DETERMINATION OF ANTIHYPERGYCEMIC EFFECT OF FLAXSEED (*L. usititatissimum*) FRACTIONS ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

Abstract

Diabetes is a metabolic disorder characterized by persistent high blood glucose level. Flaxseed is one of the functional foods used in the management of diabetes mellitus. The study aimed to evaluate the effects of different fractions of Flaxseed on albino diabetic rats, and diabetes was induced using streptozotocin. The fractions were obtained using solvents with increasing polarity, namely n-Hexane, Ethylacetate, Methanol, and Water. The presence of various phytochemicals, including alkaloids, flavonoids, tannins, saponins, balsam, carbohydrates, phenols, and resins, were identified. The phytochemical analysis revealed that the methanolic fraction contained the highest concentration of bioactive components, followed by the aqueous fraction. Significant reductions in blood glucose levels were observed across the groups treated with the Flaxseed fractions. The methanolic fraction exhibited the highest antihyperglycemic property (5.90±0.536), followed by the aqueous fraction (8.73±0.536). The hexane fraction ranked next to the aqueous fraction (20.50±1.617), while the ethylacetate fraction had the least antihyperglycemic effect (23.60±0.731). However, the protein and albumin biomarkers showed significant increase across all treatment groups. The Flaxseed fractions also demonstrated antihyperlipidemic properties, with the methanolic fraction being the most potent. Additionally, the treatment groups exhibited improved kidney function biomarkers, serum enzyme levels, and electrolyte levels. Based on the results of this investigation, Flaxseed proves to be a potent antihyperglycemic and antihyperlipidemic food. Moreover, the methanolic fraction demonstrated the greatest ameliorative effect, followed by the aqueous fraction.

Key words: Flaxseed, Diabetes, Phytochemistry, Antihyperglycaemic and Antihyperlipidemic.

1.0 Introduction

Diabetes mellitus (DM) refers to a group of metabolic disorders characterized by high blood glucose levels (hyperglycemia), and it is due to insufficient production or action of insulin or both [1, 2]. In Nigeria, the prevalence of type 2 diabetes mellitus (T2DM) has been consistently high and continues to rise. It is widely recognized that Nigeria carries the highest burden of diabetes in Africa [3]. However, there have been no comprehensive nationwide surveys or recent reports specifically estimating the extent of diabetes burden in the country. According to data [4] from 2021, DM prevalence is experiencing a worrisome surge worldwide. Specifically, the International Diabetes Federation (IDF) estimated that in the Africa Region alone, there were approximately 24 million adults aged 20 to 79 living with DM in 2021. However, projections indicate a significant escalation in the number of individuals affected by DM in the upcoming

years. By 2030, it is projected that the number of adults with DM in the IDF Africa Region will rise to 33 million. The figure is expected to reach a staggering 55 million by the year 2045 [4]. These escalating numbers carry severe consequences, not only for the affected individuals but also for healthcare systems. The growing burden of DM places increased strain on healthcare resources and necessitates enhanced efforts in prevention, diagnosis, and management to mitigate the impact on both individuals and society as a whole [5].

Long-term elevated blood sugar levels and insulin resistance are common risk factors contributing to the development of complications in DM [6]. Hyperglycemia activates various pathways such as aldose reductase, hexosamine, protein kinase C, and mitogen-activated protein kinases. Additionally, it leads to increased expression of growth factors like tumor necrosis factor-alpha, platelet-derived growth factor, insulin-like growth factor, and vascular endothelial growth factor [7].

DM is typically associated with specific complications, which can be categorized into microvascular and macrovascular complications. Microvascular complications encompass renal damage (nephropathy), nervous system damage (neuropathy), and eye damage (retinopathy). On the other hand, macrovascular complications include peripheral artery disease, cardiovascular disease, and cerebrovascular disease. It is important to note that the prevalence of microvascular complications tends to be higher than that of macrovascular complications in individuals with type 2 diabetes [8,9].

