole plant (the leaves, stems, and roots) is used traditionally in the treatment of digestive issues such as dyspepsia, anorexia, colic and stomachache, constipation, dysentery and intestinal worms [Oliver-Bever, 1986, Morebise, 2006, Nwinyi, 2008, Owu, 2012]. The plant has been reported to have been used in hyperglycaemia and hypertension [Ugochukwu, 2003], treatment of liver associated with alcoholism and viral hepatitis [Akerlele, 2008], treatment of abdominal pain after childbirth as well as in the treatment of cough, wheezing and asthmatic attacks [Essien, 2007]. This present study aims to evaluate the hypoglycemic and hepatoprotective potentials of the dichloromethane fraction of *G. latifolium* in streptozotocin-induced diabetes in rats.

2. Methodology

2.1 Plant materials

The leaves of *Gongronema latifolium* were obtained from Ogun State, Nigeria and identified by Dr. H.A Akinnibosun (Taxonomist) at the Department of Plant Biology and Biotechnology, University of Benin. The stem barks of this plant were air-dried and later pulverized to powdery form at the Pharmacognosy laboratory, Faculty of Pharmacy, University of Benin. A powdered sample (300 g) of *G. latifolium* was first soaked in 2 L of hydroethanol for a week with periodic stirring, filtered with Whatman filter paper (No. 1), and the filtrate concentrated with a vacuum concentrator at 30°C. The hydroethanol extract was then partitioned using the solvent-solvent fractionation method. First, it was defatted using n-hexane, followed by extraction with dichloromethane (DCM). The DCM fraction was subsequently concentrated to dryness using a rotary evaporator, and the concentrate was weighed and used as an experiment sample. The extracts were subsequently freeze-dried at the National Centre for Energy and Environment, University of Benin, Benin City, Edo State, and stored in a refrigerator until use.

2.2 Animals

Thirty (30) Wistar rats weighing between 90 and 120 g were purchased from the Department of Pharmacy, University of Benin, Benin City, Edo State. The animals were handled according to the Institutional Animal Guidelines (1912/PO/Re/S/16/CPCSEA) and were allowed to acclimatize to diet and environment for 2 weeks after arrival. The weights of the rats were monitored throughout the duration of the experiment. The animals were kept in clean cages in a 12-hour light/dark cycle room with daily litter changes. Type 2 diabetes mellitus was induced in twenty-four (24) animals after an overnight fast by a one-time intraperitoneal injection of streptozotocin (40 mg/kg body weight) dissolved in 0.1 M cold citrate buffer (pH 4.5). Treatment commenced after diabetes was stabilized (four days) in the animals. Animals with fasting blood glucose levels greater or equal to 150 mg/dL were considered diabetic, grouped and treated as shown below: Group 1 served as negative control; Group 2 served as diabetic untreated; Group 3 received metformin (25 mg/kg body weight); Group 4 received DCM fraction of *G. Latifolia* (200 mg/kg body weight of rat); Group 5 received DCM fraction of *G. Latifolia* (400 mg/kg body weight of rat). The plant extract was administered daily with an oral gavage for 21 days. The animals were sacrificed at the end of the 21-day administration period (on the 22nd day) after an overnight fast. Each mouse was anaesthetized in a chloroform (CHCl₃)-saturated container, blood sample collected via a heart puncture and dispensed in labelled *Lithium Heparin* and *Ethylene Diamine Tetra-Acetic Acid* (EDTA) tubes for further analysis

2.3 Biochemical assays

2.3.1 Determination of fasting blood glucose levels

Fasting blood glucose level was determined using commercially available diagnostic kits (Randox Lab., UK) with strict adherence to the manufacturer's instructions.

2.3.2 Determination of serum urea

The level of urea in the sera was evaluated using the Urease-Berthelot method [Weatherburn, 1967].

2.3.3 Determination of liver enzymes

Concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using commercially available diagnostic kits (Randox Lab., UK) following manufacturers' instructions.

2.3.4 Estimation of total protein and albumin

Albumin and total protein were evaluated using a standard Radox kit, as described by Tietz [Tietz, 1995].

2.3.5 Gene expression by RT-PCR

Total RNA was extracted from the liver using Trizol (Ambion, Austin, TX, USA), with strict adherence to the manufacturer's instructions. The RNA samples were quantified with the NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and 2 µg of RNA was reverse transcribed using oligo (dT) primers (Promega, Madison, WI, USA). Real-time quantitative PCR amplification and detection were performed on optical-grade 48-well plates in an Eco Real-Time PCR System (Illumina, CA, USA) with 20 ng of cDNA, SYBR qPCR Master Mix (Kapa Biosystems, Inc., Wilmington, MA, USA) and gene-specific primers (Table 1). To normalize mRNA expression, the expression of the housekeeping gene, β-actin, was determined. The mRNA relative quantitation was calculated using the 2^{-ΔΔCt} method.

