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Therapeutic effect of the solvent fraction of hexane leaf extract of *Tapinanthus bangwensis* (Engl. & K. Krause) (Loranthaceae) in alloxan-induced pathology in diabetic rats

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ABSTRACT

Plant-based products are gradually replacing pharmaceuticals in treating ailments, including diabetes, due to their safety, cost-effectiveness, potency, and availability. Therefore, the current study looked into the therapeutic effect of the solvent fraction of hexane leaf extract of *Tapinanthus bangwensis* (HEXETACF) (Loranthaceae) in alloxan-induced pathology in diabetic rats. The biochemical parameters were estimated using analytical grade kits via spectrophotometric method. The laboratory rats were distributed into group W (five rats on feed and water), group X (seven rats + 150 mg/kg alloxan solution only), group Y (seven rats + 150 mg/kg alloxan solution + 200 mg/kg BW silymarin for 21 days), and group Z (seven rats + 150 mg/kg alloxan solution + 250 mg/kg BW HEXETACF for 21 days). The results showed that HEXETACF and silymarin (SILY) reduced blood glucose concentration by 33.77% and 34.80%, respectively, after the 21st day of treatment ($p < 0.05$). Additionally, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activity in SILY and HEXETACF were significantly decreased compared to the diabetic group ($p < 0.05$), but no significant decrease in aspartate aminotransferase (AST) activity was observed between the test samples and the diabetic group. Furthermore, the test samples lowered malondialdehyde (MDA) levels, by improving glutathione, superoxide dismutase (SOD), and catalase (CAT) activity. The HEXETACF and SILY significantly decreased triglyceride levels (TG) compared to the diabetic group at $p < 0.05$. They also reduced low-density lipoprotein (LDL) and cholesterol levels and increased the high-density lipoprotein levels compared to the diabetic group. Additionally, no significant decrease in serum electrolytes (Na^+, K^+, and Cl^-), urea, and creatinine (including albumin and total protein) values was observed in HEXETACF and SILY, while hematological indices increased compared to the diabetic group. Histology results revealed that the test samples had normalized glomeruli, β-islet cells, and hepatocytes. However, a trace of mild congestion was noticed in the STDG. But edemic blood congestion was observed in the diabetic group. In conclusion, the current result demonstrated that HEXETACF may be a promising antidiabetic agent that could replace mSILY.

1. Introduction

The global record indicated that annually, diabetic cases rise to about 321.000 in sub-Saharan Africa and about 1.5 million worldwide. This outcome had been attributed to poor nutrition, alcoholism, a sedentary lifestyle, and drug abuse (International Diabetes Federation, 2015; Wang et al., 2015). Furthermore, the International Diabetes Federation predicted that diabetic cases in adults (18 – 90 years) would increase from 10.5% (451 million) in 2021 to approximately 12.4% (532 million) by 2045 (Cho et al., 2018; Sun et al., 2022). Considering the statistics above, researchers and funding partners are called upon to upscale the fight against diabetes mellitus, by developing effective treatment models for reducing or eradicating the disease. According to records, diabetes is the third killer disease, including a chronic metabolic

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and non-communicable disease, characterized by persistent hyperglycemia caused by defective insulin secretion or insensitivity/resistance or, in some cases, by both factors, and therefore the blood system exceeds the normoglycemic level and condenses with glucose (Ononamadu et al., 2019; Wong et al., 2024). Diabetologists categorized diabetes mellitus (DM) as type 1 diabetes (insulin-dependent diabetes mellitus, IDDM), type 2 diabetes (non-insulin-dependent diabetes mellitus, NIDDM), and pregnancy-related diabetes/gestational diabetes mellitus (PRDM or GDM). However, NIDDM is considered the most prevalent. In IDDM, the glucose level persistently increases, owing to the absence of insulin, because the islet cell has been immunologically destroyed. However, in type 2 diabetes, insulin is either not secreted sufficiently, its activity is resisted, or sometimes synergy occurs (Feyisayo & Victor, 2019; Ihegboro et al., 2020b), while gestational diabetes is triggered during pregnancy and normalizes after delivery. In the bid to find lasting solutions to this metabolic disorder, Ihegboro et al. (2022) submitted that inhibiting key metabolic pathways/enzymes, and/or the use of synthetic drugs (metfonin, glibenclamine, acarbose), may be a potent approach.

However, the fact that medicinal plants have a unique pharmacological potential compared to synthetic drugs has led to increased interest in ethnomedicine (Kolhe & Rachh, 2018; Wang et al., 2022). There are already available results comparing the antidiabetic potential of medicinal plants with standard drugs (methonin or glibenclamine), but results on silymarin are limited despite its anti-cancer, anti-inflammatory, antioxidant and antidiabetic properties (Tuorkey et al., 2015).

As far as we are aware, no result shows the antidiabetic capacity of *Tapinanthus pangenesis* with silymarin. The current research looked into the therapeutic effect of solvent fraction of hexane leaf extract of *T. bangwensis* in alloxan-induced pathology in diabetic rats, about silymarin. Briefly, *T. bangwensis*, normally found on the *Citrus* tree as a parasite, belongs to the family of the Loranthaceae. It is ecosystem-friendly in African regions and exhibits several ethnomedicinal properties. The countries where the plant is domicile have designated local names, but in Nigeria, the Hausa, Igbo, and Yoruba call it Kauci, Awurusie, and Afomo onisana, while English tacked it (all purpose herb, healing tree, life-giving tree, or bird lime), respectively (Ihegboro et al., 2020a).

2. Materials and methods

2.1. Materials

Silymarin tablets (Silybon-140 mg, India), alloxan monohydrate (Aldrich-Sigma, United Kingdom), silica gel (60-120 mesh, England), glucose strip (ACHUCHEK, USA), hexane, ethylacetate and formaldehyde solvents (BDH, England), biochemical analytical kits (Randox Laboratory Limited, United Kingdom), but not limited to the aforementioned. All the materials were of quality and analytical grade.

2.2. Plant material identification

The plant's fresh leaves were acquired in March 2022 from Mushin situated at 6°32'6.84''N and 3°20'56.28''E co-ordinate of Lagos State. The plant material was identified at the University of Lagos (Department of Pharmacognosy) by Mr. Adeleke, a taxonomist. He facilitated the issuance of the registration number (LUH 4323) and requested that a sample be kept in the institution's herbarium to ensure its traceability and authenticity.

2.3. Preparation of plant material

The leaves were washed, air-dried, and pulverized into powdered mass. About 1500 g was soaked in 5000 ml of hexane solvent and allowed to stand for 2 days, with stirring at intervals. After filtration, it was exposed to the atmosphere for evaporation, and 62.04 g of solid hexane extract was recovered. Furthermore, the recovered extract was loaded onto a column glass already packed with a mixture of silica gel and hexane solvent. After washing the column with the different combinations of the eluting solvents (hexane and ethylacetate), approximately eighty-eight fractions were collected, which were later pooled into three fractions using the TLC plate, taking into account their retention factors. The above protocol was used by Ihegboro et al. (2020a).

