



## Effects of a Fraction of the Methanol Extract of the Seeds of *Abrus Precatorius* on Malondialdehyde and Antioxidant Levels of 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, Authors ELI and OCE wrote the protocol, Authors ELI and OPA performed the statistical analysis and wrote the first draft of the manuscript. Authors ELI, OCE, OBA and ISO managed the analyses of the study. Authors ELI and OCE managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Introduction:** The aim of this study is to evaluate the effect of fraction 2 (F<sub>2</sub>) of methanol extract of the seeds of *Abrus precatorius* on malondialdehyde and antioxidant level of alloxan-induced diabetic Wistar albino rats.

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**Materials and 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 alloxan at a dose of 150 mg/kg bodyweight. The phytochemical analysis and the biochemical parameters were investigated using standard diagnostic methods.

**Results:** Both qualitative and quantitative analysis of  $F_2$  revealed the presence of high amounts of alkaloids ( $2000 \pm 80$  mg/100 g), flavonoids ( $158 \pm 17.6$  mg/100 g) and tannins ( $258 \pm 45$  mg/100 g) but low concentration of saponins ( $18.3 \pm 2.43$  mg/100 g). The malondialdehyde (MDA) level of all the test groups and the group treated with the standard drug decreased significantly ( $p < 0.05$ ) compared with that of untreated diabetic group. However, the catalase activity in all the test groups significantly increased ( $p < 0.05$ ) while the activity of superoxide dismutase increased significantly ( $p < 0.05$ ) in groups administered 20mg/kg of the fraction and the group pre-treated with 10mg/kg of the fraction compared with the diabetic untreated group. Similarly, the result revealed a significant increase ( $p < 0.05$ ) in non-enzymatic antioxidants such as reduced glutathione and vitamin E of the rats treated with graded doses of  $F_2$  while vitamin C showed significant ( $p < 0.05$ ) increase in normal control groups and pre-treated groups compared with the diabetic untreated. Studies on membrane stabilization using hypotonicity-induced red blood cell haemolysis revealed that the  $F_2$  of the methanol extract of the seeds of *A. precatorius* Linn inhibited haemolysis in a dose-dependent manner.

**Conclusion:** The results obtained from this study revealed that the  $F_2$  fraction of the methanol extract of *A. precatorius* contain important phytochemicals found in the Fraction 2 ( $F_2$ ) however showed improvement in antioxidant defence as well as has a stabilizing effect on the membrane.

**Keywords:** *Abrus precatorius*; diabetes mellitus; antioxidants; oxidative stress; malondialdehyde.

## 1. INTRODUCTION

In every population in the world and in all regions diabetes is found, including rural parts of low and middle-income countries. It describes a group of metabolic disorders that are characterized and identified by the presence of hyperglycaemia in the absence of treatment. When there is a disordered metabolic regulation leading to diabetes mellitus, it results in an abnormal fasting blood glucose concentration because although glucose is floating in the blood it cannot be transported to the cells where it is needed thereby starving and dehydrating the cells. The cells begin to lose its intracellular water to the blood in order to dilute the high glucose concentration of the blood [1]. No wonder diabetes mellitus can be referred to as starvation in the midst of plenty. It is one of the world's commonest diseases today with about 171 million people affected by the disease [2]. The number of people with diabetes is continuously increasing, with World Health Organization (WHO) estimating there were about 422 million adults with diabetes worldwide in 2014. Adults with adjusted age found in low and middle-income countries had the highest rise in diabetes from 4.7% in 1980 to 8.5% in 2004 when compared with high-income countries [3]. International diabetes federation (IDF) also estimated that type 1 diabetes mellitus was seen in 1.1 million children and adolescents with the age range of 14-18 years [4].

If there is no quick effort to stop the elevations in diabetes, by 2045 the people living with diabetes will be at least 629 million [4]. As the figure continues to increase steadily, predictions have shown that by 2030, if the current trend continues, the people living with diabetes would be over 366 million [5]. The general accepted approach of managing diabetes is faced with a lot of difficulties, partly because several of the drugs, aimed in managing diabetes pose a significant risk of inflicting heart disease. Presently no pill or injection is able to address the problem of dying pancreatic beta cells, a fundamental dysfunction in diabetes. It is now believed that the destruction or dysfunction of the  $\beta$ -cells of the pancreas is the underlying characteristic common to all forms of diabetes [6-9]. The destruction or decrease in the function of the  $\beta$ -cells are as a result of not having mechanisms through which these cells can be replaced, as the human pancreas seems incapable of renewing  $\beta$ -cells after the age of 30 years [10]. The mechanisms which can lead to the destruction of the pancreas include genetic predisposition and abnormalities, epigenetic processes, insulin resistance, auto-immunity, concurrent illnesses, inflammation, and environmental factors. Differentiating  $\beta$ -cell dysfunction and decreased  $\beta$ -cell mass could have vital implications for therapeutic approaches to maintain or enhance glucose tolerance [8]. In order to have a clear definition of

the subtypes of diabetes and also to proper a guided treatment, there is need to have a good understanding of the  $\beta$ -cells status [9].

The management of diabetes concentrates on keeping blood sugar levels as close to normal as possible, without causing hypoglycaemia. Diabetes Mellitus (DM) is a major health problem worldwide. Recently, there are some viable areas such as Asia and Africa where the disease is feared to rise in 2-3 folds [11]. According to the current classification there are two major types: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). The distinction between the two types has historically been based on age at onset, degree of loss of  $\beta$  cell function, degree of insulin resistance, presence of diabetes-associated autoantibodies, and requirement for insulin treatment for survival [12]. Increased oxidative stress is implicated in the development and progression of diabetic tissue damage and induced changes in the activities of antioxidant enzymes in various tissues [13]. Comparison with healthy controls shows that diabetics have significantly higher free-radical activity, as well as significantly lower concentrations of antioxidants [14]. These changes are of greater magnitude in patients with disease complications than in those without complications. It is possible therefore, that supplementing with foods, nutrients, and herbs that have antioxidant activity would help prevent diabetic end-organ damage [14].

