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Acute Toxicity and Hypoglycemic Activity of Aqueous Fruit Pulp Extract of *Adansonia digitata* L. (Afpead) on Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IUM, AJA and AMW designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors IKJ and MAD managed the animals, collected all data and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Oral LD_{50} and hypoglycemic activity of aqueous fruit pulp extract of *Adansonia digitata* L. (AFPEAD) were investigated in this research. A total of fourty eight (48) rats were used, twelve (12) of the rats were used for oral LD_{50} determination, and were grouped into four (4) groups of three rats (3) each. The first three groups were administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of the extract respectively, while the last group was subdivided into three groups of one rat each and were administered with 1600 mg/kg, 2900 mg/kg and 5000 mg/kg body weight of the extract respectively. Thirty (36) rats were used for the diabetic study and were grouped into six (6) groups of six (6) rats each. Group I served as normal control, group II served as diabetic control while Groups III, IV, V and VI were induced with diabetes and administered with AFPEAD at a dose

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of 1.00 g/kg, 2.00 g/kg and 3.00 g/kg body weight and standard drug (Chlorpropamide, 100 mg/kg) respectively for two weeks. The research found the oral LD_{50} of the extract to be greater than 5000mg/kg indicating that the extract was practically non-toxic and administration of the extract to test groups shows a significant ($p<0.05$) decrease in blood glucose level when compared to diabetic control after two weeks treatment with the extract. Thus indicating a hypoglycemic activity by the extract which might be due to the presence of various phytochemicals.

Keywords: Adansonia digitata; acute toxicity; alloxan; hypoglycemic activity.

1. INTRODUCTION

The plant kingdom (flora) is a rich reservoir of plant resources with variable nutritional and medicinal properties for the treatment of various diseases. Throughout the ages, herbal products are being used in the treatment of communicable and non-communicable diseases. The available literature shows that there are more than 800 plant species showing activities against many non-communicable diseases including coronary heart disease, diabetes mellitus, cancer, and hypertension [1]. The world health organization has also recommended the evaluation of the effectiveness and safety of plants used in traditional and complementary medicines [2].

Adansonia digitata L. commonly known as the baobab, belongs to the family Malvaceae and is the most widespread of the species on the African continent, found mostly in hot, dry Savannah of sub-Saharan Africa [3]. The tree has been used extensively since ancient times in traditional medicine. Several plant parts have interesting anti-oxidant, anti-microbial, anti-malarial and anti-inflammatory properties [4]. The fruit pulp is dissolved in water or milk and then used as a drink, a sauce, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking. The fruit pulp has very high vitamin C content and is a rich source of calcium [5]. The United States Food and Drug Administration generally recommended the safety of baobab dried fruit pulp as a food ingredient in 2009 [6].

Diabetes mellitus (DM), simply called diabetes, is a group of metabolic diseases in which there is persistently high glucose level in the blood over a prolonged period [7]. Diabetes is due to either pancreatic β cells not producing enough insulin, or the cells of the body are not responding properly to the insulin produced. Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscles, and adipose tissues. Therefore, deficiency of insulin and/or the insensitivity of its receptors play a central role in all forms of diabetes mellitus [8].

The classic symptoms of untreated diabetes are weight loss, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger). Other symptoms include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin [9]. Prevention and treatment involves a healthy diet, regular physical exercise, not using tobacco and maintaining a normal body weight. Blood pressure control and proper foot care are also important for people with the disease.

WHO [10] report revealed that over 1.71 million Nigerians are living with DM and this figure is projected to reach 4.84 million by 2030. The economic cost of diabetes globally was estimated in 2013 at $548 billion. *Adansonia digitata* L. fruit pulp is well known and used traditionally in northern Nigeria as anti-diabetic agent. As such, identification and utilization of anti-diabetic properties of this plant will be a way forward to confront challenges encountered by people suffering from diabetes.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Study animals

Male and female albino rats weighing between 100 g to 120 g were purchased from animal house of Biological Science Department; Bayero University, Kano. The animals were housed in well-ventilated cages in the animal house of Biological Science Department of Bayero University Kano. The rats were allowed to acclimatize for one week prior to the experiment and had access to food and clean water. Principle of laboratory animal care [11] and ethical guidelines for investigation of experimental pain in conscious animals [12] were observed during experimentation.

2.1.2 Plant material

The fruits pulp of *A. digitata* was collected in Gosa community in Abuja Municipal Area Council and Gwargwada community in Kuje Area Council of the Federal Capital Territory of Federal Republic of Nigeria (Abuja). The plant was identified and authenticated at the herbarium of the Department of Plant Biology, Bayero University Kano by a plant taxonomist, Bala'uddeen Said Adam on 31st October, 2014 with Accession Number BUK/HAN/0036.