2.4. Familiarization of animals with the new environment

Twenty-six healthy rats (males) were purchased from the University of Lagos' animal breeding laboratory. Their weight ranges were between 100 – 110 g. They were conditioned to the new environment (temperature: 25 °C, humidity: 55 °C, and illumination: 12 hours day/night cycle) for 2 weeks, before commencing the experiment, while feeding with commercially formulated rat feed and clean water (Ihegboro et al., 2020a).

2.5. Initiation of diabetic condition in the animals

The method of Emordi et al. (2018) was used. After acclimatization, the rats had become physiologically stable. Type 2 diabetes was then induced by administering a freshly prepared alloxan solution of 150 mg/kg body weight into the intraperitoneal region and leaving it for three days. When blood glucose was checked with a glucometer (ACHUCHEK, USA), a hyperglycemic condition (> 300 mg/dl) developed compared to normoglycemic rats that had 85 mg/dl.

2.6. Animal groupring and treatment

In this section, the method applied by Ihegboro et al. (2020a) was used. The animals were distributed as follows:

Group W had five rats that were fed with food and water only (normal control). Group X had seven rats that received 150 mg/kg BW alloxan solution only (diabetic group). Group Y had seven rats that were treated with 200 mg/kg BW silymarin for 21 days, after receiving 150mg/kg alloxan solution, while group Z had seven rats that were treated with 250mg/kg BW of the fraction for 21 days, after receiving 150mg/kg alloxan solution. After the 21st day of treatment, the Wistar rats were anesthetized using chloroform, and blood was collected by puncturing the jugular vein in the neck region.

2.7 Analysis of the oxidative stress markers

The liver was washed in 1.15% potassium chloride solution shortly after excision, then homogenized in phosphate buffer (pH 6.2) and then centrifuged at 1593 × g for 5 min. The collected supernatant was used to measure glutathione and malondialdehyde levels, including the activities of catalase and superoxide dismutase enzymes.

2.7.1. Quantification of reduced glutathione level

In this section, the method applied by Fatima et al. (2016) was found suitable. The method involves the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) also called Ellman's reagent, by a

sulfhydryl group to produce 2-nitro-5-mercaptobenzoic acid. The process started by diluting 50 ml of the liver homogenate into 1.0 ml of 0.1 M phosphate buffer (pH 8.0). Furthermore, the resulting mixture (3.0 ml) was added to a 20 ml of 0.01 M DTNB, and the yellow-colored product was measured spectrophotometrically at 412 nm after 5 min.

2.7.2. Quantification of superoxide dismutase (SOD) activity

In this section, the experimental protocol of Katrenčíková et al. (2021) was followed. The liver homogenate (0.05 ml) was added to a mixture of 0.186 mM methosulfate phenazine (0.1 ml), 0.3 mM nitroblue tetrazolium chloride (0.3 ml), 1.2 ml of 0.05 M sodium pyrophosphate buffer (pH 8.3), and 0.2 ml of 0.78 mM reduced nicotinamide adenine dinucleotide (NADH). After 1 min and 30 seconds, the reaction was halted by adding glacial acetic acid. In addition, 20 ml butanol was added later to remove the chromogen formed. The supernatant recovered from centrifuging the mixture at 1593 x g for 10 min was measured spectrophotometrically at 500 nm.

2.7.3. Quantification of catalase activity

To measure the ability of catalase to degrade hydrogen peroxide, the method followed by Katrenčíková et al. (2021) was used. Briefly, the liver homogenate (0.05 ml) and 1.95 ml of 0.05 M phosphate buffer (pH 7.4) were thoroughly mixed, after which 1.0 ml of 19 mM H_2O_2 was added. The entire mixture was left for a while before reading the absorbance spectrophotometrically at 240 nm.

2.7.4. Quantification of malondialdehyde level

The method of Kolagal et al. (2009) was used to measure the color intensity (pink color) formed when MDA reacted with two molecules of acidified thiobarbituric acid (TBA) at 40 °C. In a nutshell, a resulting mixture containing 0.5 ml of 20% tricarboxylic acid (TCA), 1.0 ml of 0.67% TBA, and the liver homogenate (0.5 ml) was incubated for 15 min. Later, 2.0 ml of *n*-butanol was added, followed by centrifugation at 1593 x g for 15 min. The spectrophotometric measurement of the supernatant was taken at 532 nm. To quantify the MDA level, a calibration curve was plotted by preparing different concentrations of 1,1,3,3-tetraethoxypropane, from which the MDA level would be extrapolated.

2.8. Analysis of liver enzymes activity

2.8.1. Estimation of alanine and aspartate aminotransferases

In this section, the method of Adeyemi and Orekoya (2014) was used. After mixing the both serum (0.1 ml) and 0.5 ml of the chemical reagent (containing L-alanine, oxoglutarate, and phosphate buffer, pH = 7.4), they were incubated at 37 °C. After cooling (30 min), 0.5 ml of 2 mM 2,4-dinitrophenylhydrazine was added and the entire content was mixed vigorously, and left for 25 min, before adding 0.4 mM sodium hydroxide (0.5 ml) to produce a color change, which was measured spectrophotometrically at 546 nm as against the blank.

The above procedure is also valid for estimating aspartate aminotransferase activity, except that the chemical reagent used includes L-aspartate, oxoglutarate and phosphate buffer.

2.8.2. Estimation of serum alkaline phosphatase

To estimate the serum alkaline phosphatase, the method of Tietz (2006) was used. This is a unique method in which the reaction between ALP and phenolphthalein monophosphate produces a pink colored product called *p*-nitrophenol. Shortly, after obtaining the resulting mixture, which contained the serum (0.1 ml) and 0.5 ml of the reagent [containing phenolphthalein monophosphate (63 mM) and 2-amino-2-methyl-1-propanol (pH = 7.9), it was incubated at 37 °C for 10 min. Moreover, 0.5 mL of 80 mM disodium hydrogen phosphate was added and left to stand for 20 min, before introducing 5 ml of sodium hydroxide. After 5 min, the absorbance was measured at 546 nm.

2.9. Analysis of liver function indices

2.9.1. Estimation of serum creatinine concentration

To estimate the serum creatinine concentration, the method of Jung (2008) was employed. The serum sample (0.1 ml) was mixed with 0.05 ml of the starting reagent that had 10 mM picric acid, 10 mM sodium borate, sodium hydroxide, and 240 mM surfactant, and 0.5 ml of the creatinine standard (5.0 mg/dl) was introduced and the absorbance was measured at 20^{th} and 80^{th} seconds against the reagent blank at 540 nm. Furthermore, two test tubes labeled as standards A and B were prepared, in which A contained the starting reagent (0.5 ml) and 0.1 mL of an equal volume of picric acid and creatinine standard, while B had creatinine standard (0.5 ml) and 0.1 ml of an equal volume of picric acid and creatinine standard, respectively. The absorbance was measured at 20^{th} and 80^{th} seconds at 546 nm.

