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


## Abstract

Diabetes is a metabolic disorder characterized by persistent high glucose level. Flaxseed is one of the functional foods used the in management of diabetes mellitus. The aim of this study is to assess the antihyperglycemic and antihyperlipidemic effectsof Flaxseed fractions on streptozotocin induced diabetic rats, this is to ascertain which fraction is more potent in the management of diabetes. The solvent used for fractionation was selected based on increasing polarity and the solvents are: n-Hexane, Ethylacetate, Methanol and Aqueous. Thirty (35) albino rats weighing 180-210g 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 seven groups, the first three (3) groups served as control and the other four (4) were the treatment groups. The phytochemistry analysis showed that the methanolic fraction possessed more bioactive component followed by the aqueous fraction. The phytochemical found present include: alkaloid, flavonoids, tannins, saponins, balsam, carbohydrate, phenol and resins. Across the groups that was treated with the fractions, there was significant reduction of blood glucose level, however, methanolic fraction had more antihyperglycemic property ($5.90\pm0.536^{c}$) followed by the aqueous fraction ($8.73\pm0.536^{bc}$), hexane was next to aqueous fraction ($20.50\pm1.617^{bc}$)and ethylacetate fraction had the least antihyperglycemic property ($23.60\pm0.731^{be}$)respectively.However, the protein and albumin biomarkers were significantly ($P \leq 0.005$) increased across groups. Flaxseed fractions also showed antihyperlipidemic properties, and methanolic fraction showed more antihyperlipidemic property. Across the treatment groups there was improved, kidney function biomarkers, serum enzymes and electrolytes levels. In accordance with the results of this investigation, Flaxseed is a strong antihyperglycemic and antihyperlipidemicfood; also, methanolic fraction had more ameliorative effect followed by 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, which is the most populous country in Africa, 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 from 2021, the prevalence of diabetes mellitus (DM) is experiencing a worrisome surge worldwide. Specifically, the International Diabetes

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**Comment [S6]:** "Phytochemicals"

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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. Looking even further ahead, 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 diabetes mellitus (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 diabetes, such as the use of plant extracts [10]. In line with this, the present study aimed to investigate the antihyperglycemic effect of seed fractions of Flaxseed *(Linumusitatissimum*in) onstreptozotocin-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 present study include: n-Hexane, Ethylacetate, Methanol and Aqueous,these solvents system was chosen based on their polarity.

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Plants have been a rich source of medicines for centuries, and phytoconstituents obtained from plants have been used to treat various ailments [11]. These phytoconstituents are diverse and belong to various classes, 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 diabetes and insulin resistance. Therefore, the utilization of flaxseed could have positive effects on managing diabetes 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**

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## 2.1 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, which is known for its research and breeding facilities for laboratory animals. 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.

To meet their nutritional needs, 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.

## 2.2 Preparation of Flaxseed Fractions

For this experiment, the flax plant was cultivated and collected in the Zarmaganda layout, Plateau State, Nigeria.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 then stored in an airtight container until it was needed for further use at ambient conditions [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 a sieve, and the filtrate was then concentrated at 40°C using an oven (Surgifield Medicals England SM9053A). 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 Aqueous fraction [20].