The limited effectiveness and potential adverse effects of current pharmacological therapies have led to an increased interest in alternative treatments for DM, such as the use of plant extracts [10]. Presently, DM has no cure, it can only be managed by healthy lifestyle, diet plan and medication, however, other alternative approaches can be taken to ameliorate the complication of DM and flaxseed can be considered an effective functional food for individuals seeking alternative approaches to control DM. Further research and clinical trials are warranted to explore the full potential of flaxseed fractions and their application in the development of safe and effective diabetes management strategies. In line with this, the present study aimed to investigate the antihyperglycemic effect of seed fractions of Flaxseed *(Linum usitatissimum*in) on streptozotocin-induced diabetic rats. The study seeks to understand which fraction of the flaxseed is more potent in ameliorating streptozotocin-induced diabetes. The solvent that will be

used for this study include: n-Hexane, Ethylacetate, Methanol and Water, these solvents system was chosen based on their polarity.

Plants have been a rich source of medicines for centuries, and phytoconstituents obtained from plants have been used to treat various ailments [11]. One of such plant with potential health benefits is flaxseed, which has been studied for its ability to aid in glycaemic control [12,13,14]. Additionally, the use of flaxseed may be associated with a reduction in the risk of obesity and dyslipidaemia. These risk factors are known to contribute to the development of DM and insulin resistance. Therefore, the utilization of flaxseed could have positive effects on managing DM and related conditions [15].

Flaxseed is abundant in fat, protein, and dietary fiber [16]. The main components of flaxseed include mucilage (6%), insoluble fibers (18%), proteins (25%), and oils (30-40%), with α-linolenic acid comprising the majority of the fatty acids (50-60% of oils). Flaxseed also contains lignans [17]. Studies conducted on animals and human subjects consuming flaxseed meal have demonstrated a reduction in blood cholesterol levels [13,18]

Plate 1: Image of Flaxseed

2.0 MATERIALS AND METHOD

2.1Procurement of Materials and Chemicals

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, Glibenclimide (Aviglen 5mg) and commercial kits to analyze biochemical parameters were purchased from scientific stores and local pharmacies in Jos.

2.2 Preparation of Flaxseed Fractions

For this experiment, the flax plant (*L. usititatissimum*) was cultivated in mid may 2022 and was harvested August 31st of the same year at Zarmaganda layout Jos, Plateau State, Nigeria. The Flaxseed was authenticated and identified at the Department of Plant Science and Biotechnology, University of Jos, with voucher no. JUHN21000351.

After the flaxseed was harvested, it was washed to get rid of any sand particles and dried in a room for 2-3 days. Once fully dried, the seeds were crushed into powdery form using a mixer grinder (VTCL Solitaire 4739026). The resulting powder was stored at ambient conditions, in an airtight container until it was needed for further use [19].

Fractionation of the flaxseed powder was conducted based on polarity, starting with the non-polar solvent, hexane. A total of 350g of flaxseed powder was soaked in hexane for 12 hours while being regularly stirred. After soaking, the mixture underwent filtration using whatman qualitative filter paper, grade 4. The filtrate was then concentrated at 40°C using an oven (Surgifield Medicals England SM9053A) for 6-7days. The resulting concentrated hexane fraction was preserved in an airtight container.

The residue left after the hexane extraction was subjected to evaporation for three days using an evaporating dish. The same process was then repeated with Ethylacetate, Methanol, and Water solvents, respectively. As a result, four different fractions were obtained, each named according to the solvent used for their extraction: Hexane fraction, Ethylacetate fraction, Methanolic fraction, and Water fraction [20].

2.3 Experimental Animals

In this study, male albino rats weighing 180-210g were used as experimental subjects. The rats were obtained from the animal house at the University of Jos in Nigeria and were allowed to acclimatize for two weeks before study began.

The albino rats were maintained under standard laboratory conditions in propylene cages at 25 ± 3^{o}C, relative humidity of 50±10% and under 12 hours light/dark cycle before the experimental procedure began [19]. To meet their nutritional needs, before and during the study, the albino rats were provided with access to standard pellet feed from Grand Cereal and Oil Mills Ltd, located in Jos, Nigeria. In addition to the pellet feed, the rats were also given free access to water.