Hyperglycaemia may lead to oxidative stress through the production of reactive oxygen species (ROS) or disturbed redox balance [15]. These radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defence mechanisms. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. The increase in the levels of ROS in diabetes could be due to increased production of free radicals and the attendant or decreased enzymatic antioxidants; catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). Antioxidants are biomolecules, vitamins and other nutrients that protect the cells from damages caused by radicals either by preventing or inhibiting the oxidation of other molecules. *In-vitro* and *in-vivo* studies have shown that antioxidants help to prevent the free radical damages that are associated with cancer and heart diseases. Antioxidants are usually reducing agents; e.g. thiols, ascorbic acid and polyphenols [16]. Common antioxidants include

vitamins A, C, and E, glutathione, and enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (GSH). Other known antioxidants include lipoic acid, the mixed carotenoids; several bioflavonoids, antioxidant minerals such as copper, zinc, manganese and selenium and the cofactors (folic acid, vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> and B<sub>12</sub>). They work in synergy with one another and against different types of free radicals [17].

Consequent upon the problems associated with orthodox approach in the management of diabetes, effort is now geared towards the traditional approach of treating and managing diabetes. It is assumed that this approach is safer and more natural. Most of the herbal extracts are rich in antioxidants due to their nutritional and phytochemical compositions [18-20]. The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. Research has shown that plants such as *Ocimum canum* leaves [21], *Azadirachta indica* leaves [22,23], *Averrhoa carambola* leaves [24], *Justicia carnea* leaves [25], has antidiabetic and antioxidant properties. *A. precatorius* is a twinning woody plant that is characterized with a toxic red seeds and a black mark at the base [26]. It is native to India, at altitudes up to 1200 m on the outer Himalayas. All tropical countries are now naturalized with *A. precatorius* [27]. *A. precatorius* (Rosary pea) is a wonderful herb. A small climbing tropical vine with seeds known as crab's eye was found abundant in Patalkot forest. This herb has much importance in a common tribal life. They make various ornaments by the seeds of the plant. *A. precatorius* commonly known as precatory bean or rosary pea is an endemic medicinal plant having immense pharmacological importance. The medicinal purpose of its seeds, leaves and roots are used all over Asia and Africa [28,29]. Diseases such as chronic nephritis have been treated with the seeds of *A. precatorius* [30], infestations [31]. This study aims to evaluate the effect of a fraction F<sub>2</sub> of methanol extract of the seeds of *Abrus precatorius* on malondialdehyde and antioxidant level of alloxan-induced diabetic Wistar albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical grade and they include: Absolute methanol (BDH, England); Ascorbic

acid (May & Baker, England); Bismuth carbonate (BDH, England); Chloroform (Sigma Aldrich, Germany); Alloxan (Sigma Aldrich, Germany); Biuret's reagent (May and Baker's England); Glibenclamide (Hovid BHD, Malaysia); Trichloroacetic acid (Sigma Aldrich, Germany); 2,4-dinitrophenyl hydrazine (Merck Darmstadt, Germany); Cupric Sulphate solution (Merck Darmstadt, Germany); Potassium dichromate (Sigma Aldrich, Germany); Dichromate acetic acid (May and Baker, England); Molisch's reagent (QCA, Spain); Mayer's reagent (BDH, England); Drangendorff's reagent (May and Baker, England); Wagner's reagent (Randox, USA); Picric acid (Merck Darmstadt, Germany); Lead acetate solution (Merck Darmstadt, Germany); Ethyl acetate (BDH, England); Aluminum chloride solution (BDH, England); 1% Thiobarbituric acid (BDH England).

## 2.2 Plant Material, Extraction and Fractionation Procedure

The seeds of *A. precatorius* Linn *Fabaceae* were collected from Igala Area of Kogi State and authenticated at the Bioresources Development and Conservation Programme (BDGP), Nsukka, Enugu State, Nigeria. The seed of *A. precatorius* Linn were washed with clean water. 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<sub>2</sub>O.

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 with distilled water and introduced into the column and afterward, fractions (elutions) were then collected in test tubes of about 3ml each.

## 2.3 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).

## 2.4 Visible Spectroscopy

After the development of the plate, it was spread with Drangendorff's reagent. The fractions that turned purple were pulled into a beaker as fraction (F<sub>1</sub>) while the other fractions that did not change colour were pulled together as fraction (F<sub>2</sub>). The fraction (F<sub>2</sub>) was then concentrated and afterward, a given weight was dissolved in normal saline (stock solution) which was administered to the animals based on their body weight.

## 2.5 Determination of Extract and Fraction Yield

The percentage yield of *Abrus precatorius* was calculated by weighing the seeds before extraction and after concentration of the extract while the percentage yield of fraction (F<sub>2</sub>) was calculated by weighing the fraction after concentration and weight of the crude extract after concentration. It was calculated using the formula below:

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

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

## 2.6 Phytochemical Analysis

### 2.6.1 Qualitative phytochemical analysis

The qualitative phytochemical analysis of the seed Fraction (F<sub>2</sub>) of *A. precatorius* were carried out according to the method of Harborne [32] and Trease and Evans [33] to identify its active constituents.

### 2.6.2 Quantitative phytochemical analysis

#### 2.6.2.1 Determination of alkaloid

The determination of alkaloid was carried out as described by Harborne [32]. A portion (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid and ethanol was added, covered and allowed to stand for 2 hours. This was filtered and the Fraction (F<sub>2</sub>) was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium

hydroxide was added drop-wise to the Fraction (F<sub>2</sub>) till a precipitate was formed. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

#### 2.6.2.2 Determination of flavonoid

This was determined according to the method of Harborne [32]. A quantity, 5 g of the sample was boiled in 50 ml of 2M HCl solution for 30 mins under reflux. It was allowed to cool and then filtered through whatman No. 1 filter paper. A measured volume of the Fraction (F<sub>2</sub>) was treated with equal volume of ethyl acetate starting with a drop. The solution was filtered into a weighed crucible. The filtrate was heated to dryness in an oven at 60°C. The dried crucible was weighed again and the difference in the weight gave the quantity of flavonoid present in the sample.

