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Antidiabetic Property of a Fraction of the Methanol Extract of the Seeds of *Abrus precatorius* in Alloxan-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ELI and OFCN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. 'Authors ELI, OCE and ORN managed the analyses of the study. Author ELI, OCE, VOGN and EAO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion and/or increased cellular resistance to insulin. *Abrus precatorius* has been used for the treatment of various ailments including diabetes mellitus. This study is aimed at evaluating the possible anti-diabetic effect of the methanol fraction 2 (F_2) of the seeds of *Abrus precatorius* in alloxan-induced diabetic Wistar albino rats.
Methods: The methanol extract of the seeds of *A. precatorius* Linn *Fabaceae* was fractionated by Sephadex G15. Diabetes was induced by a single intraperitoneal administration of 150 mg/kg

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bodyweight of alloxan. Acute toxicity (LD_{50}) study was done using Lorke's method. The antidiabetic activities of Fraction 2 (F_2) and the biochemical parameters were investigated using standard diagnostic methods.

Results: The value of the LD_{50} is 529.2 mg/kg bw. Oral doses (5, 10 and 20 mg/kg bw.) of the extract caused a dose-dependent significant (p<0.05) reductions in blood glucose concentrations compared with diabetic untreated controls. There were significant decreases (p<0.05) in the total cholesterol and triacylglycerol levels of rats in the treatment groups compared with the diabetic untreated group. The groups that were treated with glibenclamide, 5 mg/kg of the fraction, 20mg/kg of the fraction and the group that was pre-treated with 10mg/kg of the fraction before the induction of diabetes showed a significant decrease (p<0.05) in the concentration of low-density lipoprotein and significant increase (p<0.05) in high-density lipoprotein concentrations compared with the diabetic untreated group. The protein level increased non-significantly (p>0.05) in all the test groups compared with the diabetic untreated control group except the pre-treated group which increased significantly (p<0.05).

Conclusion: The results obtained from this study revealed that the methanol fraction of *A. precatorius* has antidiabetic property which can be as a result of the important phytochemicals found in the F_2 fraction. Fraction 2 (F_2) however showed improvement in lipid profile.

Keywords: A. precatorius; diabetes mellitus; antidiabetic effects; Fraction 2, Low density lipoprotein concentration (LDL-C).

1. INTRODUCTION

One of the mankind oldest diseases in existence is Diabetes mellitus (DM). It is observed as the body's inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycaemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis and coma leading to death. Diabetes showing abnormal increased blood sugar and with a disordered metabolism pose serious life threatening disease [1], though it can be managed very well through proper treatment and controlling.

Diabetes Mellitus is a chronic syndrome as a result of deficiency of insulin characterized by hyperglycemia [2,3]. There are several ways in which lack of insulin may arise such as destruction of β–cells of the pancreas, an organ that is responsible for the production of insulin [3]. The metabolism of carbohydrates, proteins, fats, electrolytes and water is affected as a result of deficiency of insulin leading to major organ function disorders throughout the body [3]. Although the exact cause of diabetes is uncertain, genetic and predisposing factors contribute to the onset of the disease [4].

Self-management training and education on diabetes plays an important role in the management of diabetes [5]. The management of diabetes concentrates on keeping blood glucose levels close to normal as much as possible without causing hypoglycaemia.

Consequent upon the problems associated with general accepted approach in the management of diabetes, effort is now geared towards the traditional approach of treating/managing diabetes. It is assumed that this approach is safer and more natural. The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. This practice has been recommended and encouraged by World Health Organization especially in countries where access to the conventional treatment of diabetes is not adequate [6]. However, it is vital for diabetic patients to be aware of nature, treatment, risk factors and complication of disease due to providing suitable modality to reduce the effects following complications.

There are two main division of Diabetes Mellitus, type I and type II, each with differences in pathogenesis, clinical appearance, management and treatment [7]. Insulin Dependent Diabetes Mellitus (IDDM) or Type 1 DM, is due to lack of insulin and has a peak incidence at 10-20 years [8]. The distinction between type I and type II has been based on age at onset, degree of loss of β-cell function, degree of insulin resistance, presence of diabetes-associated autoantibodies, and requirement for insulin treatment for survival [3]. There is an autoimmune destruction of beta pancreatic cells leading to insulin production inability, requiring insulin injections in insulin dependent diabetes mellitus while type II is characterized by affections to insulin action or secretion, with possible predominance of an

event, usually being both present [8]. The use of insulin may be used in the treatment with diet control, physical exercise and/or concomitant use of oral hypoglycemic agents can be used as a metabolic control of type II diabetes [8].