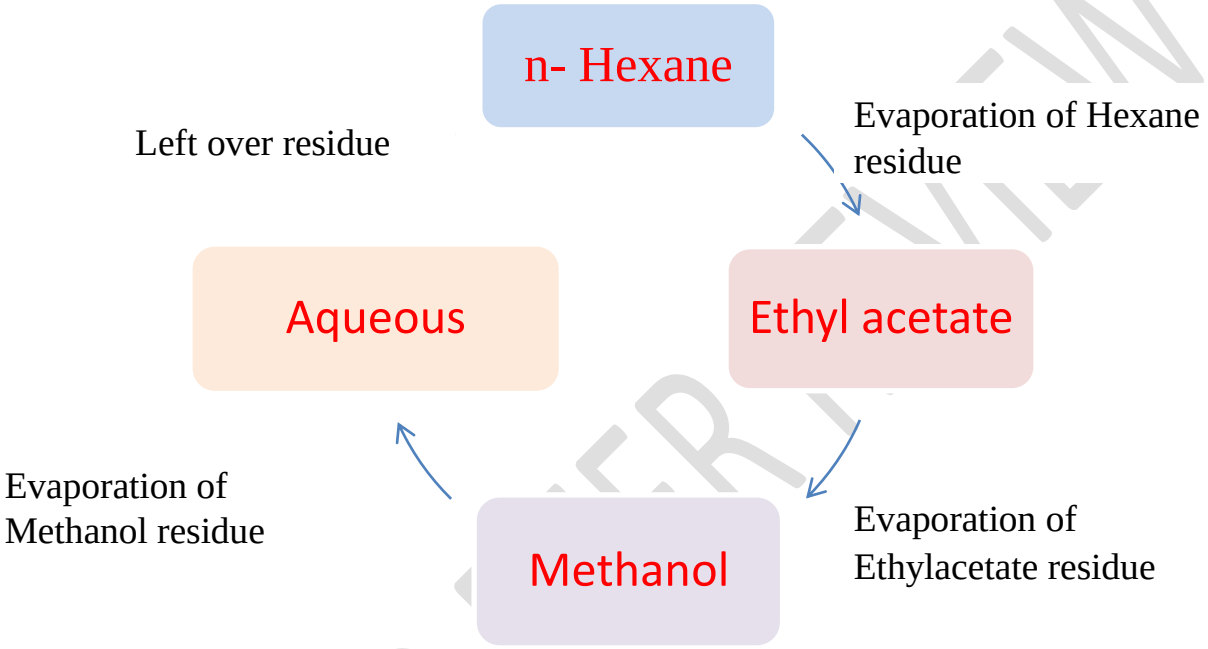

Fig 1: An overview of Fractionation process

2.4 Experimental Design

The rats in this study were divided into different groups based on their treatment conditions. All groups, including the control groups, were fed a normal diet and had unrestricted access to water for duration of 28 days which was the study period. Here is a summary of the different groups and their respective treatments:

Experimental design included 7 groups comprised of 5 rats in each group: Group A was negative control (normal rats with normal diet), Group B was positive control (diabetic rats with normal diet), Group C was orally administered standard drug (Glibenclamide 2.5mg/kg body weight) group D-G were orally administered the different fractions of flaxseed at 200mg/kg body weight.

The dosage regime was determined based on the acute toxicity studies carried out by Nanman et al., [21].

2.5 Determination of Biochemical Parameters

The biochemical parameters assay as include the following:

1. Phytochemical Screening of Fractions [22]
2. Glucose, [23]
3. Protein, Albumin [24]
4. Bilirubin [25]
5. Lipid profile [26,27]
6. Enzyme assay [28,29]
7. Electrolyte determination [30]
8. Renal function test described [31]

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 (32).

2.7 Data Analysis

Result values are expressed as mean± 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 is used to carry out the above analysis.

3.0 Result and Discussion

3.1 Percentage Yield

Below is the representation (Fig 2) of the percentage yield of the Flaxseed Fractions: Ethylacetate fraction had the highest weight 112g (32%) after the extraction, aqueous fraction accounted for 67.3g (19.43%), n- hexane extracted 48.8g (13.90%), and methanol which is the least had 15.1g (4.50%) and 100.6g(28.80%) was left over.