#### 2.6.2.3 Determination of saponins

Determination of saponins was done by weighing 1g of the sample and macerating with 10ml of petroleum ether. The sample was decanted into a beaker and washed twice with 10 ml of normal saline and filtered. The filtrate was allowed to evaporate to dryness. The residue was dissolved in 6 ml of ethanol. The solution (2 ml) was added to a test tube and 2 ml of chromogen solution was also added. The mixture was allowed to stand for 30 mins. The absorbance of the sample was read at 550 nm.

#### 2.6.2.4 Determination of tannins

Determination of tannins was done by weighing 1 g of the sample and macerating with 50 ml of methanol then filtered and 0.3 ml of 0.1N ferric chloride in 0.1N HCl was added to 5 ml of the filtrate. Also 0.3 ml of 0.0008 M potassium ferricyanide and then shaken. The absorbance of the sample was read at 720 nm.

### 2.7 Hypotonicity-induced Haemolysis Test of Red Blood Cell

The effect of fraction (F<sub>2</sub>) of the methanol extract of the seeds of *A. precatorius* on hypotonicity-induced haemolysis of red blood cell was investigated using modified method described by Shinde et al. [34].

Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, which leads to swelling and lysis. This results in the release of haemoglobin which absorb maximally at 418 nm. Hence the

optical density at 418 nm, which is an index of haemolysis, is a reflection of the stability of red blood cell membrane. Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis [34]. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde et al. [34].

### 2.8 Experimental Animal and Study Design

Twenty-eight (28) Wistar albino rats were housed in separate cages, acclimatized to laboratory conditions for one week with free access to feed and water. After acclimatization, they were distributed evenly into seven (7) groups of four (4) 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 3 days, 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<sub>2</sub>) 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 body weight of glibenclamide.  
Group 4: Diabetic rats + 5 mg/kg body weight of the *A. precatorius* fraction.  
Group 5: Diabetic rats + 10 mg/kg body weight of the *A. precatorius* fraction.  
Group 6: Diabetic rats + 20 mg/kg body weight of the *A. precatorius* fraction.  
Group 7: Normal rats + 10 mg/kg body weight of the fraction + 150 mg/kg alloxan.

### 2.9 Hypotonicity-induced Haemolysis Test of Red Blood Cell

The effect of fraction (F<sub>2</sub>) of the methanol extract of the seeds of *Abrus precatorius* on hypotonicity-induced haemolysis of red blood cell was investigated using modified method described by Shinde et al. [34].

### 2.9.1 Principle

Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, which leads to swelling and lysis. This results in the release of haemoglobin which absorb maximally at 418 nm. Hence the optical density at 418 nm, which is an index of haemolysis, is a reflection of the stability of red blood cell membrane. Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis [34]. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde et al. [34].

### 2.9.2 Preparation of erythrocyte suspension

Oxygenated blood was collected in plastic tubes containing 0.1 volume of 3.8% trisodium citrate used within 8 hours. Citrated blood samples were centrifuged at 3000x g for 10 mins and the supernatant (plasma) discarded. The pellet was washed twice by resuspending it in a volume of normal saline equal to the volume of the supernatant (plasma), i.e. Volume of normal saline = Volume of the plasma to be discarded and Centrifuge at 3000x g for 10 mins. The pellet (0.1ml) was resuspended in 2.5 ml of Normal saline and this was now used as the red blood cell (RBC).

### 2.9.3 Experiment procedure

**Chart 1. A set of eight tubes were used for the analysis. The reaction medium is shown below**

Tu be	RBC (ml)	Distilled Water (ml)	Normal Saline (ml)	Fraction (F <sub>2</sub> ) (ml)
1	0.1	-	1.9	-
2	0.1	0.8	1.1	-
3	0.1	0.8	1.0	0.1
4	0.1	0.8	0.9	0.2
5	0.1	0.8	0.7	0.4
6	-	0.8	1.1	0.1
7	-	0.8	1.0	0.2
8	-	0.8	0.8	0.4

The reaction medium was incubated at 37°C for 1 hr, the incubates were centrifuged at 3000x g for 10 mins to terminate the reaction. The absorptions of the respective supernatants were measured at 418 nm as a measure of extent of Haemolysis. In all cases, triplicate determinations were carried out. Blank containing the 1.2 ml normal saline and 0.8 ml water were used.

$$\% \text{ Inhibition of haemolysis} = 100 \times (\text{OD1} - \text{OD2}/\text{OD1})$$

Where:

OD1 = Optical density of hypotonic-buffered saline solution alone

OD2 = Optical density of test sample in hypotonic solution

### 2.10 Lipid Peroxidation (Malondialdehyde)

Lipid peroxidation was determined by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al. [35]. Lipid degradation occurs forming such products as malondialdehyde (from fatty acids with three or more double bonds), ethane and pentane (from the n-terminal carbons of 3 and 6 fatty acids, respectively). Malondialdehyde appears in the blood and urine and is used as an indicator of free radical damage. MDA is a sign of lipid peroxidation [36].

### 2.11 Antioxidant Assay

#### 2.11.1 Superoxide dismutase (SOD) assay

The Superoxide dismutase (SOD) activity was assayed according to the method of Xin et al. [37]. The principle of SOD activity assay was based on the inhibition of nitroblue tetrazolium (NBT) reduction.

#### 2.11.2 Catalase activities

This was done according to the method of Aebi [38]. The ultra violet absorption of hydrogen peroxide can be easily measured at 240 nm. On the decomposition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by catalase the absorption decreases with time and from this decrease catalase activity can be measured.