The high cost of conventional drugs used for the treatment of diabetes and its concomitant adverse effect have geared interest into the use of medicinal plants as an alternative for the treatment of diabetes. Medicinal plants are useful in the treatment of various ailments such as anemia [9], Diabetes [10] and many other diseases due to its phytochemical and nutritional composition [11]. Some plant extracts have been scientifically screened and proved to have antidiabetic activity such as *Ocimumcanum* leaves [12], *Azadirachta indica* leaves [13], *Justicia carnea* leaves [14], *Annona muricata* [15,16]. It is also very important to know that some of these herbal extracts have the potential to prevent or delay the onset and severity of diabetes mellitus [17].

Abrus precatorius (Rosary pea) is a wonderful herb. It is native to india, at altitudes up to 1200 m on the outer Himalayas. A small climbing tropical vine with seeds known as crab's eye was found abundant in Patalkot forest. In a common tribal life, this herb has much importance. It is a woody twinning plant with characteristic toxic red seeds with black mark at the base [18]. It is also an endemic medicinal plant having immense pharmacological importance. Its seeds, roots and leaves are widely used for medicinal purposes in Africa and Asia [19,20]. The seeds of *Abrus precatorius* have been used in the treatment of a variety of diseases such as chronic nephritis [21], infestations [22]. Precatory beans are certainly one of the most beautiful seeds on earth. Seeds of the plants are having reputation as one of the world's most deadly seeds. Seeds have remarkably uniform weight of 1/10th of a gram. Seeds of *Abrus precatorius*were used by goldsmiths as standard weights for weighing gold and silver in previous time [23].

The aim of this study is to evaluate the anti-diabetic effect of the methanol fraction 2 (F_2) of the seeds of *A.precatorius* in alloxan-induced diabetic Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material, Extraction and Fractionation Procedure

The seeds of *A. precatorius* Linn *Fabaceae* were collected from Igala Area of Kogi State and authenticated by a taxonomist of Bioresources Development and Conservation Programme (BDCP), Nsukka, Nigeria. The seed of *A. precatorius* Linn were washed with clean water to remove dirt and sand. They were dried under shade and then pulverized into fine powder.

Six hundred grammes (600 g) of the crushed seeds of *A. precatorius* linn were macerated in a mixture of 400 ml of methanol and 800 ml of chloroform for 24 hrs with occasional shaking. The macerate was filtered through Whatman no 4 filter paper and the filtrate shaken with 20% volume of distilled water to obtain two layers. The upper aqueous methanol layer was dried and fractionated by Sephadex column G15 beads, swollen, packed and eluted with H_20.

Fractionation was by gel filtration, using sephadex G15 which was allowed to swell for 3hrs and packed in a column of height 27 cm and diameter 2.5 cm. The extract was diluted withdistilled water and introduced into the column and afterward, fractions (elutions) were then collected in test tubes of about 3 ml each.

2.2 Thin Layer Chromatography

The fractions were spotted on a TLC plate (precoated with silica gel) and was left to dry for about one hour. Afterward, it was inserted into the chromatographic tank (made up of butanol, acetic acid and water in ratio of 65:13:22 respectively which was allowed to equilibrate for one hour).

2.3 Visible Spectroscopy

After the development of the plate, it was spread with Dragendoff's reagent. The fractions that turned purple were pulled into a beaker as fraction (F_1) while the other fractions that did not change colour were pulled together as fraction (F_2). The fraction (F_2) was then concentrated and afterward, a given weight was dissolved in normal saline (stock solution) which was administered to the animals based on their bodyweight.

2.4 Determination of Extract and Fraction Yield

The percentage yield of *A.precatorius* was calculated by weighing the seeds before extraction and after concentration of the extract while the percentage yield of fraction (F_2) was calculated by weighing the fraction after concentration and weight of the crude extract

after concentration. It was calculated using the formula below:

$$Percentage yield of extract = \frac{weight of crude extract}{weight of seeds} \times 100$$

$$Percentage yield of fraction = \frac{weight of fraction}{weight of crude extract} \times 100$$

2.5 Determination of LD_{50} of the Seeds of Methanol Extracts of *A. precatorius*

The method of Lorke [24] with some modifications to define the range of lethal dose and safe dose for the extract was used for the acute toxicity test of the seeds of *A. precatorius* Linn. Fifteen (15) albino mice were utilized in this study. The animals were divided into five (5) groups of three mice each and were administered 10, 100, 200, 400 and 700 mg/kg bodyweight of the extracts respectively. The administration of the extracts was done orally.