2.9.2. Estimation of serum urea concentration

The Ezeugwunne et al. (2017) method, which involves the use of urease Berthelot, was used. In a test tube, 10 µl of the serum was introduced alongside 100 µl of an initial reagent (containing 116 mM EDTA, 6.0 mM sodium nitroprusside, and 1.0 g/l urease) and then incubated at 25 °C for 10 min. Afterward, 2.5 ml of 12 mM phenol was added, accompanied by the addition of 2.5 ml of 27 mM sodium hypochlorite. The final solution was thoroughly mixed, and incubated at 37 °C for 15 min, and the absorbance was measured at 546 nm.

2.9.3. Estimation of serum albumin concentration

The bromocresol green method described by Macrelli et al. (2013) was used. Briefly, 10µl of the serum and 3 ml of the BCG concentrate (comprising of succinate buffer at pH 4.2 and 0.15 mM bromocresol green) were mixed vigorously and then incubated at 37 °C for 2 min. The absorbance was read spectrophotometrically at 630 nm against the reagent blank.

2.9.4. Estimation of serum total protein concentration

The biuret method as outlined by Asuk (2018) was employed. A mixture containing 20 µl of the serum and 10 ml of the biuret reagent was thoroughly mixed and kept for 10 min at 37 °C. The entire content was spectrophotometrically measured at 540 nm against the reagent blank.

2.10. Analysis of lipid profile/markers

2.10.1. Determination of triglyceride concentration

To determine the triglyceride concentration, the method of Adaramoye et al. (2013) was followed. The principle is based on the peroxidation of 4-aminophenazone in the presence of 4-chlorophenol, under which peroxidase acts to form a quinoneimine product. Briefly, 1.0 ml of trichloroacetic acid (TCA) was added to 0.1 ml of the serum sample, and centrifuged at 1106 x g for 10 min. Furthermore, three test tubes labeled as blank, sample, and standard were prepared, such that each contained water + 0.5 ml TCA, 1.0 ml supernatant, and 0.5 ml standard solution + 0.5 ml TCA, respectively. To each, 1.0 ml of cholesterol was added, and were left to stand for 20 min before measuring the absorbance at 540 nm.

2.10.2. Determining the cholesterol concentration

The method of Adaramoye et al. (2013) was good enough for estimating the serum cholesterol level. The principle involves oxidation and enzyme hydrolytic reactions in the reaction of hydrogen peroxide, 4-amino antipyrine and *p*-hydroxybenzoic acid catalyzed by peroxidase, forming a colored product called quinoneimine. 10 µl of serum and 1000 µl of cholesterol reagent, after being thoroughly mixed, was allowed to stand for 10 min. The absorbance was read within 60 min at 500 nm. Two other test tubes were prepared and labeled as standard and blank, respectively. The labeled standard contained 10 µl of standard sample and 1000 µl of cholesterol, while the blank contained 10 µl of distilled water and 1000 µl of cholesterol

2.10.3. Determining the serum low-density lipoprotein

To determine the serum low-density lipoprotein, the method of Bachorik (2000) was found appropriate. To the serum (0.02 ml), three drops of the precipitating solution were added. After being mixed and incubated for 15min, the mixture was centrifuged at 1593 x g for 10 min. The supernatant (0.01 ml) and 1.0 ml of cholesterol were put into three test tubes labeled as sample, standard 1 and standard 2, respectively, and were left for 10 min at ambient temperature. In addition, 0.01 ml of the standard reagent was pipetted into standard 1 and 2, respectively, and were left to stand for 10 min. Finally, the absorbance was measured at 505 nm.

2.10.4. Determining the serum high-density lipoprotein

To determine the serum high-density lipoprotein, the method described by Ighodaro and Ighodaro and Omole (2012) was used. The method is a catalytic reaction, in which HDL-cholesterol is degraded by PEG-cholesterol oxidase to form H_2O_2, and combines with sodium-N-(2-hydroxyl-3-sulfopropryl)-3,5-dimethoxy aniline, HSDA) and 4-amino-antipyrine catalyzed by peroxidase to form a purple/blue pigment. Briefly, the precipitating solution (0.1 ml) and the serum sample (0.3 ml) were mixed thoroughly and left for 15 min. It was centrifuged at 1106 x g for 15 min, after, the supernatant's absorbance was measured at 600 nm.

2.11. Analysis of kidney function indices

2.11.1. Estimation of serum sodium concentration

Serum sodium was estimated by the colorimetric method outlined by Igwe et al. (2020). Briefly, 1.0 ml of filtrate reagent was pipetted into test tubes labeled as blank, standard, and sample. Additionally, 50 µl of standard reagent and 50 µl of serum were added to the standard and sample tubes, respectively. The blank contained distilled water only. All the test tubes were mixed and left to stand for 3 min, followed by centrifugation at 1593 x g for 10 min. Test tubes were labeled and 1.0 ml of acid reagent was added to all tubes. Then, 50 µl of the supernatant was added to the corresponding tubes and appropriately mixed. Finally, 50 µl of color reagent was added to all tubes, and mixed, and absorbance was measured at 550 nm.

2.11.2. Estimation of serum potassium concentration

To estimate the serum potassium concentration, the turbidometric method of Egbung et al. (2020) was used. Shortly after pipetting the serum (25 µl) into the test tube, 100 µl sodium tetraphenylborate was added and the entire contents were incubated for 5 min. After incubation, the absorbance was recorded at 578 nm.

2.11.3. Estimation of serum chloride concentration

To estimate the serum chloride concentration, the method of Egbung et al. (2020) was followed. Two test tubes were prepared, labeled as calibrator and sample, respectively. 1.5 ml of chloride reagent (containing mercuric nitrate, mercuric chloride, ferric nitrate, and mercuric thiocyanate) was introduced into each test tube, while the sample test tube had an additional 0.01 ml of serum and mixed vigorously before incubation at 28 °C for 5 min. The spectrophotometric measurement of the sample was determined at 480 nm.

2.12. Analysis of haematological indices

A full package hematological analysis was carried out on the serum using a hematology analyzer (Sysmex XE-5000, SYSMEX, Japan).

2.13. Histopathological examination of some organs

The procedure outlined by Mazani et al. (2018) was used. After the organs were harvested, they were washed in a solution of physiological saline, and fixed in 10% formalin solution. In an ethanol solution (50 - 100%), the samples were dehydrated, cleared in xylene and embedded in paraffin. Afterward, hematoxylin and eosin dye were applied to a cross-sectional area of 5 µm thickness of the sample, and subsequently examined under a microscope (Olympus IX71) for possible pathological changes.

2.14. Statistical analysis

Triplicate data generated were converted to mean ± SD using SPSS (version 25.0), while both the one-way ANOVA and Tukey's post hoc tests were also conducted at a significant limit of $p < 0.05$.

3. Results and discussion

3.1. Percentage yields of fractions from crude hexane extract of T. bangwensis

Table 1 indicates that the percentage yields of the solvent fractions for CF1, CF2, and CF3 are 0.71%, 8.21%, and 4.75%, respectively.

3.2. Hypoglycemic effect of the fractions of hexane extract of T. bangwensis

According to Figure 1, the blood glucose concentration was significantly elevated (> 300 mg/dl) at $p < 0.05$, after the third day of alloxan induction, compared to the normoglycemic rats (85 mg/dl).