#### 2.11.3 Glutathione concentration

This was determined according to the method of King and Wootton [39].

#### 2.11.4 Vitamin C Concentration

Vitamin C concentration was determined using the method of Omaye et al. [40]. Ascorbic acid is oxidized and converted to diketoglutaric acid, a strong acid solution and forms a diphenyl hydrazine by reacting with 2,4 -

dinitrophenylhydrazine. The hydrazine dissolves in strong sulphuric acid solution to produce a red colour which can be measured colorimetrically at wavelength of 540 nm.

### 2.11.5 Vitamin E Concentration

Vitamin E concentration was determined according to the method of Desai [41].

## 2.12 Statistical Analysis

The data obtained were analysed using Statistical Product and Service Solutions (SPSS) IBM version 20 and the results expressed as mean  $\pm$  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, 12.51 g 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 Qualitative Phytochemical Compositions of Fractions $F_1$ and $F_2$ of the Methanol Extract of the Seeds of *A. precatorius* Linn

Table 2 depicts the results of the phytochemistry of the samples. The results show that both  $F_1$  and  $F_2$  of the methanol extract of *A. precatorius* seeds contained alkaloids, flavonoids, tannins, carbohydrates and saponins while steroids,

peptides and terpenoids were found to be also present in  $F_1$  only. Glycosides, resins and reducing sugars were not detected in both fractions of the methanol extract of the seeds of *A. precatorius*.

### 3.4 Quantitative Determination of Phytochemicals of Fractions $F_1$ and $F_2$ of the Methanol Extract of the Seeds of *A. precatorius* Linn

Table 3 shows the results of the quantitative phytochemical constituents of the different fractions of methanol extract of *A. precatorius* seed. The methanol seed  $F_2$  contained high amount of alkaloids ( $2000.00 \pm 80.00$  mg/100 g), flavonoids ( $158.00 \pm 17.60$  mg/100 g), tannins ( $258.00 \pm 45.00$  mg/100 g) and low amount of saponins ( $18.30 \pm 2.43$  mg/100 g) while  $F_1$  contained high amount of alkaloids ( $5840.00 \pm 184.00$  mg/100 g), flavonoids ( $215.00 \pm 97.50$  mg/100 g), and low amount of saponins ( $2.98 \pm 1.33$  mg/100 g) and tannins ( $6.40 \pm 0.72$  mg/100 g).

### 3.5 Effect of Methanol Fraction ( $F_2$ ) of *A. precatorius* Seeds on Hypotonicity-induced Haemolysis

When erythrocytes were suspended in water and later centrifuged, the supernatant was found to have a mean absorbance of 0.650 at 418 nm. On the other hand, suspension of the erythrocytes in normal saline, given the same treatment as in the case of water gave an absorbance of 0.321. The result showed that in the hypotonic (water) environment, there was liberation of haemoglobin and hence the high absorbance reading. Table 4 shows that when the extract was introduced, there were decreases in absorbance readings. This inhibition of haemolysis was found to be dose dependent, increasing with increased concentration of the extract in the medium.

### 3.6 Effect of Methanol Fraction ( $F_2$ ) of *A. precatorius* Seeds on Lipid Peroxidation (Malondialdehyde)

Increase in malondialdehyde (MDA) values indicates the level of lipid peroxidation in the system as shown in group 2 (diabetes untreated). The fraction ( $F_2$ ) was able to attenuate the levels of lipid peroxidation caused by the induction of diabetes to the experimental rats. Fig. 2 shows that the malondialdehyde concentrations of alloxan-induced diabetic rats (Group 2) was significantly higher ( $p < 0.05$ ) than that of normal rats (Group 1, non-diabetic rats).

The effects produced by Group 3,4,5,6 and 7 (Glibenclamide, 5 mg/kg, 10 mg/kg and 20 mg/kg respectively) were significantly lower ( $p<0.05$ ) than that of Group 2 (untreated diabetic rats). There was a significant decrease ( $p<0.05$ ) in the malondialdehyde concentration of Group 3 (Alloxan-induced diabetic rats + 2.5 mg/kg bodyweight of glibenclamide) compared with Group 2 (Positive Control-untreated diabetic rats).

### 3.7 Result of the Enzymatic and Non-enzymatic Antioxidant Assay

#### 3.7.1 Effect of methanol fraction ( $F_2$ ) of *A. precatorius* seeds on superoxide dismutase activity in alloxan-induced diabetic rats

Fig. 3 revealed that the fractions stimulated SOD activity in diabetic rats treated with the seeds fraction ( $F_2$ ). It showed that relative to the non-diabetic normal rats (Group 1), the dismutase activity of untreated diabetic rat (Group 2) was non-significantly low ( $p>0.05$ ). When compared to the dismutase activity of untreated diabetic rats (Group 2) and those of diabetic rats treated with varying concentrations of the fractions Group 3, 4, and 5 non-significantly increased ( $p>0.05$ ) the dismutase activity. Interestingly, in Group 6 and Group 7 that is diabetic rats treated with 20 mg/kg and 10mg/kg fraction, the dismutase activity was also significantly increased ( $p<0.05$ ) when compared with Group 2 (untreated diabetic rats).

#### 3.7.2 Effect of methanol fraction ( $F_2$ ) of *A. precatorius* seeds on catalase (CAT) activity

Fig. 4 revealed that the fraction ( $F_2$ ) boost the catalase level of the diabetic rats treated with the fraction ( $F_2$ ) seeds. The catalase helps to mop up oxidants in the test groups as a result of lipid peroxidation. Fig. 4 shows that the catalase activity of alloxan-induced diabetic rats (Group 2) was significantly lower ( $p<0.05$ ) than that of normal rats (Group 1, non-diabetic rats). It shows further that treatment of alloxan-induced diabetic rats with different concentrations of the fraction 5 mg/kg, 10 mg/kg, and 20 mg/kg (Group 4, 5, 6, and 7) with more effect on Group 7 increased significantly ( $p<0.05$ ) than that of Group 2. Also, treatment with standard drug (Glibenclamide) increased catalase activity significantly ( $p<0.05$ ) relative to that of untreated diabetic rats (Group 2).