To obtain the percentage yield, the following formular was used=

$$\frac{\text{Mass obtained from fraction}}{\text{Total mass of grinded Nut}} * 100$$

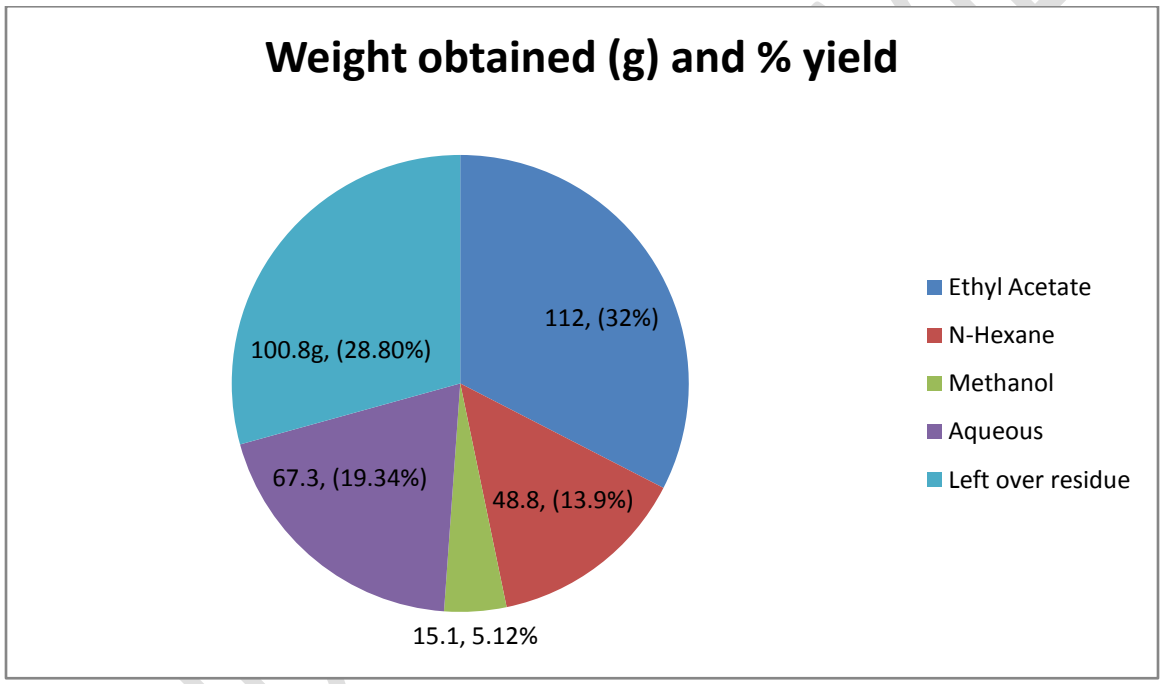

Fig 2: Percentage Yield after Extraction

3.2 Phytochemical Analysis of Flaxseed

Phytotherapy, as a form of complementary and alternative medicine, has garnered increasing attention in the treatment of diabetes over the years. The ethnomedicinal knowledge derived from traditional literature has paved the way for exploring natural remedies to manage various ailments safely, economically, and with potentially lasting effects [33,34]. Several authors have reported the antidiabetic properties of phytochemicals; Flavonoids and terpenoids were said to

possess antidiabetic activity [35,36,37,38]. Flavonoids, alkaloids, tannins, saponins, terpenoids and steroids, polysaccharides and phenolic compounds were also documented to decrease the blood glucose level [39,40]. Flavonoids are well-known for their diverse biological activities, including their antioxidant properties, which contribute to their antihyperlipidemic activity [41]. Cardiac glycosides have also been found to be beneficial in reducing diabetic complications and are used as antihypertensive agents [42]. Additionally, Luka and Istifanus [43] reported the presence of antihyperglycemic phytoconstituents such as saponins, steroidal glycosides, alkaloids, and flavonoids. Flaxseed is a rich plant endowed with various phytochemicals (Table 1) such as flavonoids, saponins, balsams, carbohydrate, tannins, cardiaglycoside, phenol and resins. Methanol fraction had more phytochemical than all other fraction followed by aqueous fraction; however, alkaloid was present in all fractions (table 1). Alkaloids are defined as small compounds with nitrogen in the form of primary, secondary, or tertiary amines. Alkaloids act as an antihyperglycemic agent by inhibiting digestive enzymes such amylase and α-glucosidase leading to lower postprandial blood glucose level [44]. Also, Flavonoids exhibit potent antioxidant properties and have the ability to inhibit the formation of advanced glycation end products, flavonoids act by neutralising free radicals, which damage the human body's cells every day; free radicals are highly unstable and reactive chemicals that antioxidants neutralize [45]. The harm caused by free radicals is regarded to be a contributing factor to many health conditions, including cancer, heart disease, diabetes, aging, and more. Saponins, on the other hand, play a role in regulating glucose and lipid metabolism, effectively controlling conditions such as hyperlipidemia and hyperglycemia [46]. The presence of tannins (Table 1) also promotes wound healing [47].

