

Evaluation Of The Antidiabetic Effect Of Aqueous Crude Extract Of Seed, Leaf And Stem Of *Linum usitatissimum* On Streptozotocin-Induced Diabetic Rats

Abstract

Diabetes is a metabolic disorder characterized by persistent high glucose level. *Linum usitatissimum* (flaxseed) which is rich in fibre and essential fatty acid is one of the functional foods used in management of diabetes mellitus. The aim of this study is to evaluate the antidiabetic effect of *L. usitatissimum* seed leaf and stem on streptozotocin induced diabetic rats to see which part is more potent in the management of diabetes. Thirty (30) albino rats weighing 170-200g were used for this study and diabetes was induced by intraperitoneal injection of 55mg/kg body weight of streptozotocin. The albino rats were randomly divided into six (6) groups which are as follows: Group A; normal control, B; diabetic control group, C; standard drug (Glibenclamide), D-F were administered different parts of crude plants extract. The phytochemistry analysis showed that the seed possessed more bioactive component followed by the leaf and lastly the stem. The phytochemical present in *Linum usitatissimum* include: alkaloid, flavonoids, tannins, saponins, terpene, steroid, balsam, carbohydrate and phenol. Across the groups that received crude aqueous extract of *L. usitatissimum* was a significant reduction of blood glucose level, however, the seed (6.60 ± 2.450^{bc}) showed more significant difference when compared to the leaf (7.65 ± 1.100^{bc}) and stem (7.95 ± 2.650^{bc}) respectively. On the other hand, the protein and albumin biomarkers were significantly ($P \leq 0.005$) increased across groups. Crude aqueous extract of *L. usitatissimum* also showed antihyperlipidemic properties, improved serum enzymes and electrolytes levels. There was also significant positive impact on hematological parameters. In accordance with the results of this investigation, *L. Usitatissimum* seed part is a more potent antihyperglycemic and antihyperlipidemic agent on streptozotocin-induced diabetic rats although, the leaf and stem also demonstrated significant positive impact on biochemical and haematological parameters. This positive result might be as result of various phytochemical component present in the plant.

Key words: phytochemistry, hypoglycaemic, hypolipidemic, hematology.

1.0 INTRODUCTION

Diabetes mellitus, a non-communicable disease (1), is one of the top five killers in the world (2). This disorder prevents the body from using sugar efficiently (3), which causes an accumulation of too much sugar in the bloodstream and its presence in the urine (4). Furthermore, abnormal lipid metabolism is usually linked to diabetes mellitus (5). Abnormalities in lipid metabolism are frequently seen in both insulin-dependent and non-insulin-dependent diabetes mellitus, and the degree of hyperlipidemia is connected with persistent hyperglycemia (6). In people with pre-diabetes, making small dietary changes and exercising frequently may help postpone or even stop the development of diabetes (7). Omega-3 fatty acids have been associated with enhancements in glycemic and insulin sensitivity (8). **Pre diabetes can be slowed down** by ingesting 1-2 grams of long-chain omega-3 polyunsaturated fatty acids daily can impaired glucose intolerance (9;10). Additionally, adding soluble and viscous fiber to the diet can reduce the glucose response to carbohydrate-rich foods by slowing down gastric emptying and glucose absorption (11). Flaxseed contains soluble fiber, α -linolenic acid (ALA), and linoleic acid, which are essential omega-3 fatty acids necessary for normal human growth and development (12).

Considering it has historically been used to treat a variety of medical ailments (13), flaxseed is recognized in Nigeria as a functional food. The objective of this study is to objectively assess the effectiveness of the flax plant in controlling diabetes mellitus. The study precisely examines the flax plant's distinct parts, including the leaves, seeds, and stems, to ascertain which one is most effective in treating diabetes.

The family *Linaceae* includes flax, also known as *Linum usitatissimum*, which is well-known for its blue blossoms flowers. It is an annual herb that resembles a little palm and has unbranched

stems that can grow up to 30 cm tall, though they usually grow shorter. The complex, pinnate leaves are touch-sensitive and close. The flax plant was grown and harvested for this investigation in Nigeria, Plateau State, and in Zarmaganda layout precisely. It was subsequently recognized, verified, and given the voucher number JUHN21000351 at the University of Jos' Herbarium Department in Plateau State.



Plate I: Fresh flax plant



Plate II: Dry flax stalk with capsule and flower



Plate III: Flaxseeds

Whole flaxseeds have a lovely nutty flavor and a crispy and chewy texture both as dietary supplements and as ingredients in different meal preparations. It contains Fats, high-quality proteins, and dietary fiber abundantly, flax plant has a sizable amount of the fiber which is water-soluble and viscous in nature (14).

Throughout history, people have gathered a variety of plant resources to satisfy their requirements (15). Numerous items that are used for healing are included in these resources, such as nuts, fruits, vegetables, spices and fibers (16). In contemporary time, many people still depend on gathering plant products for sustenance and as a source of income, especially in underdeveloped nations (17). Traditional medicine continues to be widely used and relied upon, largely because it is more affordable and it is perceived as safer than modern medical procedures. (18). Pharmaceutical companies have developed several medications as a result of the traditional medical system, which uses medicinal herbs. Despite the notable advancements made in contemporary scientific medicine (19), the use of complementary or alternative medicine has increased significantly on a global scale. This trend is especially apparent in underdeveloped nations, where traditional medicine continues to be the go-to method for treating a variety of illnesses, even among those who have access to contemporary medical procedures (20).