#### 3.7.3 Effect of Methanol Fraction ( $F_2$ ) of *A. precatorius* Seeds on Glutathione Reductase (GSH) Activity

Fig. 5 revealed that the fractions boost the GSH level of the diabetic rats treated with the *A. precatorius* seed fraction ( $F_2$ ). The GSH helps to mop up free radicals in the test groups as a result of lipid peroxidation. A significant decrease ( $p<0.05$ ) was observed in the GSH of diabetic untreated rats (Group 2) when compared with normal rats (Group 1). However, there was significant increase ( $p<0.05$ ) in GSH of rats in Group 4, 5, 6, and 7 (treated with different concentrations of the fraction ( $F_2$ )) when compared with Group 2 (Untreated diabetic rats). The effects of treatment of diabetic rats with the standard drug (Glibenclamide) were significantly increased ( $p<0.05$ ) when compared with Group 2.

#### 3.7.4 Effect of methanol fraction ( $F_2$ ) of *A. precatorius* seeds on Vitamin C concentration

Fig. 6 showed that relative to the untreated normal rats (Group 1), the vitamin C concentration of untreated diabetic rat (Group 2) was significantly low ( $p<0.05$ ). Interestingly, in Group 7 that is rats treated with 10 mg/kg fraction before induction of diabetes, the vitamin C concentration were significantly increased ( $p<0.05$ ) when compared with Group 2. When compared to the vitamin C concentrations of untreated diabetic rats (Group 2) and those of diabetic rats treated with 5 mg/kg, 10 mg/kg and 20 mg/kg (Group 4,5 and 6) respectively, the fraction treatment and standard drug (Glibenclamide) treatment non-significantly increased ( $p>0.05$ ) the vitamin C concentration.

#### 3.7.5 Effect of methanol fraction ( $F_2$ ) of *A. precatorius* seeds on Vitamin E concentration

Fig. 7 showed that in comparism with the untreated normal rats (Group 1), the vitamin E concentration of untreated diabetic rat (Group 2) was significantly low ( $p<0.05$ ). There is also a significant increase ( $p<0.05$ ) in vitamin E concentrations of the diabetic rats treated with standard drug (Group 3) when compared with that of the diabetic untreated (Group 2). Interestingly, in the diabetic rats treated with the fraction, there was a significant increase ( $p<0.05$ ) in vitamin E when compared with diabetic untreated rats (Group 2).

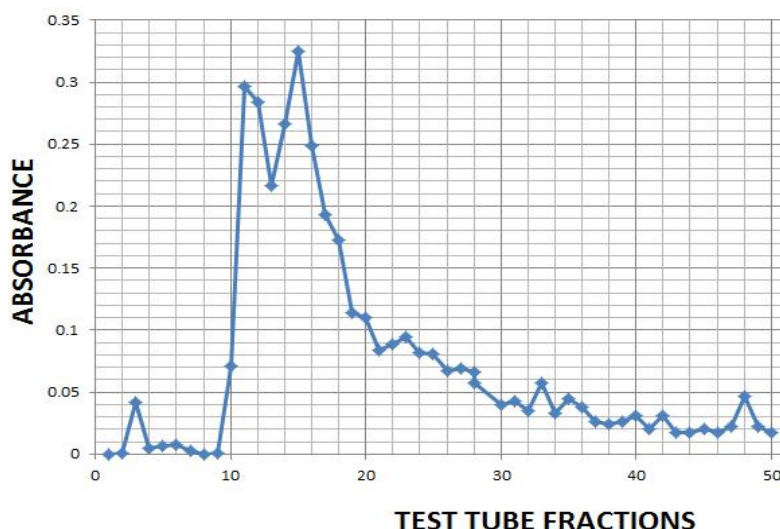


Fig. 1. Spectrophotometer reading showing the absorbance level of the eluted fractions of the methanol extract of the seeds of *A. precatorius*

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. Qualitative phytochemical compositions of fractions  $F_1$  and  $F_2$  of the methanol extract of the seeds of *A. precatorius* Linn

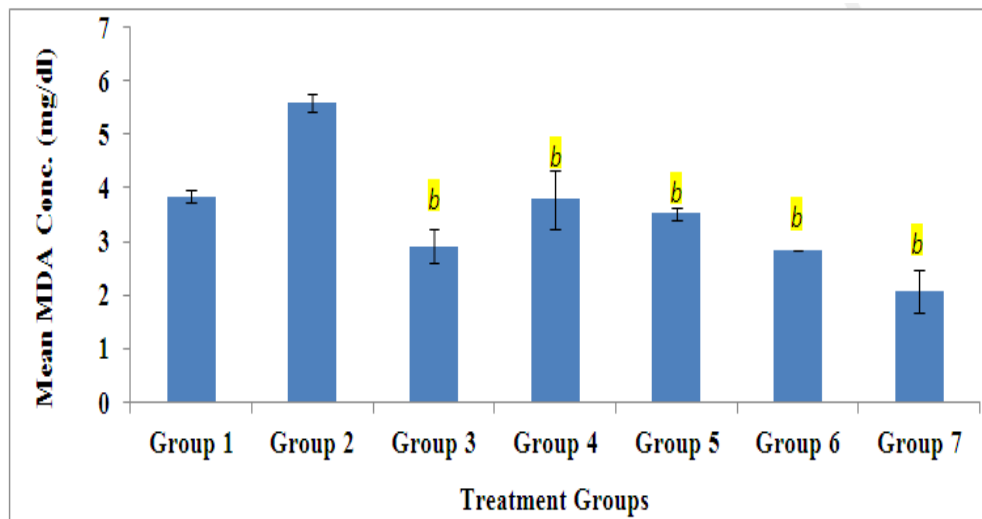
Phytochemicals	Fraction 1	Fraction 2
Alkaloids	+++	+++
Flavonoids	+++	+++
Saponin	+	+
Tannin	+	++
Carbohydrates	+	+
Steroids	+	ND
Terpenoids	+	ND
Peptides	+	ND
Glycoside	ND	ND
Resin	ND	ND
Reducing sugar	ND	ND