Table 1 : Phytochemical Screening of the flaxseed fractions

Test	**N- Hexane**	**Ethyl acetate**	**Methanol**	**Aqueous**
Alkaloids	+	+	+	+
Flavonoids	-	+	+	+
Tannins	-	+	-	-
Saponins	-	-	+	+

Cardiac glycosides	+	-	-	-
Balsam	-	-	+	+
Carbohydrates	+	-	+	+
Phenol	-	-	+	-
Resins	-	-	+	-

+ = Present - = Absent

3.3 Effect of Flaxseed Fractions on Serum Biochemistry

3.3.1 Analysis on Glucose, Total Protein and Albumin

Flaxseed has been found to possess antihyperglycemic properties without significant reported side effects [48]. The observed reduction in glycemic response seen in studies evaluating the long-term effects of flaxseed consumption is often attributed to its soluble fiber content. Fiber plays a crucial role in the process of digestion, and its main mechanisms include slowing down gastric emptying, increasing the volume and viscosity of the food bolus, and delaying the interaction between digestive enzymes and nutrients. These factors contribute to the breakdown of complex nutrients into absorbable components and slow down the absorption of glucose at the brush border, resulting in a lower glycemic peak in the blood glucose response curve [49,50]. In agreement with many other studies, there was a significant reduction ($P \leq 0.05$) of blood glucose level of diabetic rats fed with the methanolic and aqueous fraction of flaxseed, however, the ethylacetate fraction showed no significant reduction when compared with the diabetic group. It is noteworthy that the methanolic fraction of the flaxseed had more antihyperglycemic effect on streptozotocin induced diabetes rats

In normal physiological conditions, elevated blood glucose levels actually inhibit gluconeogenesis. Gluconeogenesis primarily occurs during periods of fasting, prolonged exercise, or when carbohydrate intake is limited. In the context of diabetes, the situation may be different [51,52], there can be dysregulation of glucose metabolism, leading to chronically elevated blood glucose levels, this can disrupt the normal regulatory mechanisms of

gluconeogenesis and result in increased protein breakdown and loss of nitrogen, leading to a negative nitrogen balance. Additionally, diabetics may experience decreased total serum protein levels due to factors such as oxidative stress, impaired protein synthesis, increased catabolic processes, and impaired protein absorption [53]. However, the administration of the fractions of flaxseed significantly raised protein and albumin levels compared to the diabetic and control group, this demonstrates that flaxseed positively impacts metabolism of protein in DM. Also, from table 2 shows that there was a significant increase in total protein (TP) and albumin across the treatment group, this is significant when compared with the diabetic and the normal control. Flaxseed contains protein content ranging from 20% to 30%, predominantly composed of approximately 80% globulins and 20% glutelin [54].The amino acid profile of flaxseed is similar to that of soybean, but it is gluten-free [55]. Flaxseed also contains various bioactive compounds and peptides, which contribute to reducing the risk of cardiovascular diseases, including CVD. Whole flaxseed and flaxseed meal are important sources of essential amino acids such as arginine, leucine, glutamine, valine, as well as aromatic amino acids like phenylalanine and tyrosine [55].

Table 2: Effect of Flaxseed fractions on Serum glucose, serum total protein and serum Albumin in STZ- induced Diabetic rats.