2.0 Materials and method

2.1 Procurement of materials, chemicals and animals

Thirty (30) male albino rats with 170-200 g body weight were purchased from the Animal house in the University of Jos, Nigeria, and ethical clearance was obtained with reference number: F17-00379. The rats were allowed free access to standard pellet feed purchased from Grand Cereal and Oil Mills Ltd, Jos Nigeria and water. All chemicals used in this research work were of reagent grade and purchased from the Sigma-Aldrich Company, Germany. Syringes for injections, glucometer to check blood glucose level, and commercial kits to analyze biochemical parameters were purchased from scientific stores and local pharmacies in Jos.

2.2 Preparation of the Plant Extract

After the plant *L. Usitatissimum* (Flaxseed) was collected, the seed, leaf and stem were separated and washed differently to remove the sandy particles and then air dried at room temperature under shade. When it was completely dried, the different parts were pounded to powdery form using local pestle and mortar and sieved to obtain fine powder as described by Hussain et al., (2022) with modifications. The finer powdery form of each part was stored in an air-tight plastic container at ambient conditions until when required. The preparation of the decoction was carried out using warm water at 40°C. 100g of the fine powder of each part was soaked in one (1) litre of distilled warm (40°C) water stirred frequently and allowed to stand for 30 – 60 minutes (to ensure maximum extractions of phytochemicals). The mixture was then filtered using Whatman filter paper to remove all the larger particles. The filtrate was concentrated in the oven (SM9053A England) at a temperature of 40-45°C for 9 days.

2.3 Determination of Median Lethal Dose (LD₅₀)

To evaluate the hazardous risk of impacts of the Leaf, Seed, and Stem Extract, the Median Lethal Dose was calculated, Using thirteen (13) rats for each extract as described by Chinedu, Arome & Ameh (2013) with slight modification.

The extract of *L. inumusitatissimum* Leaf, Seed, and Stem was given orally to three dosing groups of three rats each during the first part of the experiment, along with one control group that received distilled water. Within four hours of hunger, four hours after feeding, the following twenty-four hours, the following forty-eight hours, and the following two weeks, animals in each group were watched for any immediate indicators of toxicity and mortality.

In the second stage of the acute toxicity test, four rats were split into four groups, and then different higher extract doses—1200, 1600, 2900, and 5000 mg/kg bodyweight—were orally administered based on the results of phase one.

Determination of The Median Lethal Dose (LD₅₀) Of Aqueous Extract of Flax Plant

2.4 Induction of Diabetes and Administration of plant extract

Thirty (30) White albino rats (male Wistar strain) weighing 170-200g were purchased from the animal house of the University of Jos, Nigeria. Diabetes was induced by intraperitoneal injection of streptozotocin at (55mg/kg) to 25 animals in 5 groups, the animals were left for 48hours under controlled conditions after which diabetes was confirmed from the fasting blood glucose using one - touch glucometer. Blood glucose level greater or equal to 160mg/dl, accompanied with hyperglycosuria after 48 hours was considered diabetic. The 5 rats left without induction of diabetes were considered normal control (23). The plant extract was administered daily at 800mg/kg body weight of each part of the plant for 28 days and the albino rats body weight was obtained weekly using a digital weighing balance.

2.5 Experimental Design

The aqueous extract of *Linum usitatissimum* were administered through oral route. The aqueous extract of seed, leave and stem was administered orally at a dose of 800mg/kg body weight across groups daily. The grouping of the animal are as follows:

Group A_{NC} – Normal control (NC)

Group B_{DC} – Diabetic control (DC)

Group C_{Glib} – Diabetic +800mg/kg bodyweight of Standard drug (Glibenclamide)