Table 1: Primer information for qPCR experiment

Gene Name	Primer Sequence (5' – 3')
NRF2_F	CACATCCAGTCAGAAACCACTGG
NRF2_R	GGAATGTCTGCGCCAAAAGCTG
CAT_F	GTGCGGAGATTCAACACTGCCA
CAT_R	CGGCAATGTTCTCACACAGACG
β-actin_F	CACCATTGGCAATGAGCGGTTC
β-actin_R	AGGTCTTTCGGATGTCCACGT

2.4 Histological analysis

Histological analysis was done according to the method described by [Kumar, 2005]. Portions of the liver were fixed in 10 % neutral buffered formalin for histology. Thin sections of the liver were dissected and processed using a Leica TP2010 automatic tissue processor for 18 hours. The processor passed the tissues through fixation, dehydration, de-alcoholization, and paraffinization. Ultra-thin sections (5 µm) were sliced from the paraffinized sections using a Thermo Scientific semi-automated rotary microtome. The tissues were then subjected to hematoxylin and eosin staining and viewed under a microscope using 10x magnification.

2.5 Data analyses

Data were expressed as the mean ± SEM using SPSS v26 software, while GraphPad Prism 8.0 was used for the visualization of data. Statistical significance was calculated by one-way analysis of variance (ANOVA) by multiple comparisons. Differences between means were estimated by Duncan's multiple range tests, and a value of *p* < 0.05 was taken as statistically significant.

3. Results and Discussion

3.1 Administration of dichloromethane (DCM) fraction of *G. latifolium* stem bark increases body weight in streptozotocin-induced Diabetic Rats

The mean weight was significantly decreased in streptozotocin-induced diabetic rats compared with the normal control (Table 2). Administration of DCM fraction of *G. latifolium* stem bark showed a steady, significant increase in body weight in a dose-dependent manner compared to the diabetic control. Furthermore, oral administration of metformin significantly increases the weight of the rats after the 14 days period.

Table 2: Effect of DCM fraction of *G. latifolium* stem bark on body weight changes (g) in streptozotocin-induced diabetic rats

Groups	Initial Body Weight (g)	Final Body Weight (g)	Weight Loss/Gain (g)
Group 1	190.48±1.56	204.47±5.08	+13.99±0.14
Group 2	184.10±1.12	162.66±2.66	-21.44±0.11
Group 3	167.42±1.58	185.55±2.09	+18.13±0.18
Group 4	179.21±2.50	186.12±2.01	+6.91±0.11
Group 5	178.20±2.20	185.76±1.20	+7.56±0.22

Data are expressed as mean ± SEM, n = 6. Group 1 = Normal control; Group 2 = Diabetic control (STZ only); Group 3 = Positive control (Metformin); Group 4 = DCM fraction (200 mg/kg body weight); Group 5 = DCM fraction (400 mg/kg body weight)

3.2. The DCM fraction of *G. latifolium* stem bark significantly reduces fasting blood glucose levels in streptozotocin-induced diabetic rats

The effect of the DCM fraction of *G. latifolium* stem bark on fasting blood glucose in STZ-induced diabetic rats is shown in Table 3. The result indicates that the blood glucose level of the diabetic control and the DCM-treated diabetic groups increased significantly (*p* < 0.05) three days after administration of streptozotocin when compared to the normal control. Administration of the DCM fraction of *G. latifolium* reversed the STZ-induced hyperglycemia in a dose-dependent manner, comparable with

metformin administration 14 days post administration. The glucose level of the diabetic control remained significantly increased ($p < 0.05$) compared with the normal control and diabetic treated groups after the fourteen days period of study.

Table 3: Effect of DCM fraction of *G. latifolium* stem bark on fasting blood glucose in streptozotocin-induced diabetic rats

Group/Treatment	Glucose (mg/dL) Day 0	Glucose (mg/dL) Day 3 (After induction)	Glucose (mg/dL) Day 7	Glucose (mg/dL) Day 14
Group 1	85.40±2.34 ^a	86.20±2.40 ^a	84.10±1.34 ^a	84.40±2.50 ^a
Group 2	88.20±3.24 ^a	*460.00±11.43 ^b	*318.25±5.20 ^c	*348.10±10.20 ^d
Group 3	80.24±4.34 ^a	**337.25±14.50 ^b	**111.32±0.63 ^c	**108.50±6.20 ^c
Group 4	86.80±3.20 ^a	**349.21±10.12 ^b	**218.11±8.20 ^c	**213.73±4.25 ^d
Group 5	82.60±4.14 ^a	**463.40±12.00 ^b	**238.70±6.20 ^c	**122.147±3.50 ^d

Data are expressed as mean ± SEM, n=6. Values with different alphabets are statistically significant ($p < 0.05$). *Mean is significant ($p < 0.05$) when compared with normal control. ** mean is significant ($p < 0.05$) when compared with the diabetic control group. Group 1 = Normal control; Group 2 = Diabetic control (STZ only); Group 3 = Positive control (Metformin); Group 4 = DCM fraction (200 mg/kg body weight); Group 5 = DCM fraction (400 mg/kg body weight)

3.3. Administration of DCM fraction of *G. latifolium* stem bark on improves total protein and albumin concentrations in diabetic rats