Key: +: Present in small concentration  
 ++: Present in moderately high concentration  
 +++: Present in very high concentration  
 ND: Not detected

Table 3. Quantitative phytochemical composition of fractions  $F_1$  and  $F_2$  of the methanol extract of the seeds of *A. precatorius* Linn

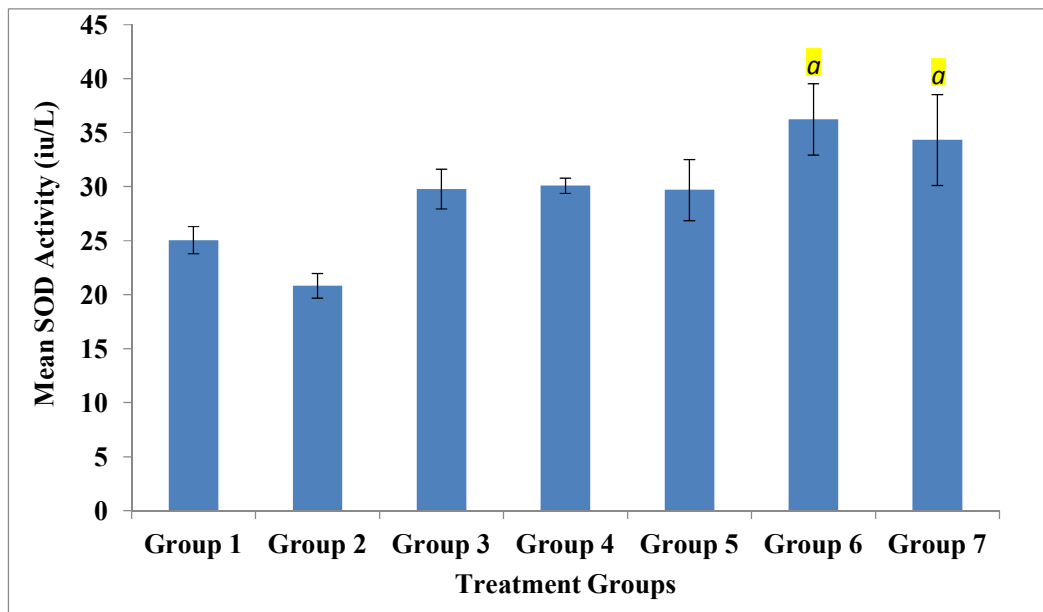
Phytochemical Constituents	Fraction ( $F_1$ ) Mean $\pm$ SD (mg/100g)	Fraction ( $F_2$ ) Mean $\pm$ SD (mg/100g)
Alkaloids	5840.00 $\pm$ 184.00	2000.00 $\pm$ 80.00
Flavonoids	215.00 $\pm$ 97.50	158.00 $\pm$ 17.60
Saponins	2.98 $\pm$ 1.33	18.30 $\pm$ 2.43
Tannins	6.40 $\pm$ 0.72	258.00 $\pm$ 45.00

Values indicate Mean  $\pm$  S.D of  $n=3$



**Fig. 2. Effect of methanol fraction (F<sub>2</sub>) *A. precatorius* on the malondialdehyde 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  
<sup>b</sup>Significant decrease with respect to Group 2 (diabetic-untreated rats)

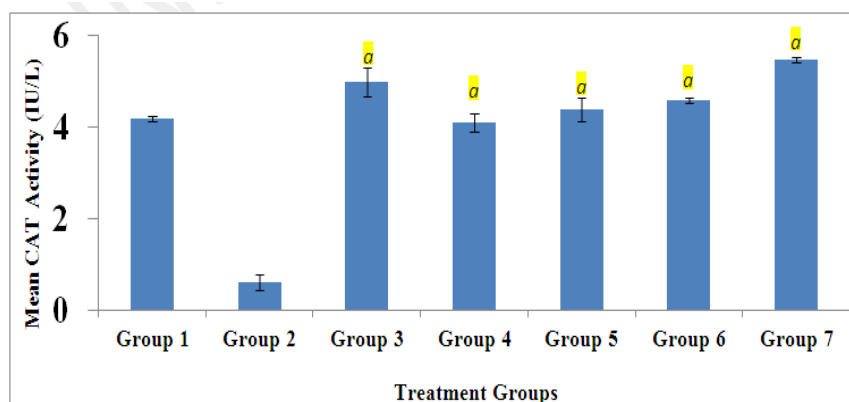


**Fig. 3. Effect of methanol fraction (F<sub>2</sub>) of *A. precatorius* seeds on the SOD activity of non-diabetic and diabetic-induced rats**

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  
<sup>a</sup>Significant increase with respect to Group 2 (diabetic-untreated rats)

**Table 4. Inhibition of hypotonicity-induced haemolysis by F<sub>2</sub> of the methanol extract of the seeds *A. precatorius***