However with consistent oral administration of the HEXETACF and silymarin (SILY) for 21 days, the blood glucose concentration reduced significantly (≥ 90 mg/dl) compared to the diabetic group. (270 mg/dl) at $p < 0.05$

Table 1. Percentage yields of the fractions of crude hexane leaf extract of *Tapinanthus bangwensis*

Samples	Eluting solvents	Collected fractions	Nature of sample after dryness	Sample yield	Prep TLC band
CF1	100% HEX	25 (F_1-F_{25})	Yellowish oily extract	0.372 g (0.74%)	-
CF2	90% HEX:10% ETAC	15 (F_{26}-F_{40})	Yellowish solid extract	4.106 g (8.21%)	1
CF3	85% HEX:15% ETAC 80% HEX:20% ETAC	47 (F_{41}-F_{88})	Brownish solid extract	2.373 g (4.75%)	6

CFs stands for column fractions, HEX represents hexane while ETAC stands for ethyl acetate

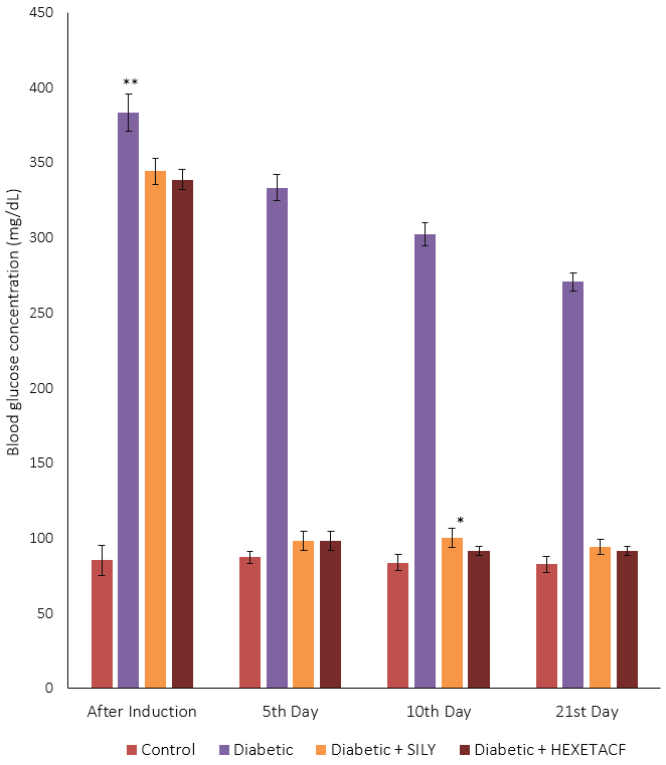

Figure 1. Hypoglycemic effect of the fraction of the hexane extract of *T. bangwensis* in alloxan-induced diabetic rats

The same * connotes no significance, while different * connotes significance at $p < 0.05$. The SILY and HEXETACF stand for silymarin and hexane-ethylacetate fraction.

Diabetes mellitus (DM), formally considered a trivial issue, has become a critical health discourse, due to the heightened annual statistical data of diabetic patients worldwide (International Diabetes Federation, 2015). Notably, DM is a severe metabolic disorder with significantly elevated plasma glucose levels that confer negative consequences on the retina, nerves, liver, kidney, heart, and reproductive cells. One key therapeutic approach to ameliorating postprandial hyperglycemia is simply to inhibit carbohydrate hydrolytic enzymes (α-amylase and α-glucosidase), thereby preventing glucose absorption after carbohydrate ingestion (Ben Salem et al., 2017; Emordi et al., 2018). Ihegboro et al. (2024) reported that the presence of 1,2-benzene dicarboxylic acid, butyl-2-ethylhexyl ester could have influenced the antidiabetic outcome, by inhibiting the metabolic activity of α-amylase and α-glucosidase (Elavarasi et al., 2020; Hassan et al., 2022; Ihegboro et al., 2024).

In addition, neophytadiene and squalene were reported to promote β-islet regeneration, thereby enhancing peripheral glucose metabolism (Alabi & Oyeku, 2017; Ferdosi et al., 2021; Ihegboro et al., 2024). The significant reduction in blood glucose concentration, after the 21st day of administration may be attributed to the hypoglycemic properties of these compounds in the plant.

3.3. Anti-Oxidative effect of fraction of the hexane extract of T. bangwensis

In Figure 2, GSH (glutathione) level improved in the treated groups compared to the diabetic untreated group (DUTG). However, the GSH level was higher in the HEXETACF-treated diabetic group (HTDG) (11.82 ± 0.5 µmol/ml) compared to the silymarin-treated diabetic group (STDG) (10.99 ± 1.50 µmol/ml). However, no significant difference was noticed between the DUTG and the treated groups. Moreover, there was no significant increase in superoxide dismutase (SOD) and catalase (CAT) activity in the treated groups. In addition, the result reveals that no significant decrease in MDA level exists in HTDG (2.21 ± 0.1 µmol/ml), and STDG (2.33 ± 0.1 µmol/ml), compared to the DUTG (2.84 ± 0.1 µmol/ml).

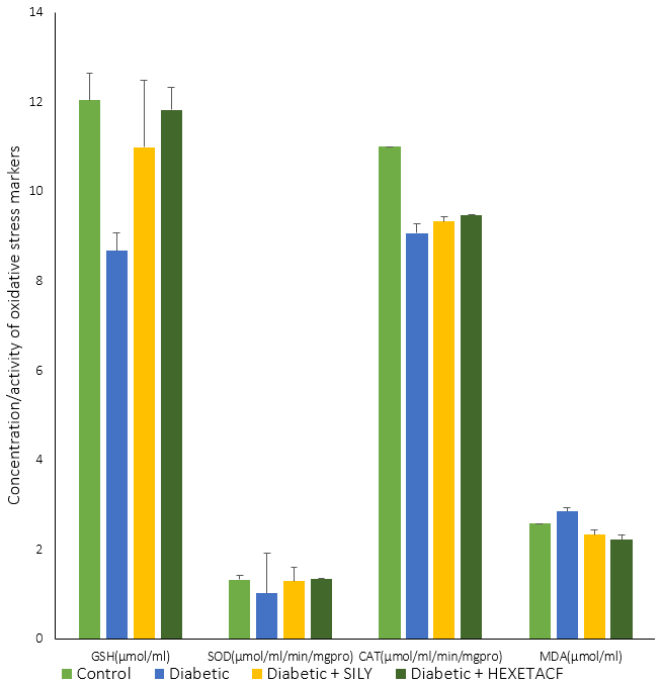

Figure 2. Anti-oxidative effect of the fraction of hexane extract of *T. bangwensis* in alloxan-induced diabetic rats

The same * connotes no significance, while different * connotes significance at $p < 0.05$. The SILY and HEXETACF stand for silymarin and hexane-ethylacetate fraction.