In the group administered streptozotocin only (Group 2), there was a decrease in the total protein and albumin levels with a significant decrease observed in total protein levels. However, treatment with the DCM extract of *G. latifolium* ameliorated the total protein ($p < 0.05$) and albumin ($p > 0.05$) levels in a dose-dependent manner. Compared to the positive control, the DCM extract was more effective in improving the total protein levels than albumin concentration (Figure 1)

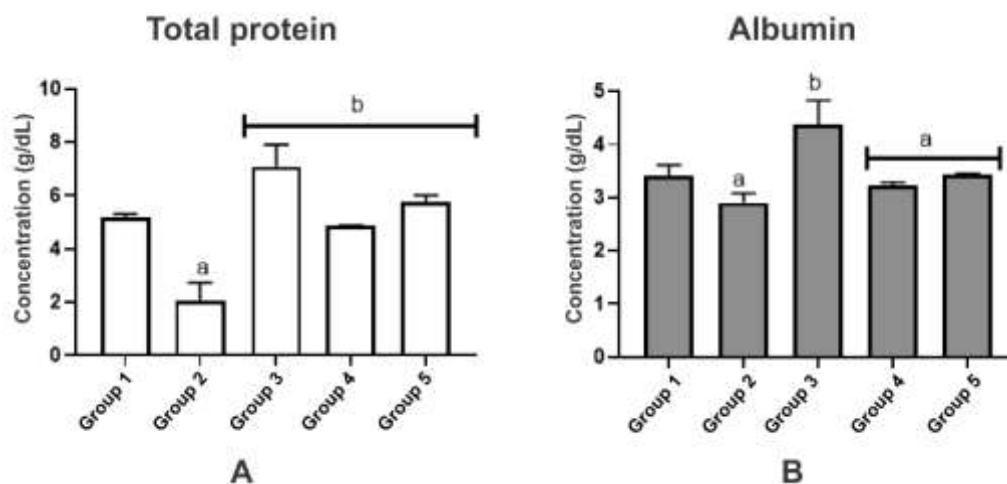


Figure 1: Effect of DCM fraction of *G. latifolium* stem bark on (A) Total protein and (B) Albumin levels in STZ-induced diabetic rats. Group 1 = Normal control; Group 2 = Diabetic control (STZ only); Group 3 = Positive control (Metformin); Group 4 = DCM fraction (200 mg/kg body weight); Group 5 = DCM fraction (400 mg/kg body weight)

3.4. *G. latifolium* stem bark DCM fraction reverses STZ-induced liver damage

The effect of the DCM fraction of *G. latifolium* on liver function parameters is depicted in Figure 2. Compared to the normal control, the diabetic untreated group (Group 2) revealed significantly increased levels of AST and ALT. Interestingly, treatment with the DCM fraction significantly reversed the STZ-induced increases in a dose-dependent manner. *Gongronema latifolium* DCM fraction was more proficient than metformin in reducing the levels of liver enzymes. In addition, the photomicrograph of the liver cells reveals features of severe vascular ulceration in the group administered STZ (Figure 3, plate II). Treatment with the DCM fraction of *G. latifolium* reversed the STZ-induced damage in a dose-dependent manner (plate V-IV).

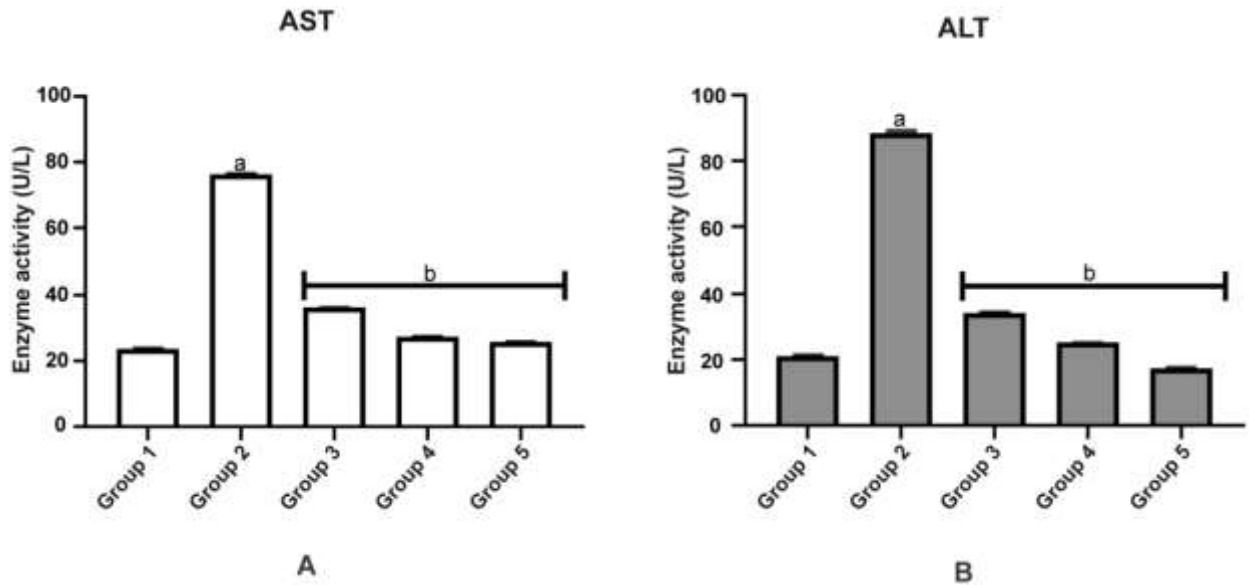


Figure 2: Effect of DCM fraction of *G. latifolium* stem bark on liver function parameters – (A) AST and (B) ALT levels in STZ-induced diabetic rats. Group 1 = Normal control; Group 2 = Diabetic control (STZ only); Group 3 = Positive control (Metformin); Group 4 = DCM fraction (200 mg/kg body weight); Group 5 = DCM fraction (400 mg/kg body weight)