2.6 Experimental Animal, Study Design and Treatment

Twenty-eight (28) Wistar albino rats were housed in separate cages, acclimatized to laboratory conditions for seven days with free access to feed and water. After acclimatization, they were distributed evenly into seven (7) groups of four rats each. The baseline glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to intraperitoneal injection of 150 mg/kg bw. of alloxan dissolved in ice cold normal saline. After 3days, rats with blood glucose levels greater than 200 mg/dl were considered diabetic and used for the investigation. The treatment lasted for eleven days (11) days during which the blood glucose concentrations of the rats were taken on day 0, 3, 7, and day 11. The route of administration of the fraction (F_2) was via oral route with the aid of an oral intubation tube. The group and doses administered are summarized below.

Group 1: (Control) – Rat received 0.2 ml of normal saline orally
Group 2: Positive control (Diabetic untreated rats) received normal saline.
Group 3: Diabetic rats + 2.5 mg/kg bodyweight of glibenclamide.
Group 4: Diabetic rats + 5 mg/kg bodyweight of the methanol fraction of*A. Precatorius* seeds.
Group 5: Diabetic rats + 10 mg/kg bodyweight of the methanol fraction of*A.precatorius* seeds.
Group 6: Diabetic rats + 20 mg/kg bodyweight of the methanol fraction of*A. Precatorius* seeds.
Group 7: Normal rats + 10 mg/kg bodyweight of the methanol fraction + 150 mg/kg alloxan.

2.7 Lipid profile Determination

2.7.1 Cholesterol determination

The method of Abell et al. [25] was followed in the determination of total cholesterol concentration. Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxidase and 4-aminoantipyrine in the presence of phenol and peroxide.

2.7.2 Triacylglycerol concentration

Triacylglycerol concentration was determined using the method of Tietz [26]. The triacylglycerols are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

2.7.3 High-Density lipoprotein (HDL)

High density lipoprotein (HDL) concentration was determined according to the method of Albers et al. [27]. LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, high density lipoproteins (HDL) present in the supernatant is determined.

2.7.4 Low-Density Lipoprotein (LDL)

Low density lipoprotein (LDL) concentration was determined according to the method of Assmann et al. [28]. LDL-C can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycol monomethyl ether.

2.8 Total Serum Proteins

The principle lies on the fact that at alkaline medium, protein peptide bonds form a stable

complex with Cu^{2+}, which is photometrically measured.

2.9 Statistical Analysis

The data generated were analysed using Statistical Product and Service Solutions (SPSS) IBM version 20 and the results expressed as mean ± standard error of mean. Significant differences of the result were established by one-way ANOVA and the acceptance level of significance was $p< 0.05$ for all the results.

3. RESULTS

3.1 Detection of Fraction

Fig. 1 shows the absorbance reading of the different test tube fractions (1-50) of the methanol extract of the seeds of *A. precatorius* at a wavelength of 265 nm fractionated using Sephadex gel G15 swollen, packed and eluted with distilled water. The fractions were spotted on a TLC plate and were spread with Dragendorff's reagent. Fractions no 19-30 were pulled together and used as fraction (F_2).

3.2 Percentage Yield of Fraction 1 and 2 of the Methanol Extract of *A. precatorius* Seeds

After fractionation, the results in Table 1 showed that further purification with Sephadex gel G15 and spraying with Dragendorff's reagent, 12.51g of the methanol seeds extracted gave 2.22g (17.75%) of Fraction 1 (F_1) while 12.51g of the methanol seeds extracted gave 1.54g (12.31%) of Fraction 2 (F_2).

3.3 Result of the Acute Toxicity (LD_{50}) Test of the Methanol Fraction of *A. precatorius*

The result of the acute toxicity (LD_{50}) test revealed that the LD_{50} value is 529.2 mg/kg bodyweightTable 2.

3.4 Effect of the Methanol Fraction (F_2) of *A.precatorius* Seeds on Rats Blood Glucose Levels before and after Induction of Diabetes with Treatments

Fig. 2 shows the mean blood glucose levels of rats treated with different doses of fraction (F2) of the methanol extract of *A.precatorius* seeds. The baseline bars indicate non diabetic glucose level. The day 0 bars indicated that all the animals in the test groups with the exception of group 1 were all diabetic. A significant decrease ($p<0.05$) was observed in the mean blood glucose values of group 3,4,5, 6 and 7 after day 11 of treatment when compared with the positive control (group 2).