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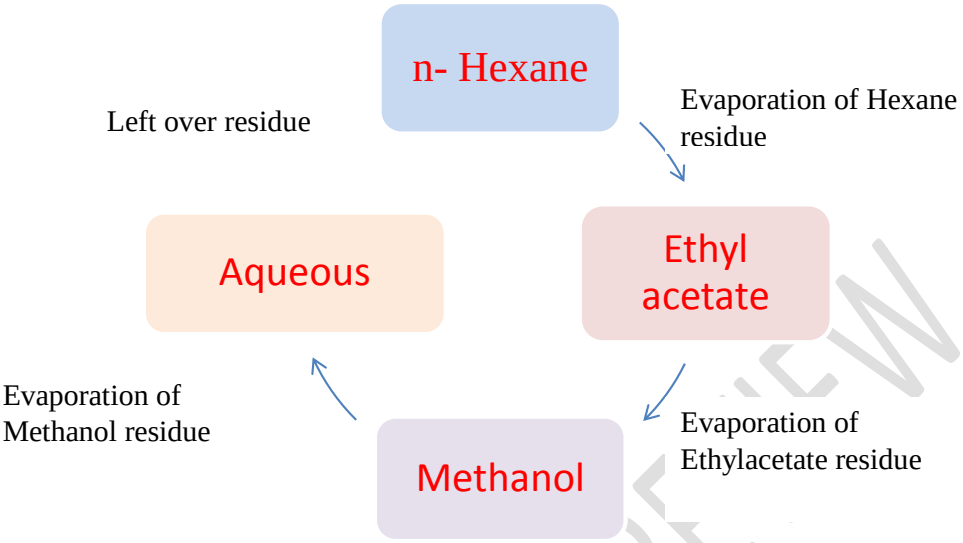


Fig 1: An overview of Fractionation process

**2.3Experimental 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. 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.

**2.4 Determination Of Biochemical Parameters**

The biochemical parameters assay as include the following:

1. Phytochemical Screening of Fractions
2. Glucose, Protein, Albumin and Bilirubin

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Dosing regimen followed?

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3. Lipid profiledescribed
4. Enzyme assaydescribed
5. Electrolyte determinationdescribed
6. Kidney function testdescribed[21]

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**2.5 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 heparinisecontainers for the analysis (22).

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**2.6 Data Analysis**

Result values are expressed as mean± standard deviation. Analysis of variance (ANOVA) was used for comparism. Differences were considered significant when values of $p \leq 0.05$. A Graph pad prism is used to carry out the above analysis.

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**3.0 Result and Discussion**

**3.1Percentage 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 formula was used=

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

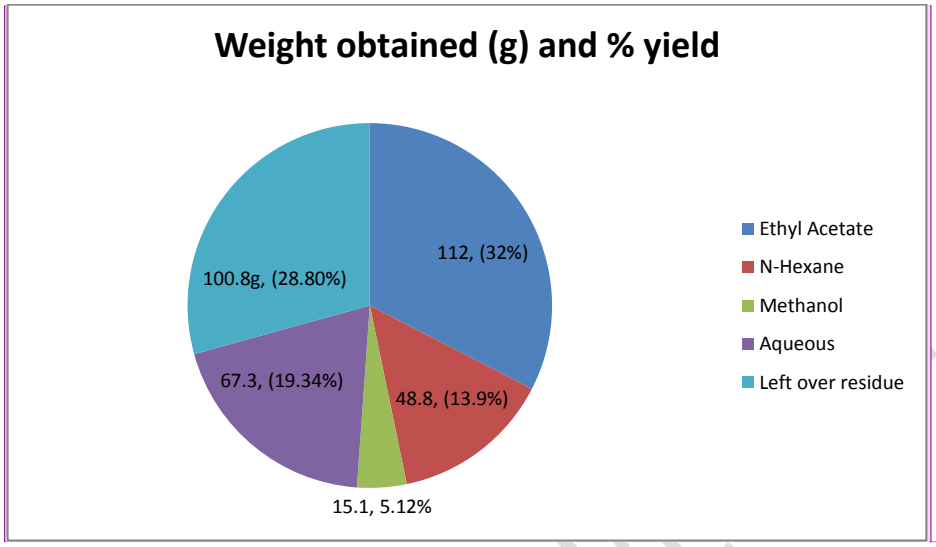


**Fig 2: Percentage Yield after Extraction**

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### 3.2Phytochemical Analysis of Flaxseed