Diabetic-associated type of oxidative stress occurs when lipid peroxyl radicals-byproducts of lipid peroxidation, protein glycation, and glucose auto-oxidation deleteriously damage the lipid-rich membrane, leading to an increase in membrane rigidity and MDA synthesis, as well as decrease erythrocytes lipid fluidity and antioxidant enzyme activity (CAT, SOD, GPx, GST) (Ananthan et al., 2004). In the current study, the HEXETACF appeared to have exerted anti-oxidative potential, by lowering MDA level and enhancing glutathione level, CAT, and SOD activity, respectively.

3.4. Effect of fraction of the hexane extract of T. bangwensis on liver enzymes cctivity

Figure 3, reveals the effect of HEXETACF and SILY on the activities of ALT, AST, and ALP. The ALT activity reduced significantly in the HTDG (89.40 ± 12.65 U/l) and STDG (62.30 ± 12.41 U/l) compared to the DUTG (140.90 ± 19.80 U/l) at $p < 0.05$. But no significant reduction was found between the STDG and HTDG, respectively.

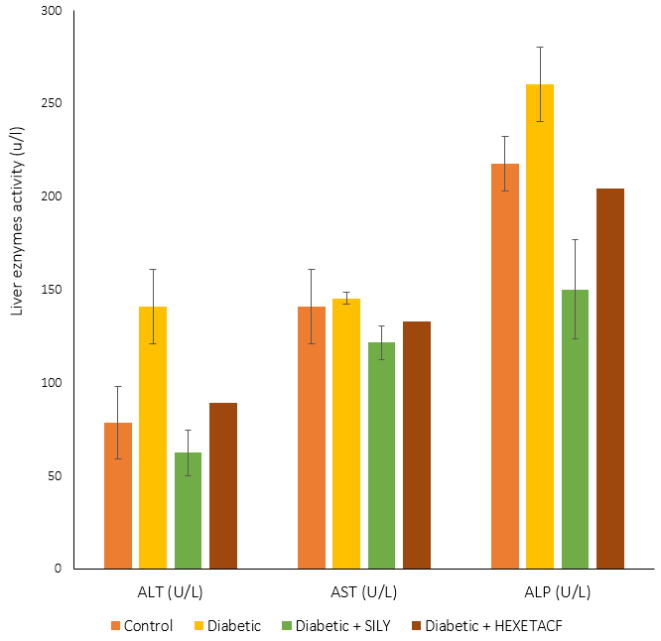

Figure 3. Effect of the fraction of hexane extract of *T. bangwensis* on the activity of liver enzymes in alloxan-induced diabetic rats
The same * connotes no significance, while different * connotes significance at $p < 0.05$. The SILY and HEXETACF stand for silymarin and hexane-ethylacetate fraction.

While AST activity increased in the DUTG (145.37 ± 3.05 U/l), it decreased in the STDG (121.43 ± 8.81 U/l) and HTDG (132.90 ± 10.41 U/l). However, no significant difference exists between the DUTG and treated groups. Finally, ALP activity significantly decreased in the STDG (150.067 ± 26.75 U/l) compared to the HTDG (204.267 ± 27.21 U/l) at $p < 0.05$, additionally significant reduction exists between the treated groups and DUTG (260.30 ± 20.10 U/l) at $p < 0.05$.

Alloxan metabolism in the liver facilitated by cytochrome P450 enzymes, produces excess radical byproducts that destroy the integrity of the pancreatic β-islet cells, affecting insulin secretion. It also has an adverse effect on hepatocyte distribution, the hematopoietic system, and the nephron (Ben Salem et al., 2017; Ihegboro et al., 2022). Interestingly, research studies have established a positive correlation between diabetes and increases in AST, ALT, and ALP activity. Notably so, because free radicals cause hepatocellular lesions, increase the permeability of the liver, and this leads to the release of liver enzymes into the circulatory system. Worthy of note, the ALT and AST perform a transamination reaction, where amino acids are converted to ketoacids, before being transformed to the corresponding amino acids. The HEXETACF's hepatoprotective potential in this study may likely be attributed to the effect of squalene (Ihegboro et al., 2024).

3.5. Hepato-Renal Effect of fraction of the hexane extract of T. bangwensis in diabetic rats

Although CREAT (creatinine) and urea levels were reduced in STDG and HTDG compared to the DUTG, no significant difference was observed between the DUTG and treated groups. Furthermore, there was no significant decrease in ALB (albumin) level, between the DUTG (≥ 30.15 g/l) and the treated groups (≥ 27 g/l). Lastly, the concentration of total protein (TP) was found to be high in the DUTG (64.30 ± 1.84 g/l) but, became reduced with 58.17 ± 9.10 g/l (HTDG) and 61.60 ± 2.43 g/l (STDG), respectively, but no significant difference occurred between the DUTG and the treated groups (Figure 4).

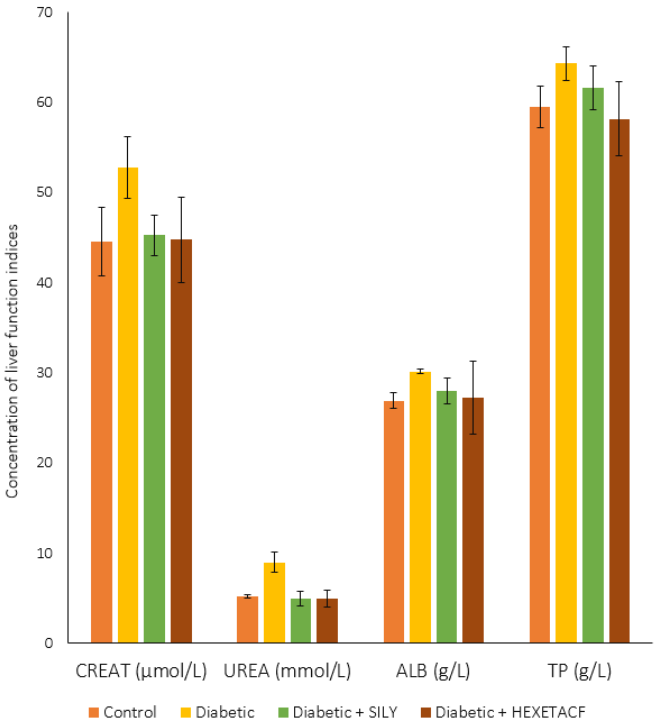

Figure 4. Anti-hepato-renal effect of the fraction of hexane extract of *T. bangwensis* in alloxan-induced diabetic rats
The same * connotes no significance, while different * connotes significance at $p < 0.05$. The SILY and HEXETACF stand for silymarin and hexane-ethylacetate fraction.

The hyperproteinemia and hyperalbuminemia observed in the DUTG had been previously reported by Num-Adom et al. (2022). However, the positive outcome shown by the HEXETACF may suggest its capacity to improve hepato-renal parameters (Emordi et al., 2018).