3.5 Result of the Lipid Profile

3.5.1 Effect of fraction (F_2) methanol extract of *A. precatorius* seeds on serum total cholesterol concentrations in alloxan-induced diabetic rats

Fig. 3 shows non-significant decrease ($p>0.05$) in the total cholesterol concentration of Group 1 rats (Normal rats) compared with the total cholesterol concentration of rats in Group 2 (diabetic untreated rats). Significant reduction ($p<0.05$) was observed in the total cholesterol concentration of Group 3 (diabetic rats treated with Glibenclamide) compared with the total cholesterol concentration of Group 1 (Normal control) and Group 2 (diabetic untreated). There is also significant decrease ($p<0.05$) in Group 7 (rats treated with F_2 before induction of diabetes) when compared to both Group 1 and Group 2. The result obtained suggests that the F_2 at lower dosage has the potential to lower blood total cholesterol concentration.

3.5.2 Effect of methanol fraction (F_2) of *A. precatorius* seeds on triacylglycerol concentrations in alloxan-induced diabetic rats

Fig. 4 shows that the triacylglycerol (TAG) concentration of alloxan-induced diabetic rats (Group 2) increased significantly ($p<0.05$) than that of normal rats (Group 1, non-diabetic rats). A significant decrease ($p<0.05$) was observed in the serum triacylglycerol in the test groups when compared to the control (Group 2). However, Group 7 showed a more effective decrease in the TAG concentration. Suggesting that the methanol fraction (F_2) at mid concentration treated with F_2 before alloxan-induction decreased the TAG concentration than those concentrations induced with alloxan before treatment.

3.5.3 Effect of methanol fraction (F_2) of *A. precatorius* seeds on High Density Lipoprotein (HDL) concentrations in alloxan-induced diabetic rats

Fig. 5 shows that the high-density lipoprotein (HDL) concentration of alloxan-induced diabetic

rats (Group 2) decreased significantly (p<0.05) than that of normal rats (Group 1, non-diabetic rats). It shows further that treatment of alloxan induced diabetic rats with different doses of the fraction produced significant increase (p<0.05) in the concentrations of HDL especially at Groups 3, 5, 6 and 7 of the methanol fraction (F_2) when compared with Group 2. There was a non-significant increase (p>0.05) in Group 4 when compared with Group 2.

3.5.4 Effect of methanol fraction (F_2) of *A. precatorius* seeds on Low-Density Lipoprotein (LDL) concentrations in alloxan-induced diabetic rats

The result of the effect of methanol fraction (F_2) of *A. precatorius* seeds on low-density lipoprotein concentrations on alloxan-induced diabetic rats showed that low-density lipoprotein concentrations increased non-significantly (p>0.05) in Group 2 (untreated diabetic rats) when compared with Group 1 (non-diabetic rats) (Fig. 6). Also shown in Fig. 6, the low-density lipoprotein concentrations of Group 3, 4, 5, and 7 rats treated with Glibenclamide (standard drug) and different concentrations of the fraction (F_2) of *A. precatorius* seeds decreased significantly (p<0.05) relative to those of the Groups 2 (untreated diabetic rats). The low-density lipoprotein concentrations of Group 5 rats treated with 10mg/kg bw. of the methanol fraction of *A. precatorius* were decreased non-significantly (p>0.05) when compared with Group 2.

3.6 Effect of Methanol Fraction (F_2) of *A. precatorius* Seeds on Total Protein Concentrations in Alloxan-Induced Diabetic Rats

Fig. 7 revealed that the fraction boosted the protein status of the treated rats. Fig. 7 showed that relative to the non-diabetic rats (Group 1), the total protein concentrations of untreated diabetic rats (Group 2) decreased non-significantly (p>0.05). When compared to the total protein concentrations of untreated diabetic rats (Group 2), the rats treated with 10mg/kg fraction (F_2) (Group 7), the fraction treatment significantly increased (p<0.05). Interestingly, in Group 3, 4, 5 and 6, that is diabetic rats treated with standard drug, 5mg/kg, 10mg/kg, and 20mg/kg, the total protein concentrations were non-significantly increased (p>0.05) when compared with Group 2 (untreated diabetic rats) suggesting that the fraction (F_2) has protein boosting potentials.

4. DISCUSSION

All over the world, this dreadful disorder called Diabetes is becoming a serious threat to humans [29]. The LD_{50} value (529.2 mg/kg bw) suggest that the methanol fraction of *A. precatorius* may be toxic although at doses below 400 mg/kg bw it can be safe for treatment.