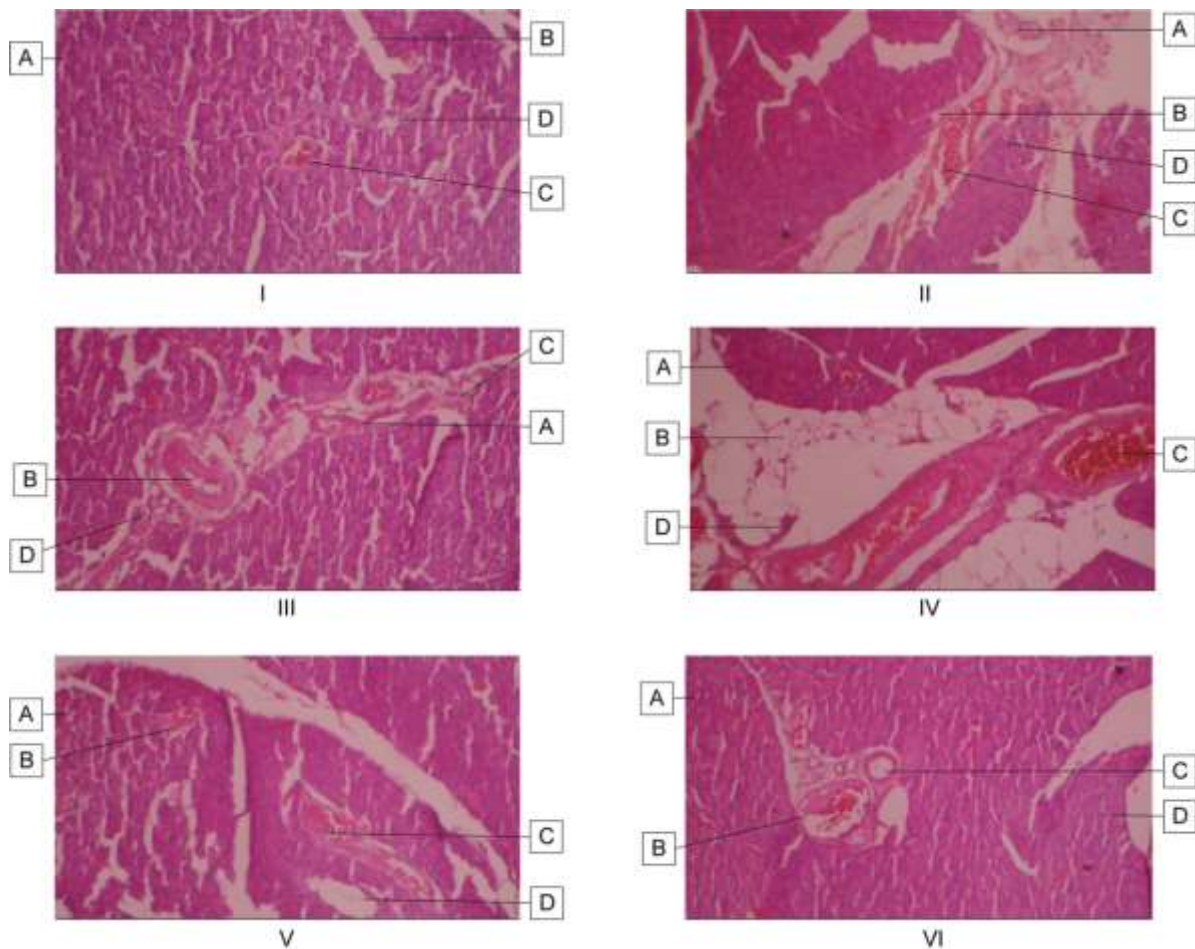


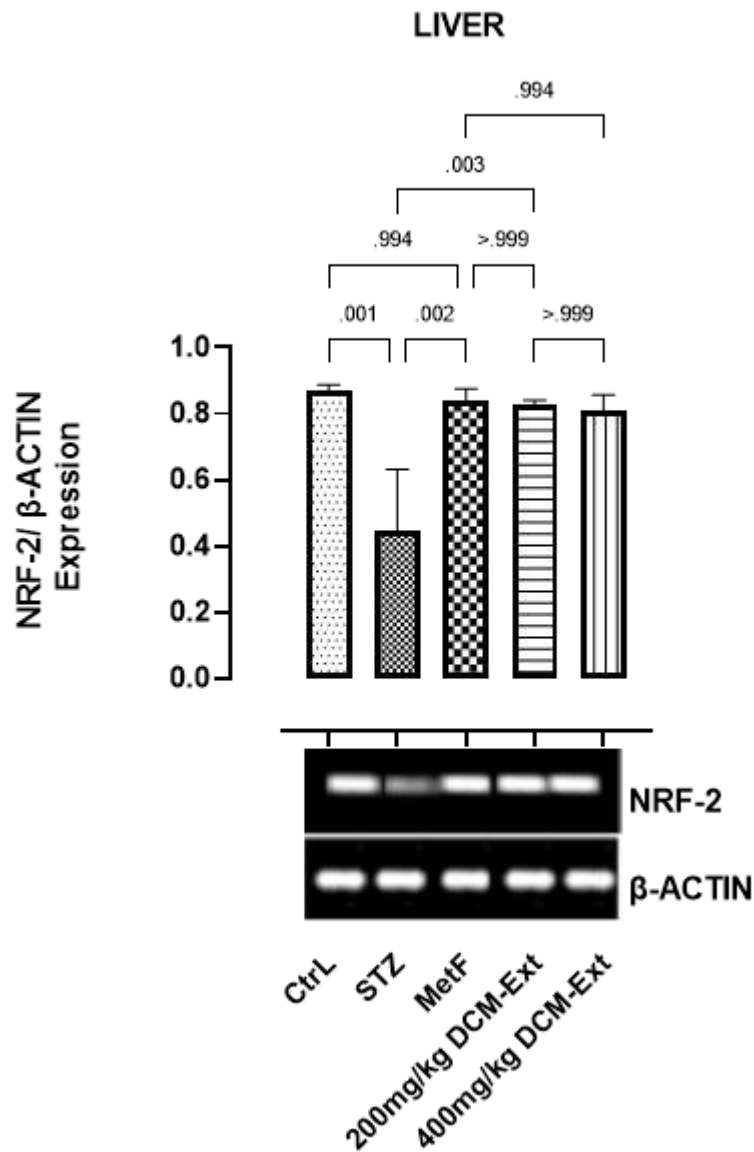
Figure 3: Effect of DCM fraction of *G. latifolium* stem bark on liver morphology (I) Rat pancreas (Control) composed of normal tissue: A. exocrine acini, B. interlobar space, C. interlobar vein, D. interlobar duct. (II) Rat pancreas given STZ only showing: A: vascular stenosis, B: severe vascular ulceration, C: congestion, D: mild infiltrates of inflammatory cells (III) Rat pancreas from diabetic control showing A: vascular obstruction, B: stenosis, C: vascular ulceration, D: infiltrates of inflammatory cells. (IV) Rat pancreas

induced and given Metformin showing normal architecture; A: acini, B: interlobar adipose tissue, C: active vascular congestion. Plate (V): Rat pancreas induced and given 200 mg DCM fraction of *G. latifolium* showing normal architecture: A. acini, B. veins, C. arteries, D. dilated duct. (VI) Rat pancreas induced and given 400 mg DCM fraction of *G. latifolium* showing normal architecture; A: acini, B: dilated vein, C: dilated artery, D: duct. (H&E, 40X).

3.5. Effect of DCM fractions of *G. latifolium* stem bark on some genetic markers

To confirm the changes induced by *G. latifolium* at the molecular level, the expression patterns of NRF-2 and CAT were determined (Figure 4). Gel electrophoresis of the PCR-amplified fragments revealed that the expression of NRF-2 and CAT was significantly repressed by the administration of streptozotocin (Group 2). However, these changes were significantly improved by the administration of *G. latifolium* DCM fraction.

A.



B.