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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[23,24].Several authors have reported the antidiabetic properties of phytochemicals; Flavonoids and terpenoids were said to possess antidiabetic activity [25,26,27,28]. Flavonoids, alkaloids, tannins, saponins, terpenoids and steroids, polysaccharides and phenolic compounds were also documented to decrease the blood glucose level [29,30]. Flavonoids are well-known for their diverse biological activities, including their antioxidant properties, which contribute to their antihyperlipidemic activity [31]. Cardiac glycosides have also been found to be beneficial in reducing diabetic complications and are used as antihypertensive agents [32]. Additionally, Luka and Istifanus[33] reported the presence of antihyperglycemicphytoconstituents 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 [34]. 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 [35]. 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 [36].The presence of tannins (Table 1) also promotes wound healing [37].

**Comment [S56]:** Give details of the tests done to identify the fractions

**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

### 3.3Effect of Flaxseed Fractions on Serum Biochemistry

#### 3.3.1 Analysis on Glucose, Total Protein and Albumin

Certain foods, such as flaxseed, have been found to possess antihyperglycemic properties without significant reported side effects [38]. 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 [39,40].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 [41,42]. In diabetes, 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 [46]. 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 a protein content ranging from 20% to 30%, predominantly composed of approximately 80% globulins and 20% glutelin(Conlin, Linin) (Hall et al., 2006). The amino

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acid profile of flaxseed is similar to that of soybean, but it is gluten-free [44]. 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 [45].

**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) | TotalProtein (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 (46). Free radical overproduction is the main cause of the elevated lipid peroxidation seen in diabetes

mellitus. Glycosylated proteins, auto-oxidation, decreased levels of the enzyme superoxide dismutase, ascorbic acid, shortage of reduced glutathione are other variables that cause oxidative stress [47]. Abnormally high amounts of cholesterol in the bloodstream define hypercholesterolemia. 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,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. It was also observed that flaxseed fractions generally showed a significant decrease in TC and TG levels in the treatment group. Elevated levels of cholesterol in the bloodstream, particularly increased LDL-cholesterol 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 [48,49]. 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 [50].

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**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 | $5.12\pm0.260^{b}$ | $1.76\pm0.296^{b}$ | $0.95\pm0.198^{b}$ | $1.24\pm0.109^{b}$ |
| C | D + Glibenclamide | $2.20\pm0.057^{c}$ | $0.43\pm0.088^{e}$ | $1.00\pm0.005^{bc}$ | $0.92\pm0.028^{ac}$ |
| D | D + Ethyl Acetate | $2.16\pm0.218^{c}$ | $0.72\pm0.089^{bc}$ | $1.09\pm0.037^{bc}$ | $1.98\pm0.011^{ac}$ |
| E | D + Methanol | $2.06\pm0.088^{c}$ | $0.48\pm0.029^{bc}$ | $2.58\pm0.020^{bd}$ | $0.98\pm0.029^{bc}$ |

| | | | | | |
|---|---|---|---|---|---|
| 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 [51]. To diagnose a condition and monitor a patient's progress during treatment, the activity of enzymes such as alanine and aspartate aminotransferase 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 [52]. 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 ($P \leq 0.05$) decreased by flaxseed fractionsas 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 [53]

**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 [54]. A kidney function test (Table 5) revealed that the untreated diabetic rats' urea, creatinine, and uric acid levels were significantly ($P \leq 0.05$) high, affecting kidney functions. When flaxseed fractions was received, the levels of urea, creatine, and uric acid significantly decreased ($P \leq 0.05$), 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 [55]. 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 [56]. The indices of renal function were significantly ($P \leq 0.05$) 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 [20]. Additionally, flaxseed has shown noteworthy hypoglycemic, hypolipidemic, and nephroprotective effects in rats with diabetes induced by streptozotocin[57,58]

**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 (59). 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 (60). The current study demonstrates that oral administration of *flaxseed* fractions significantly (P≤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 andantihyperlipidemic 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.

**Comment [S61]:** Include further prospects of the work

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