3.6. Hypolipidemic effect of fraction of the hexane extract of T. bangwensis

Figure 5 reveals the effect of HEXETACF and SILY on lipid markers in diabetic rats. Looking at the result, serum triglyceride (TG) levels decreased significantly in the treated groups compared to the DUTG at $p < 0.05$. Nevertheless, no significant difference exists between the treated groups. After treatment, serum cholesterol (CHOL) was reduced in the treated groups, compared to the DUTG, but no significance occurred between the DUTG and treated groups. The concentration of serum low-density lipoprotein (LDL) was found to be lower in HTDG (0.327 ± 0.03 mmol/l) compared to STDG (0.59 ± 0.21 mmol/l). However, no significant decrease was observed between the treated groups. Also, there was no significant difference in LDL concentration between the DUTG compared to the

treated groups. Finally, serum high-density lipoprotein (HDL) concentration decreased in DUTG but subsequently increased in the treated groups. Nevertheless, no significant difference was found between the DUTG and the treated groups.

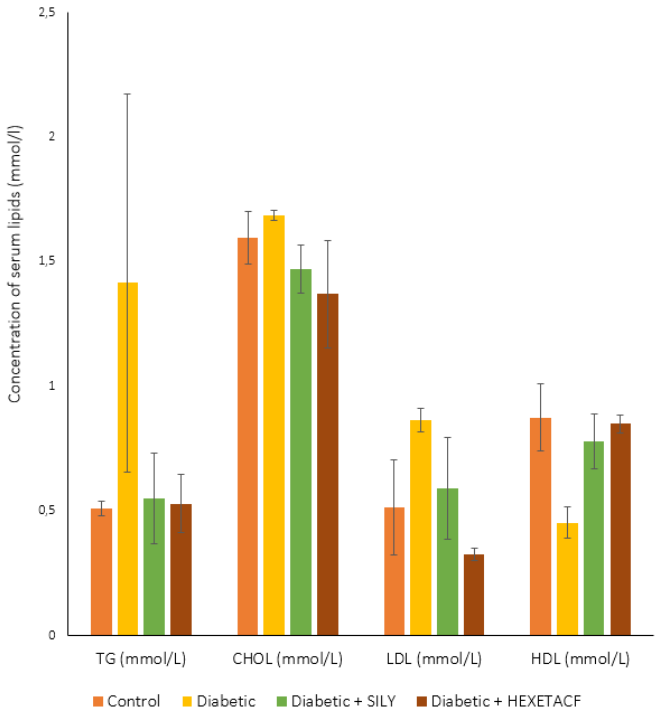

Figure 5. Hypolipidemic activity of the fraction of hexane extract of *T. bangwensis* in alloxan-induced diabetic rats

The same * connotes no significance, while different * connotes significance at $p < 0.05$. The SILY and HEXETACF stand for silymarin and hexane-ethylacetate fraction.

In diabetic conditions, a decrease in insulin secretion increases serum TG and cholesterol levels by down-regulating the activity of pancreatic lipase (lipolytic enzyme) and hydroxylmethylglutaryl-CoA reductase (HMG-CoA reductase) (Shah & Khan, 2014). This is because during insulin deficiency, free fatty acids (FFAs) are displaced from adipose tissue for biosynthesis of fatty acid and ketone bodies. But as insulin secretion improves, glucagon activity is inhibited (that is, the recruitment of FFAs from the adipose tissue), hence, depleting TG and cholesterol levels. The present results reveals that the lipid-lowering outcome may likely be attributed to the presence of hexadecanoic acid, ethyl ester, *cis*-vacenic acid, and squalene, which inhibits the activity of lipase, cholesterol esterase and HMG-CoA reductase – a key enzyme involved in LDL-cholesterol metabolism (Ihegboro et al., 2024; Lozano-Grande et al., 2018; Mirmiranpour et al., 2018; Semwal et al., 2018). Consequently, the reduction in the LDL level, and the subsequent increase in HDL level, suggests that the HEXETACF may facilitate the antiport transport of cholesterol and triacylglycerol from the liver to the peripheral tissues, and from the peripheral tissues to the liver (Donald & Judith, 1990).

3.7. Effect of fraction of the hexane extract of T. bangwensis on serum electrolytes

Considering **Figure 6**, serum sodium (Na^+) and chloride (Cl^-) increased in the DUTG. However, after treatment with HEXETACF and SILY, their concentrations were reduced. However, no noticeable significance exists between the DUTG and the treated groups. Again, serum potassium (K^+) increased in the DUTG (15.17 ± 3.20 meq/l), but decreased in both the STDG (9.23 ±1.06 meq/l) and HTDG (10.023 ±1.05 meq/l). There was no significant difference between the DUTG and the treated groups.

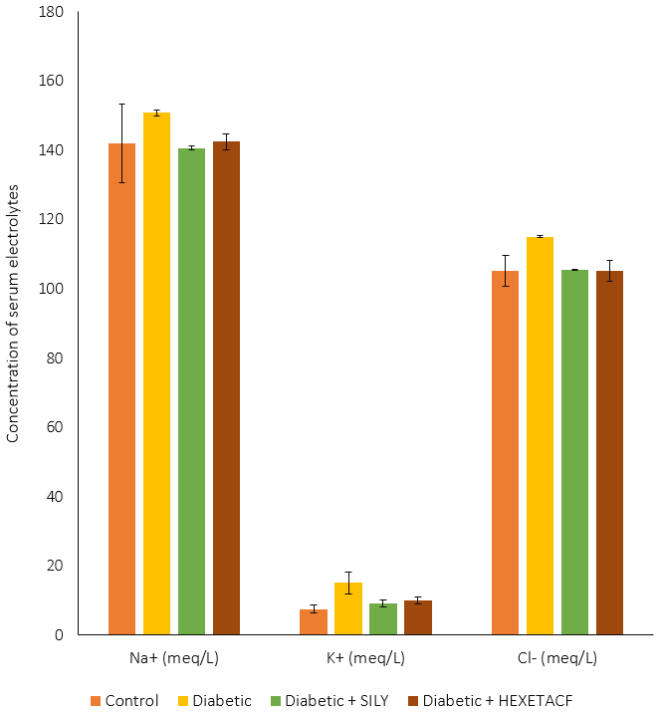

Figure 6. Effect of the fraction of hexane extract of *T. bangwensis* on serum electrolytes in alloxan-induced diabetic rats

The same * connotes no significance, while different * connotes significance at $p < 0.05$. The SILY and HEXETACF stand for silymarin and hexane-ethylacetate fraction.

Many multi-factorial reasons contribute to the electrolyte imbalance in diabetic patients. However, diabetic ketoacidosis and hyperglycemia seem to be predominant (Oyesola et al., 2020). Diabetic ketoacidosis occurs when intracellular fluid-potassium (ICF-K^+) exchanges with excess extracellular fluid-proton (ECF-H^+). Furthermore, reticulocytosis in the peripheral circulation also results in elevated K^+ concentration in the reticulocytes. Finally, in the event of diabetic acidosis, hyperchloridaemia develops via the loss of bicarbonate ions (Esievo, 2017; Navya et al., 2018). The current results indicate that the HEXETACF may improve nephritic performance in diabetic animals.