Group	Treatment	Glucose (mmol/L)	Total Protein (g/L)	Albumin (g/L)
A	Normal Control	5.76±0.088	70.00±1.732^{b}	43.00±0.173
B	Diabetic Control	23.93±2.860^{b}	55.00±1.732^{ac}	29.33±1.881^{a}
C	D + Glibenclamide	7.86±0.578^{bc}	66.66±0.764	37.33±0.881^{ad}
D	D + Ethyl Acetate	23.60±0.731^{be}	64.00±1.155^{ad}	35.33±1.155^{ad}
E	D + Methanol	5.90±0.536^{c}	63.66±7.23^{ad}	39.00±2.028^{ad}
F	D + N-Hexane	20.50±1.617^{bc}	63.66±0.881^{bd}	32.00±1.155^{ad}
G	D + Aqueous	8.73±0.536^{bc}	65.66±2.963^{ad}	38.00±1.155^{ad}

Values are expressed as mean ± SEM, n=3

[a]Values are significantly lower when compared with normal control ($p < 0.05$)

[b]Values are significantly higher when compared with normal control ($p < 0.05$)

[c]Values significantly lower when compared with diabetic control ($p < 0.05$)

[d]Values significantly higher when compared with diabetic control ($p < 0.05$)

[e]Values is almost equal to diabetic control ($p > 0.05$)

3.3.2 Analysis on Lipid Profile

Dysfunction in lipid and carbohydrate metabolism is one of many symptoms of diabetes (56), free radical overproduction is the main cause of the elevated lipid peroxidation seen in DM. Glycosylated proteins, auto-oxidation, decreased levels of the enzyme superoxide dismutase, decrease ascorbic acid, shortage of reduced glutathione are other variables that cause oxidative stress [57]. In this study, Table 3 shows the triglycerides and total cholesterol of the treatment group was experimentally significantly low when compared to diabetic control rats. However, in the methanolic and aqueous fractions, High Density Lipoprotein cholesterol (HDL) (table 3) is significantly ($P \leq 0.05$) higher when compared with both normal and diabetic control, on the other hand, LDL is also significantly low in the same fractions. It was also observed that methanolic fraction had more antihyperlipidemic property when compared to all the other fractions. Further, Flaxseed fractions generally showed a significant decrease in Total Cholesterol (TC) and Triglyceride (TG) levels in the treatment group. Elevated levels of cholesterol in the bloodstream, particularly increased High Density Lipoprotein cholesterol (LDL) levels and decreased HDL-cholesterol levels, have a significant correlation with cardiovascular diseases, as they promote the formation of plaque in arteries, resulting in atherosclerosis [58,59]. Flaxseeds are abundant in alpha-linolenic acid, an essential fatty acid that serves as a precursor to omega-3 fatty acids. Omega-3 fatty acids have been linked to improved cardiovascular health, and there is a connection between serum lipid profile and cardiovascular disease. The impact of flaxseed oil on cardiac conditions has shown consistent findings in both human and animal studies. Biochemical assessments conducted on mice, rats, and rabbits have demonstrated positive outcomes, indicating the potential antihypercholesterolemic effects of flaxseed [60].

Table 3: **Effect of *L. usititatissimum* Fractions on lipid profile in STZ induced Diabetic rats.**

Group	Treatment	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
A	Normal Control	2.00±0.088	0.39±0.088	1.71±0.032	0.70±0.105
B	Diabetic Control	5.12±0.260[b]	1.76±0.296[b]	0.95±0.198[b]	1.24±0.109[b]
C	D + Glibenclamide	2.20±0.057[c]	0.43±0.088[e]	1.00±0.005[bc]	0.92±0.028[ac]
D	D + Ethyl Acetate	2.16±0.218[c]	0.72±0.089[bc]	1.09±0.037[bc]	1.98±0.011[ac]
E	D + Methanol	2.06±0.088[c]	0.48±0.029[bc]	2.58±0.020[bd]	0.98±0.029[bc]
F	D + N-Hexane	2.23±0.115[c]	0.65±0.003[ac]	0.82±0.025[c]	1.88±0.069[bd]
G	D + Aqueous	2.40±0.305[bc]	0.86±0.202[bc]	2.13±0.025[bd]	1.17±0.199[bc]

TC – Total Cholesterol, TG – Triglycerides, HDL – High Density Lipoprotein, LDL- Low DensityLipoprotein

Values are expressed as mean ± SEM, n=3

[a]Values are significantly lower when compared with normal control ($p < 0.05$)

[b]Values are significantly higher when compared with normal control ($p < 0.05$)

[c]Values are significantly lower when compared with diabetic control ($p < 0.05$)

[d]Values are significantly higher when compared with diabetic control ($p < 0.05$)

[e]Values are almost equal to normal control ($p > 0.05$)

