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Effect of Aqueous Leaf Extract of Adansonia *digitata* (Baobab) on Some Biochemical and Haematological Indices of Alloxan-Induced Diabetic Rats

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Abstract
The study aimed to investigate the effect of aqueous leaf extract of *Adansonia digitata* on some haematological and biochemical indices of alloxan-induced diabetic rats. Twenty-five (25) male albino rats (mean weight; 130±0.1 g) were divided into five (5) groups (n=5). Diabetes was induced in rats with 150 mg/Kg of alloxan (Group 2, 3, 4 and 5). Group 1 was the normal control and received only feed and water. Group 2 was untreated and received feed and water after induction. Group 3 was treated with glibenclamide; Group 4 was treated with 200mg/kg of plant extract, while Group 5 was treated with 400mg/kg of plant extract. Treatment was done for twenty-one (21) days, after which animals were bled and blood samples collected and used for parameters analysis. Results revealed a significant ($p>0.05$) decrease in body weight, Blood Glucose level, WBC, MDA and Total Protein level of group 2 compared with the normal control, while no significant difference was observed in the parameters in group 4 and group 5 as against group 1. The result suggests that the aqueous leaf extract of *A. digitata* was capable of normalizing some haematological and biochemical abnormalities associated with diabetes in rats. It was further suggested that leaf extract of *A. digitata* could be prescribed as an adjunct to dietary therapy and main therapy for diabetes
Keywords: Adansonia digitata; Diabetes; Alloxan,Haematological and Biochemical indices

Introduction

Diabetes mellitus prevalence is increasing worldwide, and it has been projected that over 500 million adults will be affected by diabetes by the year 2030 (Sabi *et al*., 2017). Diabetes is a chronic disease that usually occurs either due to the inability of the pancreas to produce enough insulin or the inability of the body to effectively utilize the insulin it produces. According to the World Health Organization (WHO), diabetes prevalence has been rising more rapidly in low- and middle-income countries than in high-income countries. A report in 2017 by the International Diabetes Federation (IDF) reported that 425 million persons are affected by diabetes mellitus out of which more than 90% are adults and 352 million suffer impaired glucose tolerance (IGT) (IDF, 2017). Diabetes was projected to be a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation in adults. A 3% increase in diabetes mortality rates by age between 2000 and 2019 has been reported, however in 2019, diabetes and kidney disease induced by diabetes caused an estimated 2 million deaths (WHO, 2023). Medically, treatment of diabetes has been through insulin therapy, the use of oral hypoglycemic drugs (OHDs) and lifestyle modification, concerning nutrition and exercise. Considerable side effects associated with the use of insulin and oral hypoglycemic drugs have led to an increase in demand for natural medication with antidiabetic potential as plant-based medicines are perceived to be safer (Aloke, *et al*., 2022). In the quest to fight diabetes, lots of research is ongoing. The use of traditional medicine has also been since pre-historic times. The therapeutic potential of these medicinal plants is due to the presence of some phytochemicals with healing and remedial potentials (Sheraz *et al*., 2018). *Adansonia digitata is* known for its many therapeutic benefits. Its parts(roots, seeds and leaves) have been used over the years as a treatment for various ailments such as diarrhoea, malaria, and bacterial infections (Sharangi, 2009). It also has antioxidant and anti-inflammatory properties. A qualitative analysis by Chukwuma *et al* (2017), showed tha*t A. digitata* contains glycosides, saponins, steroids, and flavonoids. In Northern Nigeria, *A. digitata* has been used over the years to manage diabetes (Muhammed *et al* 2013). According to the statistical data of the World Health Organization, the prevalence of diabetes is relatively higher in low-income and middle-income countries, leading to more deaths due to the inability to

access the already existing orthodox drugs owing to poverty. Also, some of these orthodox drugs have lots of adverse effects associated with them. These problems have aroused the need for constant research to come up with cheaper and more effective drugs, with relatively minimal adverse effects. Because of the therapeutic phytochemicals contained in this plant, this study focuses on establishing its antidiabetic effects and evaluating the level of side effects relative to existing drugs. The work seeks to investigate the effect of aqueous leaf extract of *A. digitata* (baobab) on alloxan-induced diabetes mellitus in adult male albino rats.