In diabetic condition, higher levels of glucose was reported by Ogugua and Ikejiaka [30], Ogugua and Aroh [31], Etuk and Mohammed [32]. The blood glucose level in diabetes untreated rats showed a significant increase compared with the rats treated with F_2 of the methanol extract of the seeds of *A. precatorius*, which could be attributed to intrinsic oxidative stress in diabetic condition. The reduction in the blood glucose level in the treated groups suggests that the F_2 might contain antioxidant properties which possibly countered oxidative stress in the experimental animals.

Fraction 2 (F_2) of the methanol extract of the seeds of *A. precatorius* decreased significantly the levels of total cholesterol, LDL, TAG and increased significantly the level of HDL when compared with untreated diabetic rats.

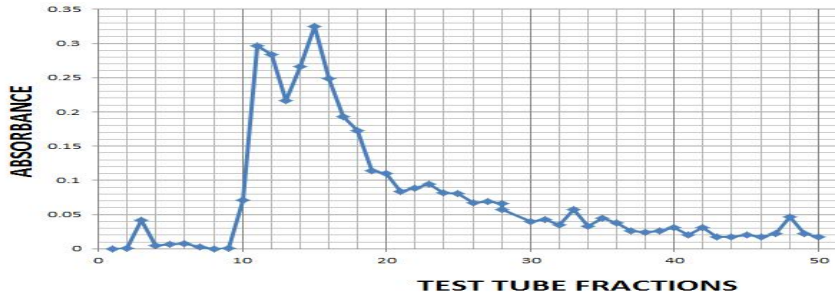

Fig. 1. Spectrophotometer reading showing the absorbance level of the eluted fractions of the methanol extract of the seeds of *A. precatorius*

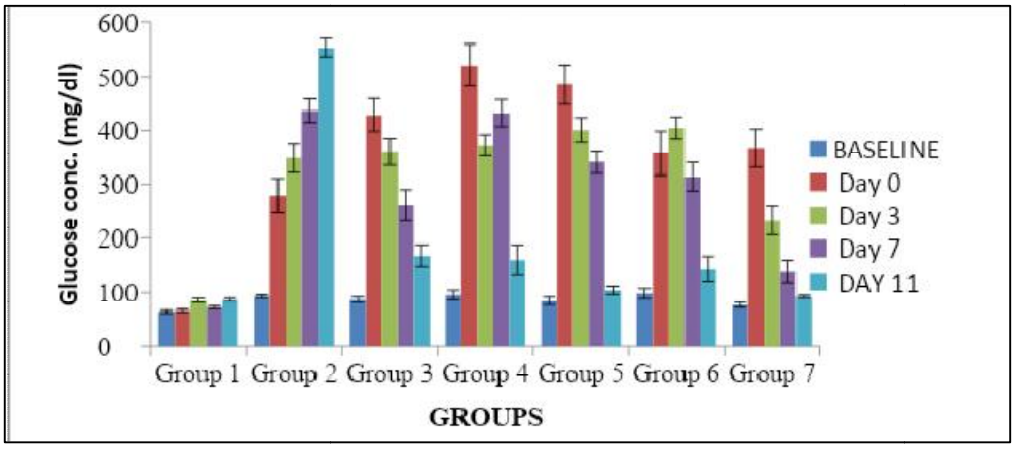

Fig. 2. The effect of fraction (F_2) of the methanol extract of *A. precatorius* seeds on rat blood glucose levels before and after induction of diabetes with treatments

Legend: Group 1: Control (Normal rats), Group 2: Positive control (Diabetic not treated rats), Group 3: Alloxan-induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan-induced diabetic rats + 5 mg/kg body weight of methanol seed fraction, Group 5: Alloxan-induced diabetic rats + 10 mg/kg body weight of methanol seed fraction, Group 6: Alloxan-induced diabetic rats + 20 mg/kg body weight of methanol seed fraction, Group 7: 10 mg/kg body weight of methanol seed fraction + Alloxan-induced diabetic rats