3.3.3 Analysis on Serum Enzymes

Alkaline phosphatase (ALP) is an enzyme that plays a crucial role in various physiological processes, including liver function and bone development. Abnormalities in ALP levels can indicate an underlying medical condition, typically related to the liver, bones, or gallbladder [61]. To diagnose a condition and monitor a patient's progress during treatment, the activity of enzymes such as alanine and aspartate aminotransferase (ALT and AST respectively) can be measured in the blood serum. This measurement can also assess the extent of organ or tissue damage and toxicity caused by chemical compounds [62]. In the case of diabetes, the concentration of these enzymes in the blood often tends to be elevated. It can be observed that

there was significant increase in serum enzyme levels in the diabetic control (Table 4) however, the serum levels of ALT, AST, and ALP were considerably (P≤0.05) decreased by flaxseed fractions as obvious in the treatment group. Administration of flaxseed resulted in depletion of serum marker enzymes and exhibited recoupment thus showing significant hepatoprotective effect. This result is also in agreement with other study [63]

Table 4: Effect of *L. usititatissimum* fractions on some serum Enzymes in STZ induced Diabetic rats.

Group	Treatment	ALP (U/L)	ALT (U/L)	AST (U/L)
A	Normal Control	190.33±18.206	126.00±13.317	114.33±6.741
B	Diabetic Control	328.67±46.976[b]	275.00±55.0139[b]	258.67±41.450[b]
C	D + Glibenclamide	239.00±33.561[bc]	208.67±25.208[bc]	173.33±15.015[bc]
D	D + Ethyl Acetate	276.00±7.371[bc]	229.33±1.453[bc]	219.67±4.485[bc]
E	D + Methanol	267.67±35.695[bc]	211.33±35.507[bc]	133.00±31.533[bc]
F	D + N-Hexane	293.67±86.335[bd]	217.33±67.966[bc]	199.37±92.543[bc]
G	D + Aqueous	310.33±60.532[bc]	258.33±60.938[bc]	194.00±59.652[bc]

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

Values are expressed as mean ± SEM, n=3

[a]Values are significantly lower when compared with normal control ($p < 0.05$)

[b]Values are significantly higher when compared with normal control ($p < 0.05$)

[c]Values are significantly lower when compared with diabetic control ($p < 0.05$)

[d]Values are significantly higher when compared with diabetic control ($p < 0.05$)

3.3.4 Analysis on Renal function

In diabetes, renal function indicators such serum creatinine, urea, and uric acid are higher s [64]. A kidney function test (Table 5) revealed that the untreated diabetic rats' urea, creatinine, and uric acid levels were significantly (P≤0.05) high, affecting kidney functions. When flaxseed

fractions was received, the levels of urea, creatine, and uric acid significantly decreased (P≤ 0.05), easing the impaired effect. Again, the methanolic and aqueous fraction showed more positive impact in renal function test when compared to other fraction. The accelerated breakdown of liver and plasma proteins that occurs with gluconeogenesis associated with chronic hyperglycemia may be to blame for the rise in urea levels seen in diabetes [65]. According to reports, poorly controlled diabetes mellitus may be to responsibility for the substantial muscle breakdown that results in increased levels of creatinine in diabetics [66]. The indices of renal function were significantly (P ≤0.05) decreased by the flaxseed fractions. This is also in line with other study, flaxseed oil has demonstrated beneficial effects in the kidney by reducing renal injury in experimental polycystic kidney disease. It has also been observed to decrease C-reactive protein levels and inflammation in patients undergoing chronic hemodialysis. Additionally, flaxseed has shown noteworthy hypoglycemic, hypolipidemic, and nephroprotective effects in rats with diabetes induced by streptozotocin[67,68]

Table 5: **Effect of *L. usititatissimum* fractions on serum Urea, serum Creatinine and serum Uric acid in STZ induced Diabetic rats.**

Group	Treatment	Urea (µmol/L)	Uric Acid (mmol/L)	Creatinine (µmol/L)
A	Normal Control	3.00±0.115	274.00±22.502	32.33±0.666
B	Diabetic Control	5.16±0.233[b]	510.00±35.000[b]	43.33±5.207[a]
C	D + Glibenclamide	3.46±0.437[e]	274.67±1.453[e]	39.66±6.386[bd]
D	D + Ethyl Acetate	3.03±0.088[e]	355.00±1.155[bc]	37.00±1.155[bc]
E	D + Methanol	2.86±0.545[ac]	320.33±48.444[bc]	36.66±8.090[bc]
F	D + N-Hexane	3.33±0.290[e]	327.00±27.319[bc]	41.66±4.910[bd]
G	D + Aqueous	2.73±0.202[ac]	262.67±13.421[ac]	35.33±0.666[bc]