Group D_{Seed} – Diabetic + 800mg/kg bodyweight of Seed extract

Group E_{Leaf} – Diabetic + 800mg/kg bodyweight of Leaf extract

Group F_{Stem} – Diabetic + 800mg/kg bodyweight of Stem extract

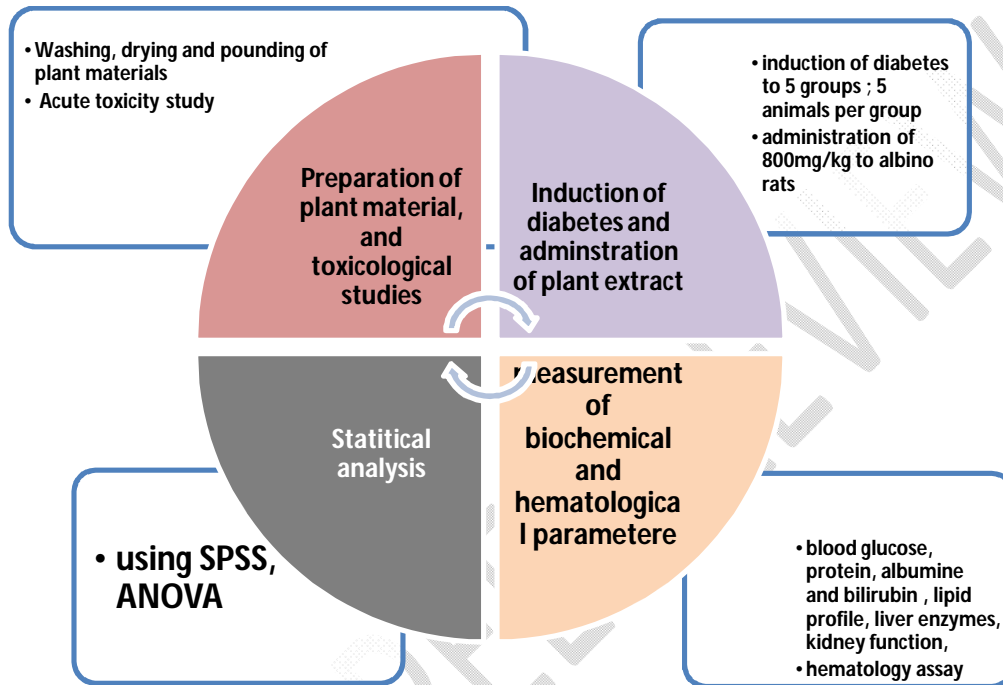


Fig1: An overview of Study Plan

2.6 COLLECTION OF BLOOD SAMPLE

At the end of the experiment, the rats were starved for 12 hours before they were sacrificed by decapitation and the blood was collected through the jugular vein in plane and heparinise containers for the analysis (24).

2.7 STATISTICAL ANALYSIS

Result values are expressed as mean \pm standard deviation. Analysis of variance (ANOVA) was used for Comparison. Differences were considered significant when values of $p \leq 0.05$. A Graph Pad prism 7.0 in triplicates ($n = 3$) was used to carry out the above analysis

3.0 Discussion

The study evaluated the antidiabetic properties of the aqueous extract and fractions of *L.usititatissimum* on STZ induced diabetic rats. According to Bhagarviet al. (2015) and Sharma (2018), diabetes is a disease condition characterized by hyperlipidaemia brought on by the uninhibited actions of lipolytic enzymes and the near absence of insulin. Prolonged hyperlipidaemia leads to serious complications that increase oxidative stress.

3.1 Acute Toxicity

According to the findings of the acute toxicity study, the LD₅₀ of flaxseed leaf, stem, and extract is **greater** than 5000 mg kg⁻¹ body weight. The limit test dose is typically employed when the experimenter has knowledge that the test substance is probably non-toxic or of low toxicity (27). As a result, the non-lethal effects caused by the large dose of this extracts suggest that acute oral exposure to plant extracts is often harmless. Therefore, it can be said that extracts are safe. Any chemical that has an estimated LD₅₀ value of more than 3000-5000 mg kg⁻¹ (by oral route) may be regarded as being low toxicity and harmless as seen in table 1 Flaxseed is observed to have low acute toxicity (28).

Table 1: LD₅₀ Toxicity Test Result *L. usititatissimum*

Group	No of Rats	Mortality Recorded	LD ₅₀
10mg/kg bw	3	0	≥ 10

100mg/kg bw	3	0	≥ 100
1000mg/kg bw	3	0	≥ 1000
1600mg/kg bw	3	0	≥ 1600
2900mg/kg bw	3	0	≥ 2900
5000mg/kg bw	3	0	≥ 5000

3.2 Phytochemical Assay

Phytochemicals are naturally occurring bioactive substances that can be found in a variety of plant sections, including fruits, vegetables, flowers, leaves, roots of medicinal plants. They provide safeguarding benefits by acting as a protective mechanism against diseases in association with nutrients and fibers (29). Table 2 presents the preliminary phytochemical composition of the aqueous extract obtained from the *L. usitatissimum* plant and its different components. It was observed that the seed part of the plant had more of the phytochemicals followed by the leaf and lastly the stem. The presence of phytochemicals such as alkaloids, terpenes, steroids, carbohydrates, resins, phenols, saponins and flavonoids was identified. The antioxidant capabilities of flavonoids are well known (30), as well as their capacity to alter how the body reacts to dangerous free radicals that are produced during the aberrant metabolic processes frequently seen in diabetes (31). Because of this, flavonoids may have therapeutic advantages (32). Flavonoids have been associated with a number of beneficial attributes on metabolic illnesses, such as cancer, diabetes, obesity, and cardiovascular disease (33). Flavonoids may be helpful in the prevention and treatment of some comorbidities (34). Additionally, they function as