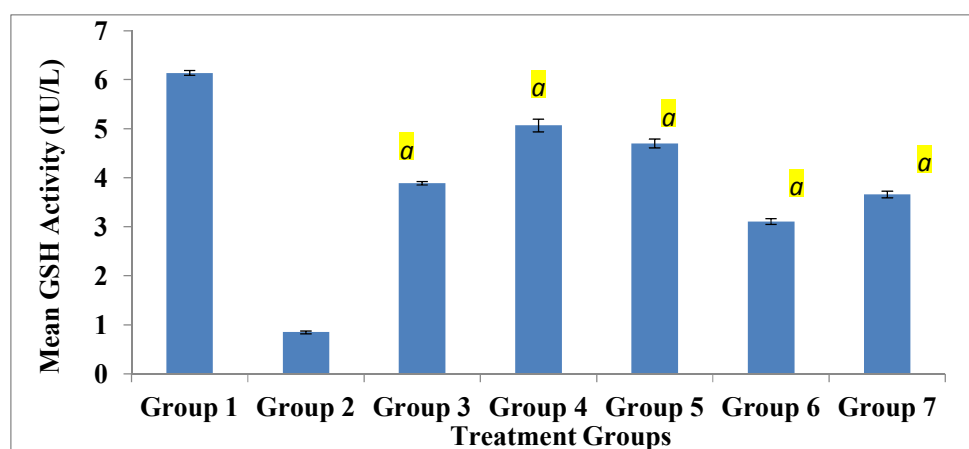
Treatment	Mean O.D at 418nm	%Inhibition of Haemolysis
1 (Isotonic solution)	0.321	-
2 (Hypotonic solution)	0.650	-
3 (Test sample)	0.121	63.2
4 (Test sample)	0.111	66.3
5 (Test sample)	0.100	69.6



**Fig. 4. Effect of methanol Fraction (F<sub>2</sub>) of *A. precatorius* seeds on the catalase activity 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

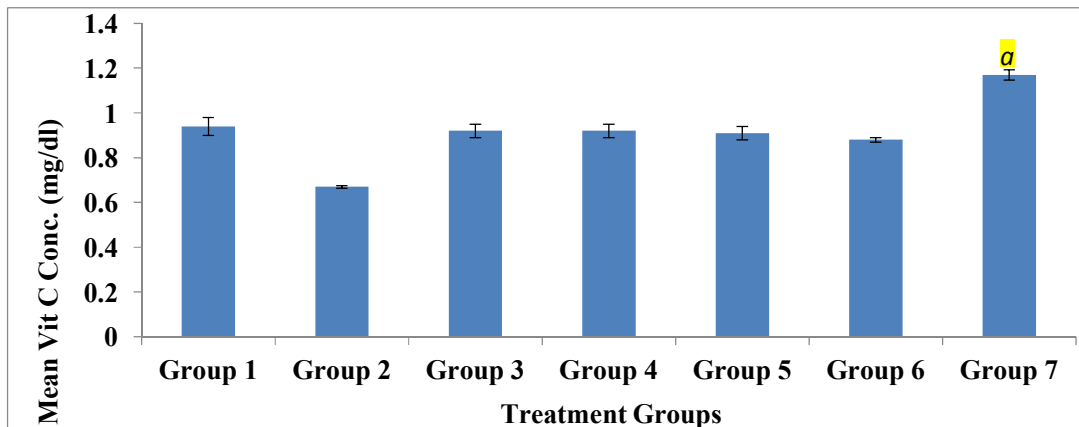
<sup>a</sup>Significant increase with respect to Group 2 (diabetic-untreated rats)



**Fig. 5. Effect of *A. precatorius* fraction (F<sub>2</sub>) on the glutathione levels 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.

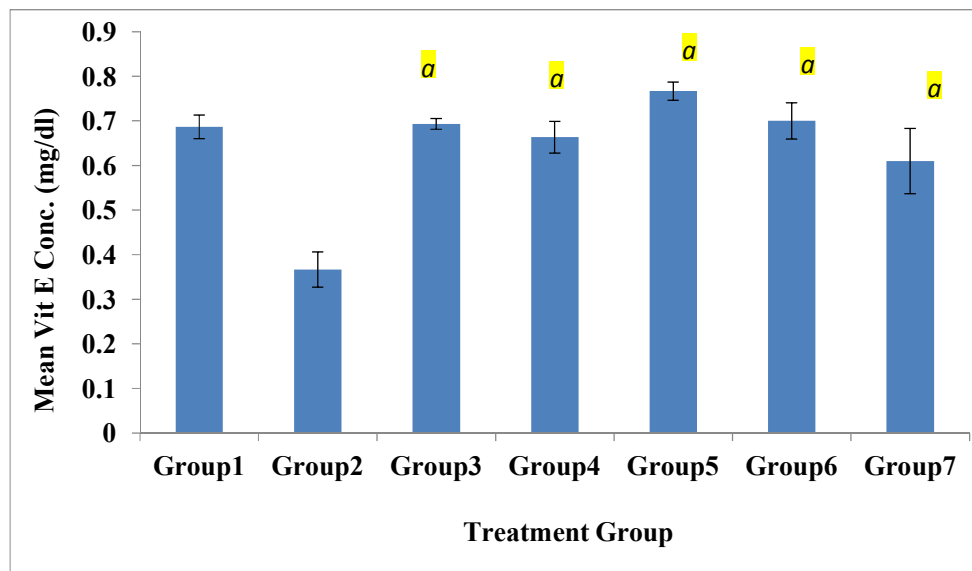
<sup>a</sup>Significant increase with respect to Group 2 (diabetic-untreated rats)



**Fig. 6. Effect of methanol fraction (F<sub>2</sub>) of *A. precatorius* seeds on the vitamin C 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

<sup>a</sup>Significant increase with respect to Group 2 (diabetic-untreated rats)



**Fig. 7. Effect of methanol fraction (F<sub>2</sub>) of *A. precatorius* seeds on the vitamin E 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

<sup>a</sup>Significant increase with respect to Group 2 (diabetic-untreated rats)

#### 4. DISCUSSION

All over the world, this dreadful disorder called diabetes is becoming a serious threat to humans

[42]. On preliminary phytochemical screening, the Sephadex G15 filtered fraction (F<sub>2</sub>) of the methanol extract of the seeds of *A. precatorius* Linn revealed the presence of alkaloids,

flavonoids, tannins, carbohydrates and saponins as major compounds that might have contributed to its anti-diabetic activity.