Values are expressed as mean ± SEM, n=3

[a]Values are significantly lower when compared with normal control ($p < 0.05$)

[b]Values are significantly higher when compared with normal control ($p < 0.05$)

[c]Values are significantly lower when compared with diabetic control ($p < 0.05$)

[d]Values are significantly higher when compared with diabetic control ($p < 0.05$)

[e]Values are almost equal to normal control ($p > 0.05$)

3.3.5 Analysis on Electrolyte

Deficiency in insulin as seen in hyperglycemia, and hyperketonemia may all contribute to subjects with diabetes having an electrolyte and water imbalance (69). Electrolytes, which are necessary for numerous body processes including controlling fluid levels, pH balance, nerve conduction, blood clotting, and muscle contraction, might become unbalanced as a result of diabetes. Electrolyte imbalances can be caused by kidney disease, dehydration, a high temperature, vomiting, and other conditions. They can also worsen the symptoms of diabetes and other endocrine problems because of the increased excretion of metabolites through the kidneys in diabetes (70). The current study demonstrates that oral administration of Flaxseed fractions significantly ($P \leq 0.05$) reduced the level of serum electrolytes (Na+, K+, and HCO3-) table 6. On the other hand, the diabetic untreated rats had significant increment when compared with the normal control. The result obtained from this work demonstrates that flaxseed fractions exhibits potent anti-diabetic activity; however, there was significantly elevated chloride across the treatment group.

Table 6 Effect of *L. usititatissimum* fractions on some serum Electrolytes Concentrations in STZ induced Diabetic rats.

Group	Treatment	Na^+(mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	HCO^-(mmol/L)
A	Normal Control	143.67±0.333	4.83±0.328	104.00±1.521	28.00±0.577
B	Diabetic Control	141.33±1.764[a]	6.43±0.088[b]	101.67±0.881[a]	25.00±0.577[a]
C	D + Glib.	143.00±0.577[e]	5.36±0.272[bd]	101.33±0.667[ae]	22.00±1.155[ac]
D	D + Ethyl Acetate	139.00±1.732[ac]	6.16±0.088[bd]	97.66±0.881[ac]	21.33±0.881[ac]
E	D + Methanol	138.33±0.333[ac]	5.03±0.088[bc]	101.33±0.881[e]	19.33±1.453[ac]

F	D + N-Hexane	138.00±1.155[ac]	6.26±0.272[b]	102.00±1.155[ad]	18.00±0.577[ac]
G	D + Aqueous	140.67±1.764[a]	5.53±0.066[bc]	102.00±0.666[ad]	19.00±0.577[ac]

Values are expressed as mean ± SEM, n=3

[a]Values are significantly lower when compared with normal control ($p < 0.05$)

[b]Values are significantly higher when compared with normal control ($p < 0.05$)

[c]Values are significantly lower when compared with diabetic control ($p < 0.05$)

[d]Values are significantly higher when compared with diabetic control ($p < 0.05$)

[e]Value is almost equal to normal control ($p < 0.05$)

3.4 Conclusion

The study shows that the flaxseed fractions have antihyperglycemic and antihyperlipidemic properties on streptozotocin induced diabetic rats. Positive impact was observed on total protein, albumin, serum electrolyte, and kidney function assay and serum enzymes. Methanolic fraction showed more ameliorative effect followed by aqueous fraction, conversely, ethylacetate fraction showed the least ameliorative potential. The presence of several phytochemicals, essential fatty acids and fibre in flaxseed could be the cause of the observed effects.

3.5 Recommendation

Flaxseed has the potential to be processed into powders and extracts, which can serve as more concentrated sources of nutrients. These concentrated forms can be utilized in the production of medicines and novel food products. By extracting the phytochemicals and proteins present in flaxseed, it becomes possible to develop safe drugs for various applications.

Ethical Approval:

Ethical clearance with reference number F17-00379 was given for this study. The ethical clearance was obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

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