antioxidants, reducing the effects of nitrogen and oxygen species on oxidative stress and reducing the risk of disease (35). The ability of flavonoids to control glucose absorption, insulin signaling, insulin secretion, and adipose deposition is related to their anti-diabetic effects (36), to reduce apoptosis and improve hyperglycemia, they focus on a number of molecules that are involved in controlling several pathways, such as those that enhance insulin production, promote β -cell proliferation, and control liver glucose metabolism. This study supports the findings of Luka et al. (2012), who noted that plants with hypoglycaemic action usually include flavonoids. Furthermore, when digestive enzymes break down dietary polysaccharides, blood glucose levels rises (38), one of these enzymes involved in this process is α -amylase, which is present in sizable amounts in saliva and pancreatic juice. Its function is to speed up the dissolution of α -1,4-glycosidic bonds found in starch, glycogen, and other oligosaccharides (39). The hydrolase class enzyme α -glucosidase, which is secreted by the epithelial cells lining the brush borders of the small intestine, is another enzyme implicated in this process. Postprandial hyperglycemia is a result of the hydrolytic degradation of oligosaccharides into absorbable monosaccharides, which is aided by α -glucosidase and amylase, and the use of secondary metabolites generated from plants to block these digestive enzymes is a popular strategy for lowering postprandial blood glucose levels (40) and alkaloids have the ability to block these enzymes. Alkaloids have the capacity to attach to either the competitive or noncompetitive sites of the digestive enzymes. This binding stops the enzyme-substrate complex from forming, which eventually lowers enzyme activity (41) Tannins have anti-inflammatory and antioxidant capabilities, tannins, a class of phytochemicals, have demonstrated neuroprotective effects in diabetes complications (42; 43). As a result of saponins' ability to block the enzymes that break down disaccharides into monosaccharides, increased blood glucose levels are reduced (44). In order to reduce high

postprandial blood sugar levels, this impact is especially important for managing both Type I and Type II diabetes (45). Saponins also have capacity to precipitate and coagulate red blood cells (RBCs), saponin-containing plants are used in the treatment of wounds (46).

Table 2: Result of the phytochemical screening of the Crude Extracts of leaf, seed and stem of *L. usititissimum*.

Test	Leaf	Seed	Stem
Alkaloids	-	+	-
Flavonoids	+	+	+
Tannins	+	+	-
Saponins	-	+	-
Terpens/Steroids	-	+	-
Cardiac glycosides	-	-	-
Balsam	+	+	+
Carbohydrates	+	+	+
Phenol	+	+	+
Resins	-	+	-

+ = Detected - = Not Detected

3.3 Biochemical Observations

The findings of this study demonstrate that treatment with *L. usititissimum* extract at a dosage of 800mg/kg bodyweight significantly ($p \leq 0.05$) reduced blood glucose levels in STZ-induced

diabetic rats across all groups when compared to the diabetic group (table 3). The decrease was notably significant compared to both the normal control group and the group treated with the standard drug Glibenclamide. These results align with previous studies (47), suggesting that the plant extract possesses hypoglycemic properties, potentially stimulating insulin release and promoting the uptake of glucose in peripheral tissues, thereby reversing streptozotocin-induced hyperglycemia. These findings indicate that *L. usitatissimum* may be acting through mechanisms such as increasing insulin secretion to enhance glucose uptake by adipose or muscle tissues and inhibiting the intestinal absorption of glucose (48). It is worthy of note to state that the crude aqueous seed part had more antihyperglycemic effect on the streptozotocin induced diabetic rats even though there was a significant reduction of blood glucose level for the leaf and stem group. Also, Albumin, a major protein present in human plasma, constitutes approximately 25% of total protein synthesis in the liver and accounts for half of the proteins secreted by the liver. Its production is impeded in various diseases, particularly those affecting the liver (49). Moreover, research indicates that an insulin deficiency leads to reduced synthesis of liver proteins, including albumin and serum proteins (50). Table 3 reveals that the extract treatment group exhibits significantly higher levels of albumin and total serum protein compared to the diabetic control groups. This finding may be attributed to the liver's improved functioning through enhanced metabolism and regulation of protein and albumin, attributable to the beneficial effects of *L. Usitatissimum*.

Table 3: Effect of Aqueous Extract of *L. usitatissimum* on Serum Glucose, Serum total Protein and Serum Albumin in STZ induced Diabetic Rats.

Group	Treatment	Glucose	Total Protein	Albumin
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		(mmol/L)	(g/L)	(g/L)
A	Normal Control	5.85 ± 0.050	74.00±4.400	44.50±1.500
B	Diabetic Control	23.35 ± 4.850 ^b	50.30±0.300 ^a	31.00±3.000 ^a
C	Diabetic + Glibenclamide	5.45 ± 0.450 ^{ac}	72.00±4.00 ^{bc}	43.00 ± 1.000 ^{bd}
D	Diabetic + Extract (Seed)	6.60 ± 2.450 ^{bc}	70.10 ± 3.000 ^{bd}	43.50±0.500 ^{bd}
E	Diabetic + Extract (Leaf)	7.65 ± 1.100 ^{bc}	69.00±1.000 ^{bd}	42.50±3.500 ^{bd}
F	Diabetic + Extract (Stem)	7.95 ± 2.650 ^{bc}	68.50±0.500	40.05±0.500

Values are expressed as mean ± SEM, n=3.

^aValues are significantly lower when compared to normal control (p < 0.05)

^bValues are significantly higher when compared to normal control (p < 0.05)

^cValues significantly lower when compared with diabetic control (p < 0.05)

^dValues significantly higher when compared with diabetic control (p < 0.05)

Elevated bilirubin levels may serve as a biomarker for oxidative stress and inflammation in individuals with pre- and new-onset diabetes (51). Studies have demonstrated that serum bilirubin levels are influenced by glucose metabolic status, with individuals with pre-diabetes and new-onset diabetes exhibiting higher bilirubin levels compared to those with normal fasting glucose (52). Consistent with other research findings, table 4 indicates that the aqueous treatment groups and the Glibenclamide treatment group exhibit significantly lower bilirubin levels compared to the diabetic control group. Similar results have been reported by (53).