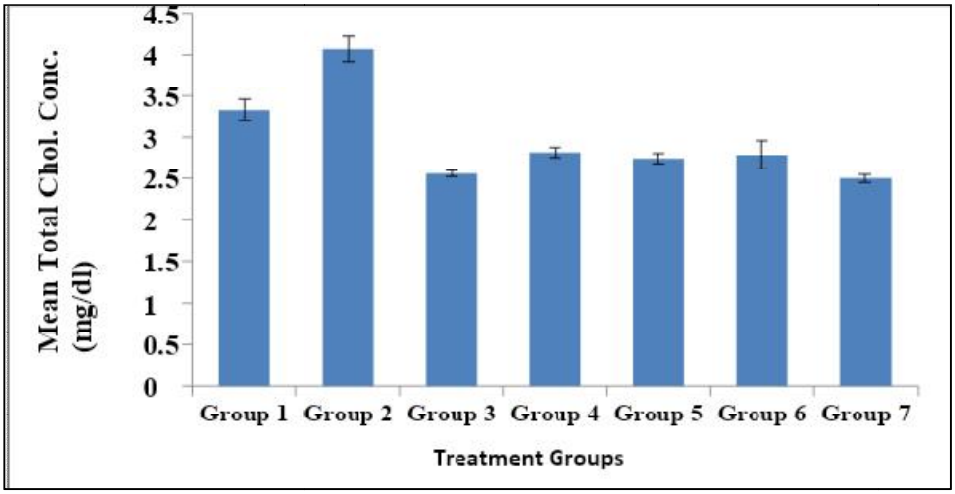

Fig. 3. Effect of fraction (F_2) methanol extract of *A. precatorius* seeds on the total cholesterol concentration of non-diabetic and diabetic-induced rats

Legend: Group 1: Control (Normal rats), Group 2: Positive control (Diabetic not treated rats), Group 3: Alloxan-induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan-induced diabetic rats + 5 mg/kg body weight of methanol seed fraction, Group 5: Alloxan-induced diabetic rats + 10 mg/kg body weight of methanol seed fraction, Group 6: Alloxan-induced diabetic rats + 20 mg/kg body weight of methanol seed fraction, Group 7: 10 mg/kg body weight of methanol seed fraction + Alloxan-induced diabetic rats

Table 1. Percentage yield of fractions (F_1 and F_2) of the methanol extract of *A. precatorius* seeds

Fractions	Extract (g)	Yield after fractionation of extract (g)	%Yield
F_1	12.51	2.22	17.75
F_2	12.51	1.54	12.31

Table 2. Result of the acute toxicity (LD_{50}) test of the methanol extract of the seeds of *A. Precatorius* Linn

Grouping	Dosage mg/kg body weight	Mortality
Group 1	10	0/3
Group 2	100	0/3
Group 3	200	0/3
Group 4	400	0/3
Group 5	700	2/3

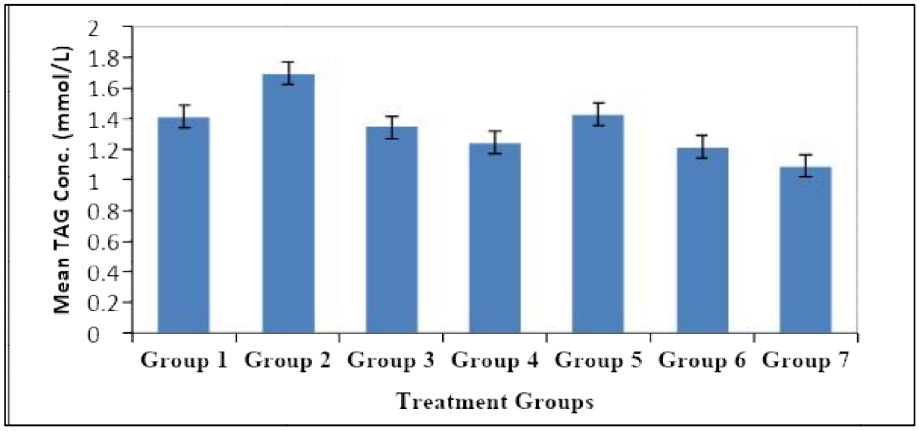

Fig. 4. Effect of methanol fraction (F_2) of *A. precatorius* seeds on the TAG concentration of non-diabetic and diabetic-induced rats

Legend: Group 1: Control (Normal rats), Group 2: Positive control (Diabetic not treated rats), Group 3: Alloxan-induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan-induced diabetic rats + 5 mg/kg body weight of methanol seed fraction, Group 5: Alloxan-induced diabetic rats + 10 mg/kg body weight of methanol seed fraction, Group 6: Alloxan-induced diabetic rats + 20 mg/kg body weight of methanol seed fraction, Group 7: 10 mg/kg body weight of methanol seed fraction + Alloxan-induced diabetic rats