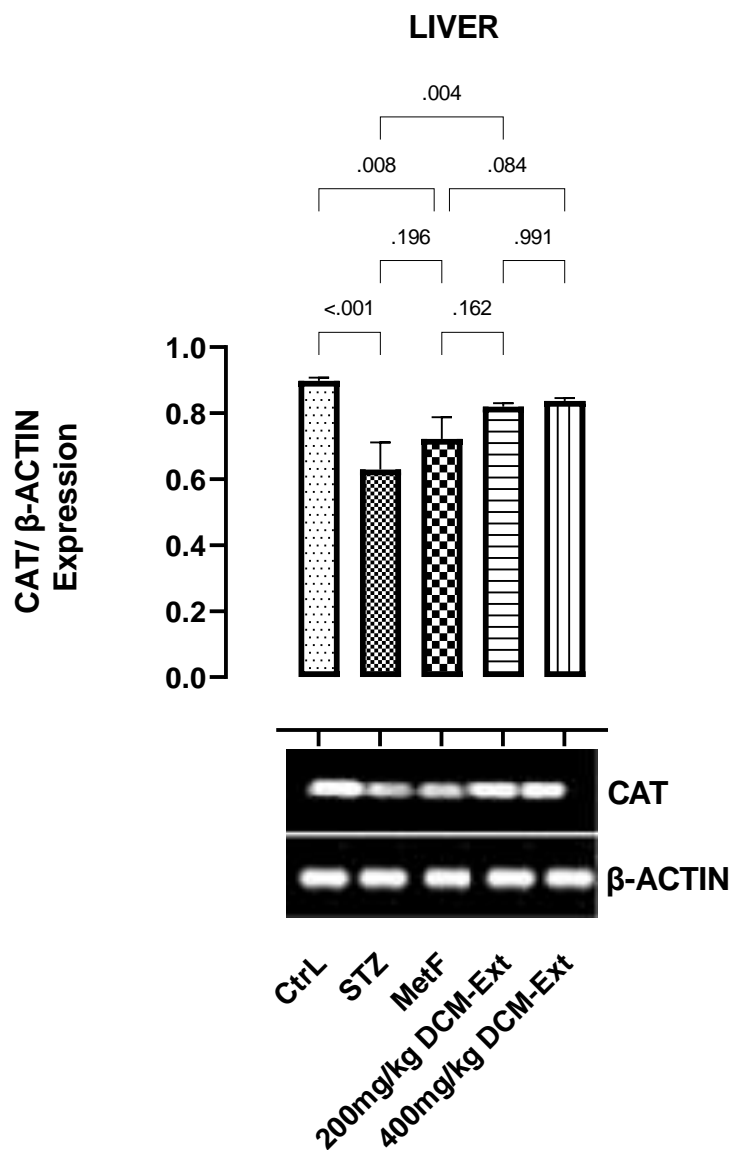


Figure 4: Effect of DCM extract of *G. latifolium* on (A) Nrf2 and (B) CAT gene expression. Group 1 = Normal control; Group 2 = Diabetic control (STZ only); Group 3 = Positive control (Metformin); Group 4 = DCM fraction (200 mg/kg body weight); Group 5 = DCM fraction (400 mg/kg body weight)

3.6 Discussion

Diabetes mellitus is a group of metabolic disorders characterized by high blood sugar levels and disturbances in insulin production and function. This study assessed the hypoglycemic and hepatoprotective potentials of the DCM fraction of *G. latifolium* stem bark in streptozotocin-induced diabetic rats. Streptozotocin-induction of diabetes is a classical experimental model to explore the potential of herbal extracts in ameliorating the disease [Rossini, 1977]. In this study, the administration of streptozotocin caused a significant amount of weight loss when compared to the control. This reduction of body weight can be due to the breakdown of tissue proteins in diabetic rats [Yanardag, 2005; Andulla, 2003]. Streptozotocin is transported to the β-cells via the GLUT2 receptors due to its structural similarity to glucose, where it selectively attacks the β-cells of the Islets of Langerhans [Schnedl, 1994]. In addition, STZ-induced necrosis of the pancreatic β-cells occurs by methylation of the DNA, leading to fragmentation, which subsequently manifests as diabetes mellitus. DNA fragmentation stimulates nuclear poly (ADP-ribose) synthetase, which depletes intracellular NAD⁺ and ATP levels, thus inhibiting the production of proinsulin [Yamamoto, 1981]. A further mechanism of action of STZ involves the release of signals that induce nitric oxide synthase, further increasing the concentration of nitric oxide (NO), increasing H₂O₂ generation and ultimately increasing the oxidative stress on the cell [Flodström, 1999; Takasu, 1991]. The result

from this study revealed that induction of diabetes mellitus by STZ precipitated a significant increase ($p < 0.05$) in the blood glucose level of the diabetic control and the DCM-treated diabetic groups three days after administration of streptozotocin when compared to the normal control. Administration of the extract reversed the STZ-induced hyperglycaemia in a dose-dependent manner, comparable with metformin administration 14 days post administration. The glucose level of the diabetic control remained significantly increased ($p < 0.05$) compared with the normal control and diabetic treated groups after the fourteen days period of study.

Administration of STZ resulted in a significant decrease in the albumin and total protein levels. Albumin, the most abundant soluble protein in the serum or plasma, constitutes 50 – 60 % of the total protein content [Rafaqat,2023]. It has been reported that low levels of albumin can serve as an indicator of an inflammatory process [Chang, 2019]. This implies sustained damage to the hepatocytes. However, treatment with the DCM extract of *G. latifolium* caused a significant increase in the total protein levels, indicating that the plant extract reversed the total protein ($p < 0.05$) and albumin ($p > 0.05$) levels in a dose-dependent manner. Compared to the positive control, the DCM extract was more effective in improving the total protein levels than albumin concentration.