Table 4: Effect of aqueous extract of *L. usitatissimum* on Serum total bilirubin, direct bilirubin and indirect bilirubin in STZ induced Diabetic rats.

Group	Treatment	TB ($\mu\text{mol/L}$)	DB ($\mu\text{mol/L}$)	IDB ($\mu\text{mol/L}$)
A	Normal Control	9.30 \pm 0.900	2.68 \pm 0.450	6.62 \pm 0.450
B	Diabetic Control	78.05 \pm 11.050 ^b	33.05 \pm 11.041 ^b	45.00 \pm 0.009 ^b
C	D + Glibenclamide	51.20 \pm 27.000 ^{bc}	22.38 \pm 7.600 ^{bc}	28.82 \pm 19.400 ^{bc}
D	D + Extract (Seed)	19.40 \pm 11.000 ^{bc}	6.68 \pm 12.850 ^{bc}	12.72 \pm 23.850 ^{bc}
E	D + Extract (Leaf)	44.25 \pm 23.850 ^{bc}	18.02 \pm 0.089 ^{bc}	26.23 \pm 23.761 ^{bc}
F	D + Extract (Stem)	56.16 \pm 11.950 ^{bc}	25.96 \pm 6.800 ^{bc}	30.20 \pm 5.150 ^{bc}

D= Diabetic, DB= Direct bilirubin, TB= Total bilirubin, IDB= Indirect Bilirubin

Values are expressed as mean \pm SEM, n=3.

^aValues are significantly lower when compared to normal control ($p < 0.05$)

^bValues are significantly higher when compared to normal control ($p < 0.05$)

^cValues are significantly lower when compared to diabetic control ($p < 0.05$)

The body produces cholesterol, which is also derived from some food sources, and it is essential for several physiological processes (54). It contributes to hormone biosynthesis, bile acid synthesis, and cell membrane composition (55). Low-Density Lipoprotein (LDL), sometimes known as "bad" cholesterol, and High-Density Lipoprotein (HDL), also known as "good" cholesterol, are both included in total cholesterol (56). Although cholesterol is essential for overall health, high amounts can be harmful since they can cause arteries to constrict or become blocked (57). A significant rise in the risk of cardiovascular disease (CVD) is unfortunately seen

in those with diabetes who are more likely to have unhealthily high cholesterol levels (58). By effectively managing cholesterol levels, individuals can lower their chances of developing CVD and premature death (59). Table 5 of this study demonstrates a considerable rise in HDL levels and, on the other hand, a significant fall in LDL levels. The *L. usititissimum* aqueous extract and the control rats were equivalent. The fact that *L. usititissimum* was able to considerably raise HDL levels and lower LDL levels in streptozotocin-induced diabetic rats suggests that it has hypolipidemic capabilities, in addition, research have shown that linoleic acid can improve insulin sensitive (60). The most common type of fat in the body, triglycerides, have normal levels that vary depending on age and gender. Atherosclerosis, or the buildup of fatty deposits within artery walls, is associated with elevated triglyceride levels, low HDL cholesterol, or high LDL cholesterol (61). Having high triglyceride levels increases the risk of heart attack, peripheral artery disease (PAD), and stroke. It is worth mentioning that elevated triglycerides do not directly cause diabetes. Rather, they indicate an impairment in the body's ability to convert food into energy (62).

Table 5: Effect of Aqueous extract of *L. usititissimum* on Lipid profile in STZ induced Diabetic rats.

Group	Treatment	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
A	Normal Control	2.30 ± 0.100	0.95 ± 0.050	1.08 ± 0.015	0.78±0.105
B	Diabetic Control	4.80 ± 0.400 ^b	1.90 ± 0.500 ^b	1.00 ± 0.335 ^a	2.81±0.190 ^b
C	D + Glibenclamide	3.25 ± 0.450 ^{bc}	1.70 ± 0.100 ^b	2.16 ± 0.165 ^b	1.73±0.065 ^{bc}

D	D + Extract (Seed)	3.25 ± 0.450 ^b	1.35 ± 0.050 ^{bc}	0.80 ± 0.115 ^{ac}	1.76±0.045 ^{bc}
E	D + Extract (Leaf)	3.10 ± 0.300 ^{bc}	1.35 ± 0.050 ^{bc}	1.03 ± 1.185 ^{be}	1.48±0.160 ^{bc}
F	D + Extract (Stem)	3.55 ± 0.550 ^{bd}	0.90 ± 0.100 ^{ac}	1.00 ± 0.150 ^{ae}	1.85 ± 0.525 ^{bc}

HDL - High Density Lipoprotein, LDL – Low Density Lipoprotein

Values are expressed as mean ± SEM, n=3

^aValues are significantly lower when compared to normal control (p < 0.05)

^bValues are significantly higher when compared to normal control (p < 0.05)

^cValues are significantly lower when compared to diabetic control (p < 0.05)

^dValues are significantly higher when compared to diabetic control (p < 0.05)

^eValues are almost equal to compared to normal control (p > 0.05)

Individuals with type 2 diabetes mellitus (T2DM) have a higher likelihood of experiencing abnormalities in liver function tests compared to those without T2DM (63). Supporting this, Mandal et al. (2018) reported elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in a diabetic population. Table 6 illustrates that the serum levels of ALT, AST, and ALP were significantly reduced (p ≤ 0.05) in groups treated with the aqueous extract of *L. usitatissimum* when compared to the diabetic control group. Similarly, decreased levels of ALT, AST, and ALP were observed in diabetic rats treated with Glibenclamide. The release of these enzymes into the bloodstream is indicative of the hepatotoxic effect of diabetes (65). Therefore, the *L. usitatissimum* aqueous extract significantly lowered the levels of ALT, AST, and ALP in the serum, suggesting its hepatoprotective properties.