2.2 Methods

2.2.1 Preparation and administration of afpead to animals

The collected fruit pulp was finely ground into powder form by using an iron mortar and pestle. The seeds were separated from the pulp using a mesh. The fineness of the powdered pulp was achieved by sieving through a clean white muslin cloth allowing the fibres to be completely removed. The powder was stored in a clean air-tight plastic container at room temperature until use. Twenty (20) gram of the powder was accurately weighed and dissolve in 100 ml of distilled water to prepare a concentration of 200 mg/ml of AFPEAD for administration to experimental animals.

The volume of the extract for administration into the laboratory rats was determined based on the weight of the animals by the following relationship.

$$\text{Volume to be administered (ml)} = \frac{\text{weight of rats (kg)} \times \text{dose (mg/kg)}}{\text{concentration of the extract (mg/ml)}}$$

2.3 Preparation of Alloxan

One (1) gram of Alloxan Hydrate was dissolved in 20 ml of distilled water and swirled to mix to give a concentration of 50 mg/ml

2.4 Induction of Diabetes Mellitus with Alloxan

Rat to be induce with Diabetes mellitus were fasted overnight for a period of 12 hours, diabetes was induced by injecting alloxan hydrate intraperitoneally at dose of 120 mg/kg using a sterile 1ml syringe. The volume of the solution containing 120 mg/kg given to each experimental albino rat was determined by the following relationship:

$$\text{Volume to be administered (ml)} = \frac{\text{weight of rats (kg)} \times \text{dose (mg/kg)}}{\text{concentration of the extract (mg/ml)}}$$

2.5 Experimental Protocol

2.5.1 Acute toxicity

The LD_{50} was determined by the method [13].

In the initial phase, nine (9) rats were divided into three (3) groups. Each group contained three (3) rats. The rats in group I, II and III were administered orally with 10, 100, and 1000 mg/Kg of AFPEAD respectively via cannula and were monitored for mortality and general behavior for twenty (24) hours.

In the last phase, three rats were grouped into three containing one rat each. The rats were administered with 1600 mg/kg, 2900 mg/kg and 5000 mg/kg doses of AFPEAD respectively. The rats were monitored for signs of toxicity which include: paw licking, salivation, rubbing of nose on floor, loss of appetite, change in body weight and death within 24 hrs.

2.5.2 Hypoglycemic activity

To investigate of the effect of oral administration of aqueous fruit pulp extract of *A. digitata* on blood glucose concentration in diabetic rats, thirty six (36) rats were grouped into six (6) of six (6) rats each. In this research work, large dosage of the extract were adopted in order to mimic the human habitual dosage.

Group I: normal control
Group II: diabetic control
Group III: diabetic rats, administered with 1.00 g/kg of AFPEAD daily for two weeks
Group IV: diabetic rats, administered with 2.00 g/kg of AFPEAD daily for two weeks
Group V: diabetic rats, administered with 3.00 g/kg of AFPEAD daily for two weeks
Group VI: diabetic rats, administered with conventional anti-diabetic drug (Chlorpropamide, 100 mg/kg body weight) daily for two weeks.

Fasting blood glucose concentrations of rats was determined at the end of first and second week of extract administration.

2.6 Statistical Analysis

Results were expressed as mean ± standard deviation and analysed using ANOVA, with p value <0.05 considered significant, using of GraphPad Instat3 Software [14].

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the corresponding results of phase 1 and phase 2 of oral LD_{50} determination. No mortality in animals at all doses of the extracts up to 5000 mg/kg was observed. Table 2 shows the effect of oral administration of AFPEAD on fasting blood glucose concentrations of diabetic rats at the interval of seven (7) and fourteen (14) days of oral treatment. At 48^{th} hours after alloxan injection, the blood glucose levels of group II (diabetic control group) and test groups (groups III – VI) increase significantly ($p<0.05$) compared to normal control (group I), thus confirming induction of diabetes in the diabetic control group and test groups. No significant difference was observed in fasting blood glucose of diabetic rats following seven days of oral administration of AFPEAD. However, blood glucose levels of all test groups decreases significantly ($p<0.05$) compared to diabetic control group following fourteen days of extract administration (group III-V) and treatment with standard drug (group VI).

3.2 Discussion

Acute toxicity is an adverse or undesirable effect that is manifested within a relatively short time interval ranging from almost immediately to within few days following exposure to a foreign compound [15]. In the safety evaluation of compounds, acute toxicity tests are those that evaluate effects occurring within about 24-48 hours of a single dose, usually quantified using the LD_{50} concept. The study established the oral LD_{50} (Table 1) of AFPEAD to be greater than 5000 mg/kg body weight which is interpreted as practically nontoxic according to scale of classification [16]. The finding is in accordance with the finding of [17] who reported the LD_{50} of aqueous extract of *Terminalia avicennioides* to be above 5000 mg/kg. The low toxicity obtained may have been responsible for its widespread use by humans as nutritional source and as a remedy for different diseases, as popularly speculated or proclaimed in Northern parts of Nigeria where the plant is abundantly distributed and utilized.