The liver is central to the process of metabolism, detoxification, and excretion of harmful metabolites [Mori, 2003]. In this present study, diabetes impacted liver function by increasing the levels of AST and ALT. Liver enzymes such as ALT and AST are commonly used as indicators of liver health. Elevated levels of liver enzymes indicate damage to liver cells and are associated with T2DM and insulin resistance. These enzymes are elevated in the serum due to cellular leakage into the blood stream [Kesari, 2007]. West and co-workers demonstrated that diabetes raises the risk of chronic liver disease, and blood alanine transaminase levels serve as a sensitive indicator of liver-related mortality [West, 2006]. Treatment with the DCM fraction significantly reversed the STZ-induced increases in a dose-dependent manner. This indicates that the fraction was proficient in the repair of the STZ-induced damage. Interestingly, the result of the histopathological examination of the liver confirms the repair potential of the DCM fraction, as evidenced by the near normal architecture of the liver. The induction of diabetes experimentally has been reported to cause pathological changes in other tissues aside from the pancreas [Ali, 2023]. Pathological changes were observed in tissues such as the small intestines and the liver of experimentally-induced diabetic animals. In the study under consideration, vascular congestion, necrosis and periportal infiltration of inflammatory cells were observed in the liver of the diabetic control animals. This observation agrees with the study carried out by Ali and Mustafa [2023], who reported necrosis in the liver of STZ-induced diabetic animals. Administration of the DCM fraction of 400 mg *Gongronema latifolium*/ kg bodyweight of animal restores the changes observed in the tissue, though 200 mg *Gongronema latifolium* /kg bodyweight of animal could not reverse the alterations. Treatment with metformin gave rise to vascular congestion but with normal hepatocytes. In addition, normal morphology was observed in the pancreas of the control animals. Obstruction of the blood vessels, stenosis, ulcer and infiltration of inflammatory cells were observed in the pancreas of the diabetic animals. The diabetic animals treated with metformin had normal architecture of pancreatic acinar with vascular congestion. Treatment with 200 mg- and 400 mg *Gongronema latifolium*/ kg bodyweight of animals ameliorated the morphological alterations in the pancreas of diabetic animals.

Oxidative stress and inflammation play an important role in the pathophysiological changes of liver diseases. Nuclear factor erythroid 2-related factor 2 (NRF-2) is a transcription factor that positively regulates the basal and inducible expression of a large battery of cytoprotective genes, thus playing a key role in protecting against oxidative damage [Fuentes-Agudo, 2023]. NRF2 drives the expression of the main antioxidant enzymes in the cell for oxidative stress detoxification. Superoxide dismutase (SOD) catalyzes the conversion of $O_2^{\cdot-}$ into H_2O_2 and molecular oxygen. Subsequently, the enzyme catalase (CAT) reduces H_2O_2 to water [Rosa, 2021]. Therefore, NRF-2 deficiency results in an increase in ROS and oxidative stress in cells. In this study, the expression of NRF-2 and CAT were significantly repressed by the administration of streptozotocin implying an increase in the ROS and subsequent oxidative damage of the liver cells. Expression of both NRF-2 and CAT were restored to normal levels by the administration of the DCM fractions. This result indicate a significant restoration of the liver cells in the rats administered the DCM fraction of *G. latifolium*.

4. Conclusion

The DCM (dichloromethane) fraction of *G. latifolium* stem bark possess hypoglycaemic and hepatoprotective effects and ameliorates other adverse diabetic condition imposed by streptozotocin-induced diabetes on the experimental rats. Further pharmacological and biochemical investigations will elucidate the mechanism of action and be helpful in projecting this plant as a therapeutic target in diabetes research.