Table 6: Effect of aqueous extract of *L. usitatissimum* on some Serum Enzymes in STZ induced Diabetic rats.

Group	Treatment	ALT(U/L)	AST (U/L)	ALP(U/L)
A	Normal Control	68.50 ± 4.500	56.50 ± 3.500	95.50 ± 2.000
B	Diabetic Control	258.83 ± 95.500 ^b	202.50±70.500 ^b	300.50 ± 80.500 ^b
C	D + Glibenclamide	125.00 ± 23.000 ^{bc}	99.50 ± 21.500 ^{bc}	149.00 ± 19.000 ^{bc}
D	D + Extract (Seed)	113.00 ± 10.000 ^{bc}	87.50±17.500 ^{bc}	140.50 ± 17.500 ^{bc}
E	D + Extract (Leaf)	181.50 ± 86.500 ^{bc}	128.50±58.500 ^{bc}	196.50 ± 92.500 ^{bc}
F	D +Extract (Stem)	162.00 ± 54.500 ^{bc}	116.50±45.500 ^{bc}	184.00 ± 49.000 ^{bc}

AST – Aspartate transaminase, ALT – Alanine Aminotransferase, ALP – Alkaline

Phosphatase

Values are expressed as mean ± SEM, n=3

^aValues are significantly lower when compared to normal control (p < 0.05)

^bValues are significantly higher when compared to normal control (p < 0.05)

^cValues are significantly lower when compared to diabetic control (p < 0.05)

^dValues are significantly higher when compared to diabetic control (p < 0.05)

In diabetes, markers such as urea, uric acid, and creatinine tend to increase due to kidney impairment, which can be caused by diabetes. This can result in the leakage of these biomarkers into the urine (66). In diabetic rats, the levels of urea, creatinine, and uric acid were significantly higher compared to normal rats, suggesting that diabetes can lead to kidney dysfunction. However, when diabetic rats were treated with different parts of an aqueous extract from *L. usitatissimum*, there was a significant reduction in the levels of urea, creatinine, and uric acid compared to untreated diabetic rats. This suggests that the extract has a protective effect on the kidneys. These findings are consistent with the studies conducted by Dinko and Edward (2015)

and Dirk et al. (2015). Diabetes mellitus profoundly affects the kidneys and is considered the main culprit behind diabetic nephropathy, (69). In addition to oxidative stress and the presence of advanced glycation end-products, disrupted lipid metabolism and the build up of lipids in the kidneys have been proposed as factors that contribute to the development of diabetic nephropathy. Several research studies have reported the observation of lipid accumulations in the kidneys of both diabetic patients and animal models, suggesting that these deposits may play a significant role in the progression of diabetic kidney disease (70).

Table 7: Effect of aqueous extract of *L. usitatissimum* on serum urea, serum creatinine and serum uric acid concentrations in STZ induced Diabetic rats.

Group	Treatment	Urea (mmol/L)	Creatinine (μ mol/L)	Uric Acid (μ mol/L)
A	Normal Control	312.50 \pm 54.500	13.50 \pm 2.000	1.00 \pm 0.200
B	Diabetic Control	548.00 \pm 60.000 ^b	42.00 \pm 9.000 ^a	3.20 \pm 0.400 ^b
C	D + Glibenclamide	446.50 \pm 31.500 ^{bc}	33.00 \pm 8.000 ^{ab}	2.60 \pm 0.600 ^{bd}
D	D + Extract (Seed)	375.50 \pm 3.500 ^{bc}	20.50 \pm 4.500 ^{bc}	1.80 \pm 0.400 ^{bc}
E	D + Extract (Leaf)	436.50 \pm 17.500 ^{bc}	28.50 \pm 2.500 ^{bc}	2.55 \pm 0.250 ^{bd}
F	D + Extract (Stem)	505.00 \pm 101.500 ^{bd}	27.00 \pm 1.00 ^{bc}	2.55 \pm 0.500 ^{bd}

Values are expressed as mean \pm SEM, n=3

^aValues are significantly lower when compared to normal control (p < 0.05)

^bValues are significantly higher when compared to normal control (p < 0.05)

^cValues are significantly lower when compared to diabetic control (p < 0.05)

^dValues are significantly higher when compared to diabetic control (p < 0.05)

The increased volume and metabolites excretion via the kidneys, usually in excess of normal thresholds give rise to imbalance in homeostasis with respect to electrolytes (71). Usually measured electrolytes are Na^+ , K^+ , Cl^- and HCO_3^- . These four ions in plasma exert the greatest influence on water balance and acid-base relationship (72). From table 8, when compared to the normal and diabetic control, Treatment with *L. usititissimum* aqueous extract showed that there was a significant decrease in serum concentration of Na^+ , Cl^- and HCO_3^- . The analysis plant extracts administration has impact on the electrolytes and exhibited properties capable of boosting the buffering function of the body system (73).