Material and Methods*Chemicals*

All reagents used were of analytical grade and were prepared using glass-distilled water.

Plant material

A. digitata leaves were obtained from, Zaria, in Kaduna State Nigeria. Identification was carried out in the Department of Plant Science at Michael Okpara University of Agriculture Umudike.

Preparation of the plant extract

Air drying of leaves was done for 14 days at room temperature. The dry leaves were milled using an electric grinder. Five hundred grams (500 g) of the milled sample was soaked in distilled water for 72 hours on a magnetic starrier. After 72 hours, it was filtered using filter paper (Whatman No.4). The filtrates were concentrated using a Rotary evaporator at 40°C. The percentage yield was 16.5 g.

Furthermore, it was heated in a water bath for 48 hours. It was further subjected to oven drying for 48 hours until the extract was completely free from moisture.

Experimental Design

Diabetes was induced with 150 mg/Kg of alloxan. After three days (72 hours) of induction, it was confirmed with Accu-Check. Machine. Twenty (20) induced rats were chosen for further experimentation. Diabetic rats were grouped into four (4) of 5 rats each excluding the control group which was five rats only. Grouping was as follows:

Group 1;Normal control group received feed + H_2O

Group 2; received 100mg/kg Alloxan + feed + normal saline

Group 3 received100mg/kg Alloxan + Standard drug (Glibenclamide) + feed + H_2O

Group4; received100mg/kg Alloxan + 200mg/kg extract + feed + H_2O

Group 5; received100mg/kg Alloxan + 400mg/kg extract + feed + H_2O

Treatment was for twenty-one (21) days after which blood samples were obtained for use in parameters analysis.

Determination of Parameters

All the chosen parameters were determined using the appropriate bio-diagnostic kits/methods and the manufacturer's instructions were duly followed.

Statistical Analysis

IBM SPSS Statistic 25 Computer package (USA) was used. Data were subjected to a one-way analysis of variance (ANOVA) followed by the Tukey test. Results were expressed as a standard deviation of five determinations (n=5) and were considered statistically significant at $p \leq 0.05$.

Results and Discussion

Diabetes mellitus is a serious metabolic disorder with impaired glucose metabolism. (Mediani *et al*., 2018). The high prevalence of morbidity and mortality in diabetic patients is due to the complications that are associated with the disease. The evidence of this is seen in the derangements in the biochemical and haematological indices as diabetes progresses (Rashid *et al*., 2019). Considerable side effects associated with the use of insulin and oral hypoglycemic drugs have led to an increase in demand for natural medication with antidiabetic potential as plant-based medicines are perceived to be safer (Aloke, *et al*., 2022). The use of traditional medicinal plants is however proving to be a potential remedy to the research of Scientists. Past studies on the phytochemical composition of *A. digitata* (baobab) leaf extracts suggest the presence of glycosides, saponins, steroids, and flavonoids. The presence of these important phytochemicals, including alkaloids in the leaf in previous in-vivo studies (Sa'id *et al*., 2020) suggests its use in traditional medicine.

The present study investigated the biochemical and haematological parameter assessment of alloxan-induced diabetic rats treated with aqueous leaf extract of *A. digitata* (baobab). The parameters; haemoglobin (HB), white blood cell (WBC), red blood cell(RBC), glucose level, body weight, total protein and Malondialdehyde (MDA) of rats represented above suggesting that the plant's leaf extract were effective in normal the level of all the parameters to a concentration near the normal control (group 1). Values differ significantly ($p<0.05$) with the untreated group 2 and comparatively no significant ($p<0.05$) difference with the Diabetic rats group treated with 5mg of glibenclamide (group 3). This result agrees with the existing literature strongly suggesting appreciable medicinal potentials of *baobab* treatment of different ailments (Abiona, *et al*., 2015; De Caluw´e, *et al*., 2010; Kapusta, *et al*., 2018).