3.8. Histological effect of fraction of the hexane extract of T. bangwensis on some organs

Figure 7, indicates that in STDG and HTDG, there were normal hepatocytes with central vein (CV), portal vein (PV), basophilic portion with nuclei, and acidophilic cytoplasm of acinar cells, without any abnormalities. It also showed normocellular glomeruli disposed on a background containing renal tubules **(Figure 8)**, while the pancreas had normal exocrine acini with islets and no inflammatory cells **(Figure 9)**. However, the DUTG had congested blood vessels with edema observed in the organs.

3.9. Erythrocytopoietic effect of fraction of the hexane extract of T. bangwensis

According to **Table 2**, the HEXETACF and SILY improved the levels of RBC, MCH (mean cell hemoglobin), HGB (hemoglobin), mean cell hemoglobin count (MCHC), and as well as the MCV (mean cell volume), HCT (packed cell volume) and MPV (mean platelet volume) compared to the diabetic rats. Furthermore, the DUTG (18.75 ±

3.89%) had a higher % RDW-CV compared to the HTDG (16.77 ± 1.70%) and STDG (17.43 ± 3.13%). Finally, the result highlighted that the HEXETACF had substantial haematologic activity compared to the SILY.

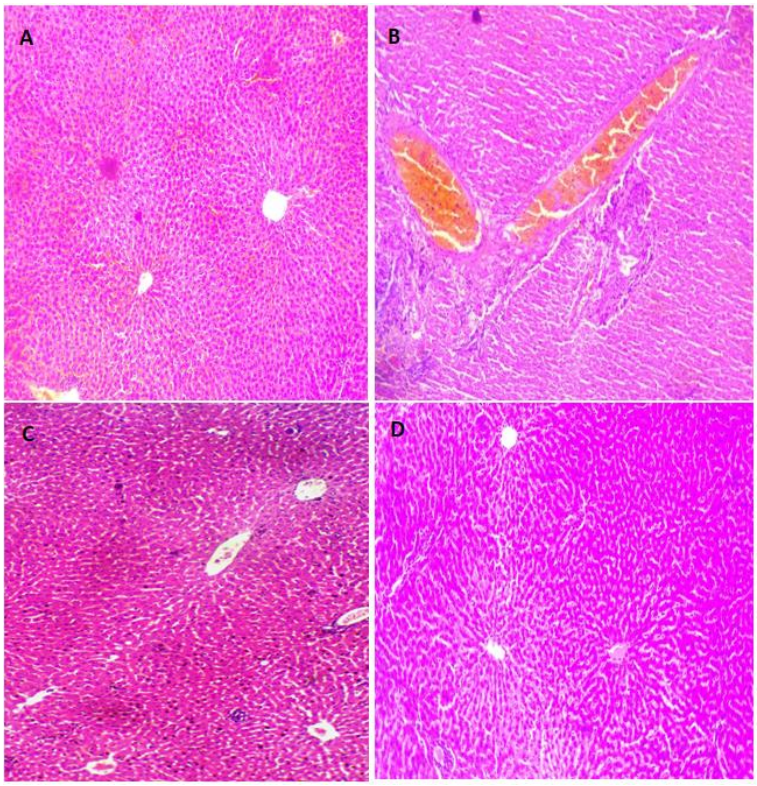

Figure 7. Histological sections of the liver tissue of the different groups
A) Control group (normal hepatocytes), B) Diabetic group (edemic vascular congestion), C) Diabetic + SILY (normal hepatocytes), D) Diabetic + HEXETACF (normal hepatocytes)

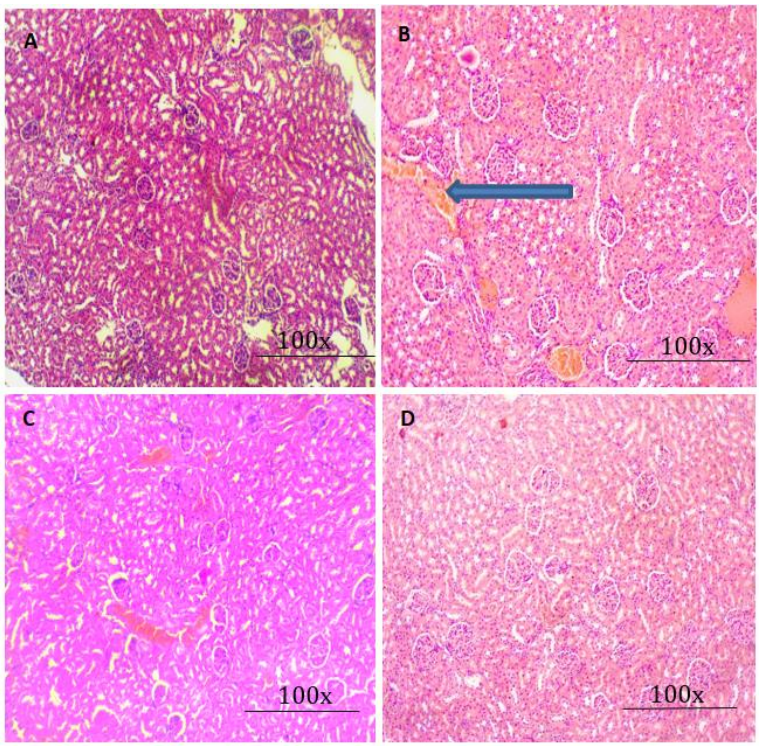

Figure 8. Histological sections of the kidney tissue of the different groups
A) Control group (normal glomerular), B) Diabetic group (edemic vascular congestion), C) Diabetic + SILY (normal glomerular with mild congestion), D) Diabetic + HEXETACF (Normal glomerular)

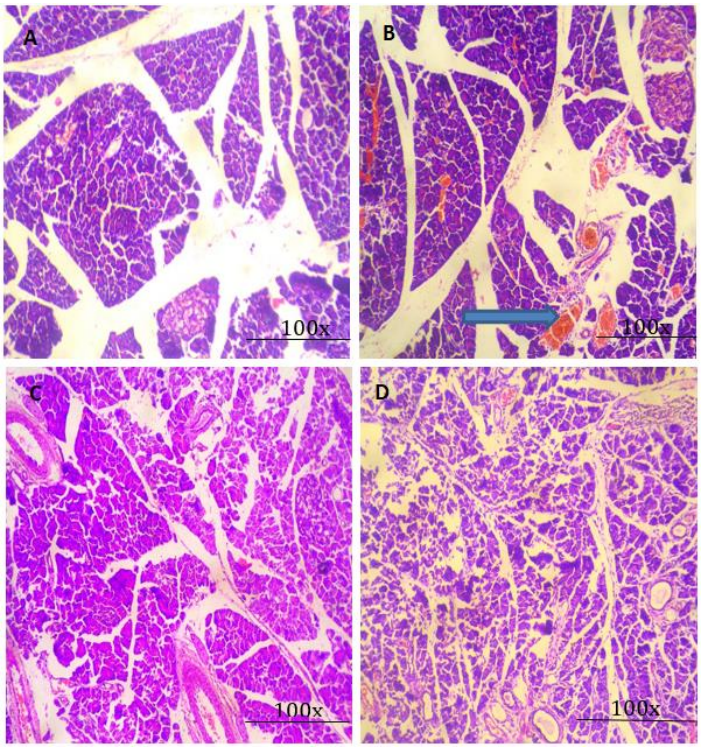

Figure 9. Histological sections of the pancreatic tissue of the different groups
A) Control group (normal islet cells), B) Diabetic group (edemic vascular congestion), C) Diabetic + SILY (normal islet cells), D) Diabetic + HEXETACF (normal islet cells)