Table 8: Effect of aqueous extract of *L. usititissimum* on some serum electrolytes Concentrations in STZ induced Diabetic rats.

Group Treatment		Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
A	Normal Control	148.00 ± 0.00	4.50 ± 0.250	103.50 ± 0.159	28.00 ± 1.000
B	Diabetic Control	145.00 ± 1.000 ^a	4.85 ± 0.100 ^e	104.50 ± 1.500 ^b	25.00 ± 1.000 ^a
C	D + Glibenclamide	134.27 ± 2.000 ^{ac}	5.40 ± 0.500 ^{bd}	96.00 ± 4.000 ^{ac}	23.00 ± 2.000 ^{ac}
D	D + Extract (Seed)	134.00 ± 0.000 ^{ac}	4.90 ± 0.200 ^{ae}	98.50 ± 0.500 ^{ac}	24.50 ± 1.500 ^{ac}
E	D + Extract (Leaf)	136.00 ± 4.000 ^{ac}	5.00 ± 0.200 ^{bd}	99.00 ± 3.000 ^{ac}	25.50 ± 1.500 ^{be}
F	D + Extract (Stem)	137.00 ± 7.000 ^{ac}	5.55 ± 0.350 ^{bd}	100.50 ± 6.500 ^{ac}	22.00 ± 1.000 ^{ac}

Values are expressed as mean ± SEM, n=3

^aValues are significantly lower when compared to normal control (p < 0.05)

^bValues are significantly higher when compared to normal control ($p < 0.05$)

^cValues are significantly lower when compared to diabetic control ($p < 0.05$)

^dValues are significantly higher when compared to diabetic control ($p < 0.05$)

^eValues are significantly almost equal when compared to normal control ($p < 0.05$)

3.4 Analysis of haematological studies

Assessing the hematological parameters of Red Blood Cells (RBCs) and White Blood Cells (WBCs) can provide valuable information about the adverse effects on the blood components in animals, (74). Additionally, these parameters can help elucidate the blood-related functions of chemical compounds found in plant extracts (75). In individuals with diabetes, various factors like advanced glycation end products (AGEs), oxidative stress, angiotensin II, and cytokines can stimulate WBCs. This activation of WBCs in diabetic individuals leads to the release of cytokines and transcription factors that significantly contribute to inflammation, (76). When evaluating the effects of *L. usitatissimum* aqueous extract derived from the leaf, stem, and seed (as presented in Table 9), a noteworthy reduction in WBC count was observed across the experimental groups.

Table 9: Effects of Aqueous extracts of *L. usitatissimum* on White blood count and its parameters Concentrations in STZ induced Diabetic rats.

Treatment	WBC	NEU(%)	LYM(%)	MON(%)	EOS(%)	BAS(%)
A Normal Control	6.35±0.158	10.86±0.318	65.36±3.351	0.53±0.088	0.36±0.033	0.00±0.000
B Diabetic Control	19.99±1.669 ^{bd}	65.70±13.884 ^b	45.93±14.856 ^a	6.13±0.348 ^a	2.73±0.202 ^b	1.36±0.441 ^b
C D + Glib	7.03±2.116 ^{ac}	30.63±1.746 ^{bc}	60.90±0.907 ^{bd}	3.13±0.866 ^{ad}	0.53±0.120	0.76±0.202

D	D + Extract (Seed)	7.51±1.370 ^b	37.76±1.281 ^{bc}	61.23±1.501 ^{bd}	0.63±0.088 ^{ac}	0.56±0.033 ^{bc}	0.52±0.000 ^{ac}
E	D + Extract (Leaf)	12.88±0.904 ^{bd}	51.26±1.648 ^{bc}	30.93±1.411 ^{ac}	1.10±0.057 ^{ac}	1.30±0.321 ^{bc}	1.31±0.600 ^{bd}
F	D + Extract (Stem)	13.94±1.280 ^a	49.20±0.550 ^{bc}	48.83±0.977 ^{ad}	2.73±2.783	0.93±0.371 ^{bc}	1.10±0.057 ^{bd}

WBC- White blood cells, NEU- Neutrophil, LYM- Lymphocyte, MON- Monocytes, EOS- Eosinophil, BAS- Basophile

Values are expressed as mean ± SEM, n = 3.

^aValues are significantly lower when compared with normal control (p < 0.05)

^bValues are significantly higher when compared with normal control (p < 0.05)

^cValues are significantly lower when compared with diabetic control (p < 0.05)

^dValues are significantly higher when compared with diabetic control (p < 0.05)

Erythrocytes, being a crucial element of the bloodstream, serve as a highly responsive indicator of an individual's overall health condition (77). The dangers that diabetic patients face include hyperglycemia, hyperosmolarity, oxidative stress, inflammation, and problems with lipid metabolism. These hazards can all have an impact on their red blood cells (erythrocytes). These elements influence the aggregation, deformability, and fluidity of the erythrocyte membrane. In the end, these modifications disturb microcirculation and aid in the emergence of diabetic problems (78) Table 10 shows that administration of the extracts had a positive change in the haematological parameters suggesting that it may have contributed to the ameliorative effect that is observed from the treatment group.