Treatment of the animals with baobab leaf extracts presented a dose-dependent significant reduction in blood glucose levels. Table I shows a significant increase ($p<0.05$) in the glucose level of the untreated diabetic rats (group 2) compared to normal control (group 1). There was a significant decrease in the glucose level of diabetic rats treated with 400mg/kg compared with the untreated diabetic rats (group 2) suggesting that the extract possesses a potential antidiabetic effect. The antidiabetic potential is attributed to the phytochemical constituents of the baobab leaf extracts. Abiona, *et al*., (2015) confirmed the positive presence of phytochemical constituents such as polyphenols, flavonoids, terpenoids and other constituents of baobab leaf. However, the actual mechanism by which the plant extract brings about its anti-diabetic effects is yet to be fully elucidated. However, Alloxan induces diabetes in animals such as

rats by destroying the beta cells of the Islet of Langerhans in the pancreas which leads to the reduction in the synthesis and release of insulin, causing the blood glucose to rise. The aqueous leaf extract of baobab may have exerted its action on the beta cells of the Islet of Langerhans in the pancreas of the rats. The secondary metabolites present in the leaf extract of baobab could be responsible for the antidiabetic action. Due to the rich flavonoid and alkaloid contents of baobab, these components may be the main antidiabetic principles of the extract. Emerging experimental evidence suggests that alkaloids and flavonoids possess antioxidative properties that could protect pancreatic islets and help in the regeneration of beta cells leading to an increase in insulin production. In addition, alkaloids and flavonoids exert their effect either by promoting the entry of glucose into cells, thus stimulating glycolytic enzymes or by inhibiting the liver glucose-6-phosphatase, thus causing a reduction in the release of glucose in the blood (Tang *et al*., 2017).

The results from this study (Table II) also indicate a progressive weight loss in the untreated diabetic rats (group 2) when compared with normal control (group 1) as shown in Table 2. Loss of weight could be due to the excessive breakdown of tissue protein and fatty acids occasioned by the observed decrease in plasma insulin concentration resulting in the loss in muscle and adipose tissue. It was suggested that the lack of insulin causes inhibition of protein synthesis and increased degradation which increases amino acid levels in the blood to be subsequently used for gluconeogenesis (Qian *et al*., 2015). The body weights of rats in group 2 decreased significantly ($p<0.05$) when compared to group 1. But when treated with 400mg/dl of the baobab extract, increased significantly when compared to group 2 Nondiabetic animals. A similar loss in weight has been observed in STZ-diabetic rats by Mestry, *et al*., (2017). The results from this study suggest that the extract is effective in preventing muscle wasting and protein turnover.

Table III shows the haematological indices of alloxan-induced diabetes in rats administered aqueous baobab leaf extract. Red blood count (RBC) and Hemoglobin (HB) of the diabetic benchmark (group 2) contrasted insignificantly with the normal control (group 1) in reduction proportion. In the same way, HB and RBC showed an insignificant increase in all test groups ($p<0.05$) compared to group 2 (untreated group). Alteration of the immune system and various haematological parameters during diabetes has been reported (Mansi and Lahham, 2008). It has also been reported that ingestion of medicinal plants or drugs can as well alter the normal haematological indices (Ajagbonna *et al*., 1999). Of interest is the insignificant reduction in HB of group 2 (untreated diabetic group) due to a reduced red blood cell count in which there might be a decreased haemoglobin concentration or hypothermia. As seen in Table III of the haematological parameters of this study, a direct relationship exists between RBC and HB where an increased RBC is usually proportional to an increase in haemoglobin concentration(and vice versa) (Essiet*et al*., 2003)

The present study also significantly shows an increase in the WBC of group 2 compared with group 1. However, there was a significant reduction in WBC of groups 4 and 5 upon treatment with the leaf extract when compared with group 2. Increased WBC count is associated with type 2 diabetes mellitus as well as other diseases and is also a marker of inflammation (Asgary *et al*., 2005). The significant ($P<0.05$) rise in WBC count of the diabetic control rats compared to normal controls is in line with earlier reports by Demirtas *et al*., (2015). The reduction in WBC count upon treatment of diabetic rats with *baobab leaf* extract could be attributed to the anti-inflammatory action (Baba *et al*., 2018), antioxidant effect (Atiko *et al*., 2016) and free radical scavenging activity (Essiet*et al*., 2020) in baobab.