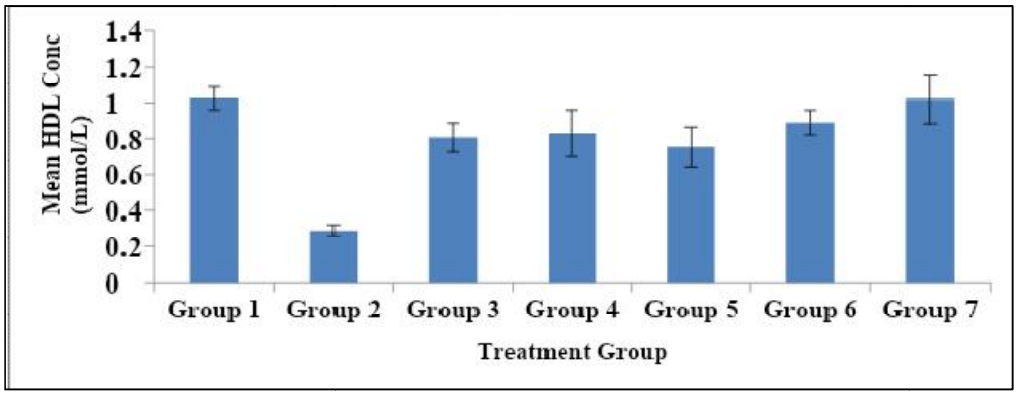

Fig. 5. Effect of fraction (F_2) of the methanol extract *of A. precatorius* seeds on the HDL concentration of non-diabetic and diabetic-induced rats

Legend: Group 1: Control (Normal rats), Group 2: Positive control (Diabetic not treated rats), Group 3: Alloxan-induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan-induced diabetic rats + 5 mg/kg body weight of methanol seed fraction, Group 5: Alloxan-induced diabetic rats + 10 mg/kg body weight of methanol seed fraction, Group 6: Alloxan-induced diabetic rats + 20 mg/kg body weight of methanol seed fraction, Group 7: 10 mg/kg body weight of methanol seed fraction + Alloxan-induced diabetic rats

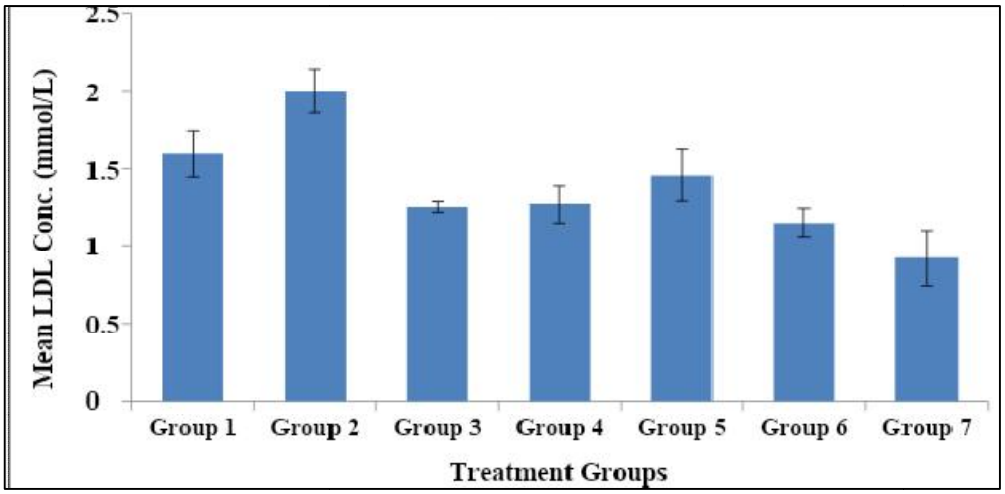

Fig. 6. Effect of methanol fraction (F_2) of *A. precatorius* on the low-density lipoprotein concentration of non-diabetic and diabetic-induced rats

Legend: Group 1: Control (Normal rats), Group 2: Positive control (Diabetic not treated rats), Group 3: Alloxan-induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan-induced diabetic rats + 5 mg/kg body weight of methanol seed fraction, Group 5: Alloxan-induced diabetic rats + 10 mg/kg body weight of methanol seed fraction, Group 6: Alloxan-induced diabetic rats + 20 mg/kg body weight of methanol seed fraction, Group 7: 10 mg/kg bodyweight of methanol seed fraction + Alloxan-induced diabetic rats