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References

- [1] Akerele JO, Obasuyi O, Ebomoyi MC, Oboh IE and Uwumarongie OH. (2008). Antimicrobial activity of ethanol extracts and fractions of the seeds of *Gascinia kola* Hackel (Guttiferae). *Afr. J. Biotech.* 7(2): 169-172.
- [2] Ali M.A and, Mustafa NG. (2023). A comparative histopathological study of pancreas, intestine and liver of experimentally induced diabetes in rats. *IJVS.* 37 (4): 971-978
- [3] Andulla B and Varadacharyulu NCH. (2003). Antioxidant role of mulberry leaves in streptozotocin-diabetic rats. *Clinica Chimica Acta.* 338: 3-10.
- [4] Chang DC, Xu X, Ferrante AW and Krakoff J. (2019). Reduced plasma albumin predicts type 2 diabetes and is associated with greater adipose tissue macrophage content and activation. *Diabetol Metab Syndr.* 11: 14.
- [5] Eleyinmi AF. (2007). Chemical composition and antibacterial activity of *Gongronema latifolium*. *J. Zhejiang Universal Sci.* 8: 352-358.
- [6] Essien JP, Ebong GA and Akpan EJ. (2007). Antioxidant and antitussive properties of *Gongronema latifolium* leaves used locally for the treatment of fowl cough in Nigeria. *J Appl Sci Environ Manage.* 11(4): 47- 50
- [7] Fiskin G and Sahin N. (2021). Nonpharmacological management of gestational diabetes mellitus: Diaphragmatic breathing exercise. *Altern Ther Health M.* 27: 90-96.
- [8] Flodström M, Tyrberg B, Eizirik DL and Sandler S. (1999). Reduced Sensitivity of Inducible Nitric Oxide Synthase-Deficient Mice to Multiple Low-Dose Streptozotocin-Induced Diabetes. *Diabetes.* 48(4):706-713.
- [9] Fuertes-Agudo M, Luque-Tévar M, Cucarella C, Martín-Sanz P and Casado M. (2023). Advances in understanding the role of NRF2 in liver pathophysiology and its relationship with hepatic-specific cyclooxygenase-2 expression. *Antioxidants.* 12(8): 1491. <https://doi.org/10.3390/antiox12081491>
- [10] Kumar V, Abul KA and Fausto KN. (2005). Robbins and Cotran Pathologic Basis of Disease. 7th ed. Elsevier Saunders, Philadelphia, PA.
- [11] Kesari AN, Kesari, S., Singh, S. K., Gupta, R. K and Watal, G. (2007). Studies on the glycemic and Epidemic effect of *Murraya koenigii* in experimental animals. *J Ethnopharmacol.* 112: 305 - 311.
- [12] Mori DM, Baviera AM, Ramalho LTDO, Vendramini RC, Brunetti IL and Pepato MT. (2003). Temporal response pattern of biochemical analytes in experimental diabetes. *Biotechnol. Appl. Biochem.* 38: 183 – 191.
- [13] Morebise O, Fafunso MA, Makinde JM and Olajide OA. (2006). Evaluation of bioactivity of *Gongronema latifolium* leaf extract in rodents. *Sci. Focus.* 11(1): 27-30.
- [14] Nwinyi OC, Chinedu NS and Ajani OO. (2008). Evaluation of antibacterial activity of *Pisidium guajava* and *Gongronema Latifolium*. *J Med Plants Res.* 2(8): 189-192.
- [15] Okafor JC, Okolo HC and Ejiogor MAU (1996). Strategies for enhancement of utilization of potential edible woody forest species of South-Eastern Nigeria. In: L.J.G. van der Maesen et al. (eds.). *The biodiversity of African plant.* Kluwe Academic Publishers. The Netherlands; 1996: 684-695.
- [16] Okafor JC. (1975). The role of common edible (wild and semi-wild) wood plants in the native diets in Nigeria. *Agr. Nat. Res.* 40.
- [17] Onike R. (2010). A survey of medicinal values of *Gongronema latifolium* (madumaro) in African Alternative Medicine. *Nig. J. Physiol. Sci.* 24(1): 79-83.
- [18] Oliver-Bever, B. (1986). Medicinal plants in tropical West Africa. Camb. Univ. Press. London. pp. 89-90.
- [19] Owu DU, Nwokocha CR, Obembe AO, Essien AD, Ikpi DE and Osim EE. (2012). Effect of *Gongronema latifolium* ethanol leaf extract on gastric acid secretion and cytoprotection in streptozotocin-induced diabetic rats. *West Indian Med J.* 6(9): 853-860.
- [20] Rosa AC, Corsi D, Cavi N, Bruni N and Dosio F. (2021). Superoxide dismutase administration: A review of proposed human uses. *Molecules* 26: 1844
- [21] Razaqat S, Sattar A, Khalid A and Razaqat S. (2023). Role of liver parameters in diabetes mellitus - a narrative review. *Endocr Regul.* 57(1): 200-220. doi: 10.2478/enr-2023-0024. PMID: 37715985.
- [22] Rossini AA, Like AAA, Chick WL, Appel MC and Cahill Jr GF. (1977). Studies of streptozotocin-induced insulinitis and diabetes. *Pro. Natl. Acad. Sci. USA.* 74 (6): 2485–2489.
- [23] Schnedl WJ, Ferber S, Johnson JH and Newgard CB. (1994). STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. *Diabetes.* 43 (11): 1326–1333.
- [24] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. (2023). Erratum to "IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045" [Diabetes Res. Clin. Pract. 183 (2022) 109119]. *Diabetes Res. Clin. Pract.* 204:110945. <https://doi.org/10.1016/j.diabres.2023.110945>
- [25] Tan SY, Mei Wong JL, Sim YJ, Wong SS, Mohamed Elhassan SA, Tan SH, et al. (2019). Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. *Diabetes Metab Syndr.* 13 (1):364–372. doi: 10.1016/j.dsx.2018.10.008.
- [26] Takasu N, Asawa T, Komiya I, Nagasawa Y and Yamada T. (1991). Alloxan-induced DNA Strand Breaks in Pancreatic Islets; evidence for H₂O₂ as an intermediate. *J Biol Chem.* 266 (4): 2112-2114
- [27] Tietz NW. (1995). *Clinical Guide to Laboratory Tests.* 3rd ed. W.B. Saunders, Philadelphia.
- [28] Ugochukwu NH, Babady NE, Cobourne M and Gasset SR. (2003). The effect of *Gongronema latifolium* leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. *J. Biosci.* 28:1–5.
- [29] Weatherburn MW. (1967). Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem.* 39(8): 971-974.
- [30] West J, Brouil J, Gazis A, Jackson L, Mansell P, Bennett A, et al. Elevated serum alanine transaminase in patients with type 1 or type 2 diabetes mellitus. *QJM.* 2006; 99: 871–876.
- [31] Yanardag R, Ozsoy-Sacan O, Bolkent S, Orak H and Karabulut-Bulan O. (2005). Protective effects of metformin treatment on the liver injury of the streptozotocin-diabetic rats. *Hum Exp Toxicol.* 24:129–135.
- [32] Yamamoto H, Uchigata Y and Okamoto H. (1981). Streptozotocin and alloxan-induced DNA strand breaks and poly (ADP-ribose) synthetase in pancreatic islets. *Nature.* 294: 284-286.