Table 10: Effects of Aqueous extracts of *L. usitatissimum* on Red Blood Count and its Parameters Concentrations in STZ induced Diabetic rats.

Group	Treatment	RBC	HGB	HCT(PCV)	PLT	PCT
A	Normal Control	8.01±0.500 ^{bd}	15.78±1.019 ^{bd}	47.50±0.458	279.67±7.446 ^{ac}	0.21±0.014
B	Diabetic Control	3.48±0.274 ^b	6.70±0.550 ^b	39.83±1.328 ^b	623.67±110.900 ^b	0.45±0.087 ^e
C	D + Glibenclamide	7.45±0.183 ^{bc}	14.80±0.450 ^{bd}	45.93±0.845 ^{bc}	306.00±5.568 ^{ac}	0.23±0.015 ^{ac}
D	D + Extract (Seed)	7.25±0.119 ^{bc}	13.83±0.120 ^{bc}	44.93±0.338 ^{bc}	373.67±41.289 ^{ac}	0.35±0.040 ^{ac}
E	D + Extract (Leaf)	6.96±0.136	12.10±0.152	43.60±2.635 ^{bd}	549.67±1.453 ^{ac}	0.40±0.045 ^{ac}
F	D + Extract (Stem)	7.03±0.063 ^{bc}	13.53±0.202 ^{bc}	41.50±0.556 ^{ec}	511.33±14.847	0.42±0.036 ^{ac}

RBC-Red Blood Cells, HGB-Haemoglobin, HCT-Haematocrit, PLT-Platelets and PCT-Plateletricit

Values are expressed as mean ± SEM, n = 3.

^aValues are significantly lower when compared with normal control (p < 0.05)

^bValues are significantly higher when compared with normal control (p < 0.05)

^cValues are significantly lower when compared with diabetic control (p < 0.05)

^dValues are significantly higher when compared with diabetic control (p < 0.05)

^eValue is equal to normal control (p < 0.05)

3.5 Conclusion

On the basis of the current research carried out, it can conclude that the plant *L. usititissimum* possesses antihyperglycaemic and antihyperlipidemic effect in streptozotocin-induced diabetic rats. The traditional use of the plant to manage diabetes is supported by this laboratory finding. The progressive decrease in the blood glucose levels of rats and improvement of biochemical parameters shown by the treatment with the aqueous extract of *Linum usititissimum* may be due to the presence of phytochemicals found in abundance in the plant.

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APPENDIX

ETHICAL CLEARANCE

UNIVERSITY OF JOS, NIGERIA
FACULTY OF PHARMACEUTICAL SCIENCES



Private Mail Bag 2084
Jos, Nigeria, Private
Tel: 079-55930, 559431-4

DEPARTMENT OF PHARMACOLOGY ETHICAL COMMITTEE ANIMAL EXPERIMENTAL UNIT
TO: MR./MRS./MS/DR./PROF.

REF/UJ/FPS/PCL/AEU/2 NAME: VONGDIP, DANMAN GODWIN
DATE: 12th November, 2021


REFERENCE: ASSURANCE APPROVAL
UJ/FPS/F17-00379

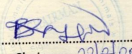
ETHICAL CLEARANCE CERTIFICATE

We write on behalf of the Ethical committee and wish to convey the Committee's approval to you; based on the Protocol form you filled for the research that you are about to embarked upon.

We wish to clearly state that, having made the conditions of the Institutional Animal Care and Use (IACU) in collaboration with the Office of Laboratory Animal Welfare (OLAW), Reference number: F17-00379, you are hereby cleared and by this, your need for animal (s) in research is JUSTIFIED.

Please NOTE that the procedures you have indicated are to be strictly adhered to. Accept our Congratulations and wishing you success in your research.


Secretary 22/11/2021
Mr. Wazoh Luka


Chairman 22/11/2021
Dr. B. B. Bukar

PROTOCOL FORM

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IAACUUC)
ANIMALEXPERIMENTAL UNIT
DEPARTMENT OF PHARMACOLOGY
FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF JOS, NIGERIA.

PROTOCOL FORM FOR RESEARCH WORK/PROJECT

- Name of student: VONGDIP, DANMAN GODWIN
- Name of supervisor: DR. C. P. LUKA
- Topic of research/project: Evaluation of antidiabetic effect of Linum usitatissimum
- Rationale for animal use and consideration of alternative: Similar physiological features to humans and plant is still in discovery phase
- Justification for the choice of the species and number of animals: To find anti-diabetic treatment that is safe and most effective
- Research procedure involving animals: Induction of Diabetes and Intervention with plant extract
- Procedures to minimize pain and stress: Use of Anesthesia before sacrifice
- Names of the Technologist and Technical staff that will perform the work with the animals: Mr. Iungfo, Iahaku Lory And Mus Nany Lazonin
- Method of euthanasia: Use of Chloroform
- End of point criteria/expected results and benefits to human and animals: Plant extract resulted antidiabetic with histotoxic effects

OFFICIAL USE

COMMENT AND RECOMMENDATION OF THE COORDINATOR: Recommended and forwarded for your approval
SIGN: COORDINATOR Shummar

COMMENT AND RECOMMENDATION OF THE CHAIRMAN: Approved
SIGN: CHAIRMAN 22/11/2021