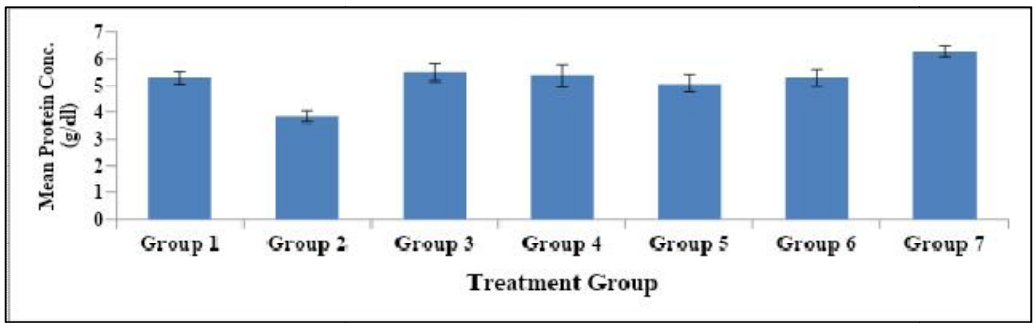

Fig. 7. Effect of methanol fraction (F_2) of *A. precatorius* seeds on the total protein concentration of non-diabetic and diabetic-induced rats

Legend: Group 1: Control (Normal rats), Group 2: Positive control (Diabetic not treated rats), Group 3: Alloxan-induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan-induced diabetic rats + 5 mg/kg body weight of methanol seed fraction, Group 5: Alloxan-induced diabetic rats + 10 mg/kg body weight of methanol seed fraction, Group 6: Alloxan-induced diabetic rats + 20 mg/kg body weight of methanol seed fraction, Group 7: 10 mg/kg body weight of methanol seed fraction + Alloxan-induced diabetic rats

The reduced serum cholesterol level as shown in the present investigation on F_2 of the methanol extract of *A. precatorius* could be attributed to the inhibition of endogenous synthesis of lipids probably by potentiating the secretion of insulin. Lipids play a vital role in the pathogenesis of diabetes mellitus. An increase in the levels of

cholesterol and triacylglycerol that was observed in untreated diabetic rats could be as a result of increased mobilization of free fatty acids from peripheral depots. In insulin-deficient diabetes, the concentration of serum free fatty acids is increased as a result of free fatty acids out flows from fat depots where the balance of the free fatty acid esterification triacylglycerol lipolysis cycle is displaced in favour of lipolysis [33]. The increased level of serum HDL level in diabetic rats could suggest that the F_2 might be considered as a substitute of drugs to protect diabetes-associated and hypercholesterolaemia complications.

The fraction (F_2) of the methanol extract of the seeds of *A. precatorius* showed an increase level in serum protein in diabetes treated groups. This increase could be attributed to the bioactive

ingredients contained in the F_2 that possess insulin-like property which enhanced normal protein metabolism. Serum is the path through which the proteins and amino acids removed from the peripheral tissues are transported in the body and this is the reason for the elevation of serum protein in diabetics [34]. Treatment with F_2 removes the free radicals and so there is an increase in the protein content of *A. precatorius* treated diabetic rats. However, the decrease in total protein concentrations in the serum of diabetic rats may also be attributed to a decreased amino acid uptake or a greatly decreased concentration of variety of essential amino acids.

In order to develop and construct knowledge about the antidiabetic effect of F_2 of *A. precatorius,* further studies should be performed using different solvents in the extraction of the seeds of *A. precatorius* in order to compare the different active components of the different solvents that may possess potency. Also, higher doses below 100 and 200mg/kg bodyweight of methanol fraction (F_2) of *A. precatorius* linn should be used and different fractions should also be used to ascertain if there is another fraction different from fraction 2 that may possess anti-diabetic properties.

5. CONCLUSION

The results obtained from the lipid profile, total protein concentrations and hypotonicity-induced heamolysis analysis led to the conclusion that the F_2 (Fraction 2) of the methanol extract of the seeds of *A. precatorius* Linn in the administered doses and through the oral route of administration were able to reduce blood glucose concentrations in alloxan-induced diabetic rats. The F_2 fraction of the methanol extract of the seeds of *A.precatorius* will be a good substitute to conventional drugs as it increases the good cholesterol (HDL) and lowers the bad cholesterol in alloxan-induced diabetic rats

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocol was examined and approved by the ethics committee of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria in accordance with the Institutional Animal Care and Use policy in Research, Education and Testing as recommended for institutions. The experiment was carried out in strict compliance with the principle for the use and handling of laboratory animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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