Table 2. Effect of the fraction of the hexane leaf extract of *T. bangwensis* on erythrocytic indices in alloxan-induced diabetic rats

Parameters	Control	Diabetic only	Diabetic + SILY	Diabetic + HEXETACF
RBC (x 10^{12}/l)	8.66 ± 0.74^{a}	7.59 ± 2.32^{ab}	7.70 ± 0.47^{a}	8.32 ± 0.61^{a}
MCH (pg)	22.90 ± 0.28^{a}	18.80 ± 1.41^{ab}	19.67 ± 1.41^{a}	21.87 ± 0.76^{a}
MCHC (g/l)	33.30 ± 0.71^{a}	31.65 ± 1.63^{ab}	31.77 ± 0.78^{a}	32.13 ± 1.12^{a}
HCT (%)	45.10 ± 0.14^{a}	44.60 ± 8.06^{ab}	44.80 ± 1.04^{a}	46.23 ± 4.22^{a}
HGB (g/dl)	15.55 ± 1.06^{a}	14.20 ± 3.25^{ab}	14.43 ± 0.50^{a}	14.90 ± 1.54^{a}
MCV (dl)	54.00 ± 1.98^{a}	49.80 ± 7.50^{ab}	58.40 ± 3.31^{a}	55.60 ± 0.95^{a}
MPV (dl)	7.20 ± 1.13^{a}	6.70 ± 0.71^{ab}	6.83 ± 0.12^{a}	7.03 ± 0.31^{a}
RDW-CV (%)	15.45 ± 1.77^{a}	18.75 ± 3.89^{ab}	17.43 ± 3.13^{a}	16.27 ± 1.70^{a}

Values were in triplicates and were expressed as mean + standard deviation. ^{a} indicates no significant difference exists between the control group and the other groups. ^{b} indicates a significant difference exists between the diabetic group and the treated groups at $p < 0.05$. RBC: Red blood cell, MCH: Mean cell hemoglobin, MCHC: Mean cell hemoglobin count, HCT: Packed cell volume, HGB: Haemoglobin, MCV: Mean cell volume, MPV: Mean platelet volume, RDW-CV: Red blood cell distribution

Table 3. Effect of the fraction of the hexane leaf extract of *T. bangwensis* on leucocytic indices in alloxan-induced diabetic rats

Parameters	Control	Diabetic only	Diabetic + SILY	Diabetic + HEXETACF
WBC (x 10^{9}/l)	11.55 ± 1.485^{a}	9.45 ± 4.031^{ab}	9.93 ± 4.46^{a}	10.30 ± 5.23^{a}
LYMP (%)	37.70 ± 0.42^{a}	30.60 ± 6.36^{ab}	34.37 ± 6.87^{a}	36.73 ± 5.28^{a}
GRAN (%)	42.05 ± 4.88^{a}	47.35 ± 3.47^{ab}	40.47 ± 6.60^{a}	45.83 ± 7.77^{a}
MID (%)	20.25 ± 5.30^{a}	18.55 ± 6.29^{ab}	25.17 ± 3.53^{ac}	19.43 ± 2.63^{a}
PLT (x 10^{9}/l)	776.00 ± 24.04^{a}	612.50 ± 71.41^{ab}	700.00 ± 123.24^{a}	675.33 ± 83.72^{a}

Values were in triplicates and were expressed as mean + standard deviation. ^{a} indicates no significant difference exists between the control group and the other groups. ^{b} indicates a significant difference exists between the diabetic group and the treated groups at $p < 0.05$. WBC: White blood cell, LYMPH: Lymphocyte, GRAN: Granulocyte, MID: Combined values of other WBCs not classified as lymphocytes or granulocytes, PLT: Platelet count

The decrease in RBC count and HGB, suggests insufficient production of erythropoietin – a glycoprotein hormone (Ohlsson & Aher, 2006; Thomas, 2008; Thomas et al., 2003). Moreover, the decrease in mean HGB concentration per erythrocyte and per volume of packed red blood cells (MCH and MCHC), HCT, and MCV – which represents the average volume of RBC indicate pathological condition with anemia (Hoffman et al., 2013). Our finding implies that the HEXETACF, enhanced erythropoietin production, inhibits ROS-induced RBC hemolysis, or reduces RBC osmotic fragility (Ben Salem et al., 2017; Muhammad et al., 2012).

3.10. Effect of fraction of the hexane extract of T. bangwensis on leucocytic indices

Table 3 reveals that WBCs (white blood cell count) and the % LYMPH (lymphocyte) were higher in the HTDG compared to the STDG, but lower in the DUTG. Also, the result indicates that the DUTG (18.55 ± 6.29%) and STDG (25.17 ± 5.33%) had the lowest and highest % MID, respectively. However, the HTDG (19.43 ± 2.68%) had a higher % MID compared to the DUTG. Finally, the STDG (700.00 ± 123.24 x 10^{9}/l) had platelet count (PLT) higher when compared to the HTDG (675.33 ± 83.72 x 10^{9}/l), while the DUTG (612.50 ± 71.41 x 10^{9}/l) had the lowest PLT count. Considering all the parameters, no significant difference exists between the DUTG and the treated groups.

Queiroz et al. (2021) reported decreased WBCs and LYNF and increased GRAN (granulocytes) level in alloxan-induced animals, while Ben Salem et al. (2017) reported a decrease in PLT count in diabetic states. The study further established that these pathological changes affect immunity and blood homeostasis (Uhuo et al., 2022). Interestingly, our results indicate that HEXETACF could improve the immune system and thrombocytopoietic activity, by reversing these pathological abnormalities in the diabetic rats.

4. Conclusions

The current result reveals that blood glucose concentration reduced between 33-34%, indicating an improved insulin secretion. Moreover, the hepatocellular membrane appears to have been restored, considering the decreases in the activity of the liver enzymes, compared to the diabetic rats. Additionally, the plant exhibited antioxidative potential, by increasing GSH concentration, and SOD and CAT activity, which culminated in MDA reduction. In comparison with the diabetic rats, the serum TG, cholesterol, and LDL decreased, with an increase in HDL level. Finally, the haematologic indices increased, suggesting an improvement in the secretion of the haematopoietin hormones. In conclusion, the results demonstrate that HEXETACF looked promising as an alternative antidiabetic agent in the absence of SILY.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

Ethical approval for this study was obtained from the Nigerian Police Academy, Kano, Ethics Committee (Date: March 15, 2023, Number: NPA/ETC/03/2023).

Availability of data and materials

All data generated or analyzed during this study are included in this published article. On request, the associated author can provide more information.

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Supplementary File

None.

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