Table 1. Acute toxicity studies of aqueous fruit pulp extract of *A. digitata* administered orally to albino rats

Experiment	Dose of AFPEAD (mg/kg)	Number of rats/group	Mortality
Phase I	10	3	0/3
	100	3	0/3
	1000	3	0/3
Phase II	1600	1	0/1
	2900	1	0/1
	5000	1	0/1

The pancreas is an endocrine organ in vertebrates containing α-cells, β-cells, δ-cells and γ-cells; secreting glucagon, insulin, somatostatin and peptide proteins respectively. It also function as an exocrine organ producing α-amylase, lipases, peptidases and ribonuclease which catalyze the hydrolysis of starch, fats, peptides and ribonucleic acids in the duodenum. Insulin action in the uptake of glucose by the cells is a critical step in glucose homeostasis and in clearing the postprandial glucose load. Insulin stimulates the uptake of glucose into cells of the liver, skeletal muscle and adipose tissue [18]. Destruction or dysfunction of this glandular organ that inevitably play vital role in energy metabolism profound great effect on the organism, the primary metabolic outcome is hyperglycaemia, which translates to diabetes when it remains persistent [19]. Alloxan is a toxic glucose analogue which selectively destroy β-cells of pancreas when administered to animals. This causes an insulin dependent diabetes mellitus known as alloxan-induced diabetes in the animals, which is characteristically similar to Type 1 diabetes in humans. Alloxan is selectively toxic to insulin producing pancreatic β-cells because it preferentially accumulates in β-cells through uptake via glucose transporter-2 (GLUT2) [20]. For all animals a single dose of alloxan, 140 – 180 mg/kg is administered [21]. In this study, a milder approach to induce diabetes was adopted using a dose of 120 mg/kg body weight of alloxan at a concentration of 50 mg/ml was used to achieve induction of diabetes to rats. Also, a rest period of 48 hours was allowed before the commencement of extract administration. The result showed slow but steady induction of diabetes within 48 hours, this might be as a result of low dose and concentration of alloxan used. These finding is in accordance with the research of [22] who

Table 2. Blood glucose level (mg/dl) of rats before and after alloxan induction, and at seven and fourteen days following treatment with AFPEAD and chloropropamide

Groups	No ALX	ALX 2 Hrs	48 Hrs	Day 7	Day 14
G I	97.83±1.94	92.50±6.38[a,b]	92.00±2.43[a,b,c,d,e]	96.33±3.56	97.33±1.97
G II	92.33±9.14	101.67±6.15	125.67±2.07[a]	138.67±3.51	151.17±2.32[a,b,c,d]
G III	91.50±3.99	101.17±3.31	128.33±1.75[b]	106.67±6.02	93.17±3.55[a]
G IV	94.67±4.13	112.67±4.13[a]	129.50±2.59[c]	105.33±4.55	92.33±2.73[b]
G V	83.67±2.23	104.67±7.67	127.16±1.17[d]	106.33±3.14	92.83±5.46[c]
G VI	97.33±3.82	116.83±2.04[b]	128.83±1.94[e]	104.83±2.86	93.17±2.04[d]

Results are expressed as mean ± SD (n=6). Values in the same column bearing similar superscripts are significantly different at P<0.05

reported the induction of diabetes using 120 mg of alloxan but commenced treatment after five days diabetes induction.

Administration of the extract for one week shows a steady decrease in blood glucose of both test groups (Group III- V) and Chlorpropamide treated group (group VI), but it was unable to reverse the marked hyperglycemia in diabetic test rats. Considerable normalcy in glucose levels became obvious after two weeks of extract administration in both test groups and standard drug treated group. However, the decrease in glucose levels in test groups was not in a dose dependent pattern implying that AFPEAD at doses 1.00 g/kg, 2.00 g/kg and 3.00 g/kg is equally efficacious in diabetic control following two weeks of extract administration. This is in accordance with the findings of [23] who reported "that the methanolic fruit pulp extract of *Adansonia digitata* possess antidiabetic effect on alloxan induced diabetic rats". The significant decrease ($p<0.05$) in the level of blood glucose in the group treated with Chlorpropamide when compared with the diabetic control rats may be attributed to its ability in lowering blood glucose or retarding the necrosis caused by alloxan [24].

The hypoglycemic ability exhibited by the extract may not be unconnected with the presence of phytochemicals. It was reported that *Adansonnia digitata* pulp is rich in flavanoids, vitamin C and Procyanidins [25] that serves as antioxidants which scavenge free radical species generated by alloxan, thus preventing the destruction of pancreas beta cells and to maintain physiological functions of body organs [26]. It may also acts by stimulating insulin release from beta cells; this is because the diabetic produced from this instance was moderate rather than severe.

4. CONCLUSION

The research concludes; firstly, aqueous fruit pulp extract of *A. digitata* is practically nontoxic with an LD_{50} greater than 5000 mg/kg body weight via oral administration route. Secondly, the extract was found to possess some hypoglycemic activity because it significantly lowers blood glucose, thus supporting the traditional claim.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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