It is a well-established and evidence-based scientific fact that plasma protein levels could suffer changes due to disease (Suzuki, 2006). In the current study plasma total protein concentration was found markedly reduced in the untreated diabetic groups when compared with the normal control group (Table III). The changes in the concentration of plasma total protein could be due to changes in the rate of their catabolism, changes in the rate of their anabolism or the volume of distribution (Marshall, *et al*., 2004). Each protein has a characteristic half-life in the circulation for instance, in normal healthy adults the half-life of albumin is approximately 20 days and in certain diseases, the half-life of the protein could be markedly altered (Murry, 2012). This can be added to the cause of the observed decreased protein concentration.

Malondialdehyde (MDA, the end product of lipid peroxidation is one of the most reliable indexes of oxidative stress. The level of MDA increased in diabetic rats (table III) compared to the normal control which indicated an overproduction of free radicals. The significantly decreased MDA level ($p<0.05$) in the group treated with 400mg/kg extracts as compared with the group that received 200mg/kg of the extract indicates that the extract has antioxidant properties and this could be one of the mechanisms by which the extract alleviate the toxic effect of diabetes in the animals in group 4 and 5. The antioxidant effect of A. *digitata has* been attributed to the presence of phytochemicals such as flavonoids known for their chelating properties and protection against free radical attacks. The leaf extract of Adansonia *digitata* has been reported to possess ten times more potent anti-oxidative capacity than vitamin C (Ayele *et al*., 2013).

Conclusion

The findings of the present study suggest that the administration of dose-dependent of A. *digitata* leaf extract has antidiabetic, and antioxidant properties. The antioxidant properties of the plant are a result of the abundant flavonoid and alkaloid contents and this could be responsible for its antidiabetic effect. Finally, the leaf

extract of A. *digitata* is safe and potent to normalize the haematological abnormalities associated with diabetes mellitus and, therefore, could be prescribed as an adjunct to dietary therapy for the management of diabetes.

Ethics statements

All animal experiments were approved by the Ethics Committee of Michael Okpara University of Agriculture, Umudike, Nigeria and carried out by the National Institutes of Health guide for the care and use of Laboratory animals.

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Declarations of Competing Interest

There are no conflicts of interest to declare.

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Table I: Mean Glucose Levels of Normal and Test Groups

Groups (g)	Glucose Concentration (mg/dl)
Group 1	82.4000±7.26636
Group 2	115.2000±5.93296*
Group 3	80.4000±8.23408
Group 4	82.6000±8.01873
Group 5	84.6000±6.98570

Values are Mean ± Standard Deviation (SD) of five determinations. Value with asterisks (*) represents a significant increase difference from the control (P<0.05)

Table II: Mean Body Weights of Normal and Test Groups

GROUPS	Body Weight(g)
Group 1	176.0000±10.27132
Group 2	108.4000±5.98331*
Group 3	156.4000±7.76531
Group 4	158.6000±4.72229
Group 5	130.8000±23.28519*

Values are Mean ± SD of five determinations. Values with asterisks (*) are significantly decreased (P<0.05). from the control

Table III: Biochemical and Haematological Indices of Normal and Test

Group /Treatment	HB(g/dl)	RBC (x1012/L)	WBC (x109/L)	MDA(mg/ml)	Total protein (g/dl)
Group1	14.7400±2.230	5.1600±.4159	7.8800±.2280	3.0700±.5054	4.0200±.3271
Group2	12.5000±.5050	5.0600±.3362	9.0000±.6782*	4.9920±.7034*	2.9400±.2881*
Group3	12.8600±1.6502	4.9600±.2967	8.2400±.8295	2.0880±1.1789	3.6400±.8019
Group4	12.6000±1.3528	4.7600±.16733	8.2000±.4242	2.3120±1.0144	4.1000±.5050*
Group5	12.9200±.7823	5.0800±.0837	7.9600±.6066	2.3700±.9960	4.2600±.4561*
P-Value	0.128	0.255	0.053	0.000	0.004

Values are Mean ± Standard Deviation of five determinations. Values with asterisks (*) are statistically significant (P<0.05) in their differences from the control