The quantitative phytochemical composition showing that the  $F_2$  contained high amount of alkaloids, flavonoids, tannins and even low amount of saponins indicates that the  $F_2$  possesses some biologically active compounds which could serve as potential source of drugs. Phytochemicals are said to possess medicinal properties which play important roles in animals [43] and when ingested by animals, it may also exert some biological activities [44]. This might suggest that  $F_2$  may have the ability to scavenge free radicals due to the presence of alkaloids and flavonoids which are the chief sources of antioxidants in plants with a known key function of playing some roles in free radical scavenging ability. Therefore, the antioxidant activities of *A. precatorius* seed extract could be attributed to flavonoid component as observed. The LD<sub>50</sub> value (529.2 mg/kg bw) suggest that the methanol fraction of *A. precatorius* may be toxic although it can be safer for treatment at doses below 400 mg/kg bw.

The fraction ( $F_2$ ) of *A. precatorius* seed was found to exhibit high membrane stabilization against hypotonicity-induced haemolysis of the red cells as shown in the result on Table 4 by the percent inhibition of haemolysis. The high membrane stabilizing activity of  $F_2$  of *A. precatorius* seed observed in this study, may be due to its flavonoids and tannin contents. There is a relationship between the protection against hypotonicity-induced haemolysis to membrane stabilization which is an anti-inflammatory index [45]. This inhibition of haemolysis was found to be dose-dependent, increasing with elevated amount of the  $F_2$  in the medium. The anti-haemolytic properties of  $F_2$  of the methanol extract of the seeds of *A. precatorius* may be due to the presence of some active constituents of the fruit like flavonoid, tannin and Saponins. In experimental animals, studies have shown that flavonoids exert profound stabilizing effects on lysosomes both *in vitro* and *in vivo* [46,47] while tannin and saponins have the ability to bind cations and other biomolecules, and are capable of stabilizing the erythrocyte membrane [48,49].

The mean malondialdehyde (MDA) activity showed significant reduction in the treatment groups compared with the untreated diabetic groups. The decrease in the mean MDA levels of the treated groups could be attributed to the

ability of the seed  $F_2$  to scavenge free radicals generated by the inducing agent (alloxan) thereby preventing lipid peroxidation and sparing the cell. In the pathogenesis of diabetes mellitus, free radicals have been reported as a result of their severe cytotoxic effects, such as lipid peroxidation and protein denaturation of the cell membrane followed by alteration of the membrane receptor and fluidity properties [50].

The mean catalase (CAT) and superoxide dismutase (SOD) activities of the test groups increased significantly compared with the untreated diabetic groups. These increases in both catalase and superoxide dismutase activities of the rats treated with different doses of the seed  $F_2$  could be attributed to the antioxidant defensive mechanisms by some phytochemicals such as alkaloids, flavonoids, tannins and saponins which could prevent oxidative cell damage by scavenging the free radicals in the system. In tissues, CAT and SOD has been known to play a vital role as protective enzyme against free radical formation [51].

The treatment with the fraction ( $F_2$ ) of the methanol extract of the seeds of *A. precatorius* in diabetic rats effectively restored the depleted level of glutathione, a non-enzymatic antioxidant, caused by alloxan induction. This decrease in the glutathione status of the diabetic untreated rat can be attributed primarily to the alloxan (a xenobiotic) that was administered intraperitoneally into the rats. Xenobiotics are known to deplete antioxidants as they are consumed in the course of scavenging reactive oxygen species generated. Also, the decrease in glutathione level could lead to a devastating decrease in the total antioxidant status of the rats because glutathione helps in recycling cellular antioxidants and plays a key role in the detoxification of harmful compounds.

*A. precatorius* fraction ( $F_2$ ) was shown to decrease the concentration of vitamin C in untreated diabetic rats. This decrease in concentration of vitamin C in diabetic condition may be due to augmented utilization as an antioxidant defence system. In streptozotocin-induced diabetes, vitamin C scavenges free oxygen radicals that are toxic by-product of many metabolic process [52,50].

The fraction ( $F_2$ ) of *A. precatorius* also increases vitamin E concentration significantly in the diabetic treated groups compared with the untreated diabetic groups. The  $F_2$  effectively restored the depleted level of this non-enzymatic

antioxidant caused by alloxan induction after day 11. The increase could be as a result of the F<sub>2</sub> having a scavenging role in preventing, inhibiting and delaying reactive oxygen species (ROS) mediated reactions. Vitamin E prevents cell structure from rupture (damage) and also during the process of peroxidation reduces the lipid peroxides generated [53]. The decrease in vitamin E in diabetic untreated rats could be as a result of oxidative stress/injury or increased susceptibility to free radicals mediated lipid peroxidation.

In order to develop and construct knowledge about the antidiabetic effect of F<sub>2</sub> 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 potent in the management and treatment of diabetes. Also, higher doses above 100 and 200mg/kg bodyweight of methanol fraction (F<sub>2</sub>) 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 antidiabetic properties.

## 5. CONCLUSION

The results obtained from the antioxidant activities and hypotonicity-induced hemolysis analysis led to the conclusion that the F<sub>2</sub> (Fraction 2) of the methanol extract of the seeds of *A. precatorius* was able to reduce blood glucose concentrations in alloxan-induced diabetic rats. This supports scientific evidence to the claim that the seeds of *A. precatorius* are used in the management of diabetes. The F<sub>2</sub> can be used also to manage diabetes due to its improvement in antioxidant properties. The antioxidant defensive mechanism of F<sub>2</sub> could be attributed to the increased activities of catalase, superoxide dismutase and decrease in malondialdehyde concentration. The observed antioxidant activities may be due to the presence of alkaloids, flavonoids and tannins which might stimulate cell survival by strengthening of the defence system and restoring the antioxidant capacity.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and

producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## ETHICAL APPROVAL

The experimental protocol was 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. 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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