Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.89, 2021

Acute Toxicity and Antidiabetic Studies of Adansonia digitata Stem Bark Extracts on Streptozotocin Induced Diabetic Rats

Abubakar Bilyamini Maazu[1]* Muhammad Ibrahim Usman[1] Madu Adamu Gadaka[2]
1.Department of Biochemistry, Faculty of Science, Yobe State University, Damaturu, Nigeria
2.Department of Biochemistry, Faculty of Science, University of Maiduguri, Nigeria

Abstract
The study investigates acute toxicity and anti-diabetic potential of solvents extract (n-hexane, Chloroform, Ethyl acetate and Methanol) of *Adansonia digitata* stem bark. For each solvent, twelve (12) 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 2500mg/kg, 3500mg/kg and 5000mg/kg body weight of the extract respectively. Thirty (35) rats were used for the diabetic study and were grouped into seven (7) groups of six (5) rats each. Group I served as normal control, group II served as diabetic control while Groups III, IV, V, VI and VII were induced with diabetes and administered with standard drug, n-hexane, Chloroform, Ethyl acetate and Methanol extract respectively for two weeks. The research found the oral LD_{50} of all the extract to be greater than 5000mg/kg indicating that the extract was practically non-toxic. Administration of the extract to test groups shows a significant ($p<0.05$) decrease in blood glucose level in chloroform, ethyl acetate and methanol extract administered groups compared to diabetic control with ethyl acetate extract having the highest activity. It also ameliorate liver toxicity by decreasing the serum level of liver enzymes. Thus indicating a hypoglycemic activity by the extract,
Keywords: Acute toxicity; *Adansonia digitata*; Antidiabetic; Solvents Extract and Streptozotocin.
DOI: 10.7176/ALST/89-04
Publication date: August 31st 2021

1. INTRODUCTION

Diabetes mellitus, a chronic non-communicable disease is ranked 7th killer disease in the world with an estimated of over 420 million people affected as reported by. The report also revealed that 1.81 million Nigerians are living with Diabetes mellitus and this figure is projected to reach 4.84 million by 2030 with the current prevalence rate estimate of 2.5%. Diabetes is due to either the pancreas is 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 or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus [1].

Type 1 DM results from the body's failure to produce enough insulin. Also referred to as insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes. It is a chronic autoimmune disorder in which the immune system attacks the insulin-secreting cells in the pancreas resulting in massive death of β-cells. Type 1 diabetics have to be on daily insulin therapy for life. Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. Also known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes [2]. The primary cause is excessive body weight and inadequate exercise. Gestational diabetes is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level. Symptoms of untreated diabetes includes weight loss, frequent urination, increased thirst, increased hunger, blurry vision, headache, fatigue, slow healing of cuts, and itchy skin [3].

Adansonia digitata; baobab; Monkey-bread tree (called kukah by many ethnic groups in Nigeria), belongs to the family Malvaceae and is the most widespread of the Adansonia species on the African continent, found in hot, dry savannah of sub-Saharan Africa and semi-arid regions of the world [4]. Regarded as the largest plant in the world. They usually grow as solitary individuals, and distinctive trees. The specie is slow growing, and blossoms on well-drained soil, fairly intolerant to frost and waterlogged conditions, the main stem of larger baobab trees may reach enormous proportions of up to 28 m in girth. Although baobab trees seldom exceed a height of 25m. The stem is covered with a bark layer, which may be 50-100 mm thick. The bark is grayish brown and normally smooth and succulent but can often be variously folded and seamed from years of growth. The leaves are hand-sized and divided into 5-7 finger-like leaflets. Being deciduous, the leaves are shed during the winter months and grow again in late spring or early summer [4].

With the current increase in prevalence rate of diabetes mellitus, research for alternative drugs of higher efficacy, safety and cost effectiveness to replace and/or support the currently used drugs is necessary. Various researches have reported Adansonia digitata pulp to possess anti-hyperglycemic activity [5]. However there are

little studies or no study on the anti-hyperglycemic activity of the stem bark as well as isolation and identification of the bioactive ingredient(s) responsible for the medicinal properties. This study therefore will investigates the antidiabetic potential of Stem bark extract of the plant against STZ induced diabetic rats.

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 *ad libitum*. Principle of laboratory animal care [6] and ethical guidelines for investigation of experimental pain in conscious animals [7] were observed during experimentation.

2.1.2 Collection and extraction of the plant material

Adansonia digitata stem barks was collected from Yobe State University, Damaturu. The plant was identified and authenticated by a taxonomist at the Plant Biology Department, Bayero University, Kano with Accession Number BUK/HAN/0036. The sample was shade dried and ground to powder.

Five hundred grams of the powder were weighed and soaked in two liters of different solvents of increasing polarity viz-a-viz hexane, chloroform, ethyl acetate and methanol. The solution was shaken vigorously and left to stand at room temperature for 24 hours for each solvent and filtered by passing through Whattman's Filter No.1. The filtrate thus obtained was concentrated by complete evaporation of the solvent to yield solvents free extracts labelled **E1, E2, E3** and **E4** for Hexane, Chloroform, Ethyl acetate and Methanol extracts respectively.

The powder was stored in a clean airtight plastic container at room temperature until use. Twenty (10) gram of the powder was accurately weighed and dissolve in 100 ml of DMSO to prepare a concentration of 100 mg/ml. The volume of the extract for administration into the laboratory rats was determined based on the weight of the animals by the following relationship.

$$Volume\ (ml) = \frac{Dose\ (mg/kg) \times weight\ of\ rat\ (kg)}{Concentration\ of\ extract\ (mg/ml)}$$

Induction of diabetes

Diabetes mellitus was induced according to the method of Muhammad *et al* by injecting STZ hydrate intraperitoneally at dose of 40 mg/kg using a sterile 1ml syringe. The volume of the solution containing appropriate volume to be given to each experimental albino rat will be determined by the following relationship [8].

$$Volume\ (ml) = \frac{Dose\ (mg/kg) \times weight\ of\ rat\ (kg)}{Concentration\ of\ alloxan\ (mg/ml)}$$

After 48 hours window period, rats with fasting glucose levels of > 200mg/dL will be considered to be diabetic.

Acute toxicity

LD_{50} of the extracts (n-hexane, chloroform, Ethyl acetate and methanol) was determined using the method of Lorke [9]. For each fraction, nine rats were divided into three (3) groups of 3 rats each. The rats were orally administered with 10, 100, 1000mg/Kg of the respective fraction. The rats were monitored for general behavior and mortality for 24 hours.

In the absence of any toxicity, three rats were grouped into three groups of one rat each, and were orally administered with 2500mg/kg, 3500mg/kg and 5000mg/kg body weight of the respective fraction. They were observed for signs of toxicity which include: paw licking, salvation, rubbing of nose on floor, change in body weight and death within 24 hours. The number of death in each group within 24 hours was recorded and LD_{50} was calculated from the relation

$LD_{50} = \sqrt{}$min conc. full death × max conc. no death

Screening of extracts for hypoglycaemic activities

Thirty five rats were grouped into seven groups of five rats each. Extracts were administered for a period of two weeks at a dose less than one tenth of the raised LD_{50} according to Sadish *et al* [10].

Group I: Normal control: non-diabetic, No extract was administered
Group II: diabetic control: diabetic, No extract was administered
Group III: standard drug (Metformin, 100mg/kg bodyweight)
Group IV: diabetic, administered with 250mg/kg body weight of n-hexane extract (E2)
Group V: diabetic, administered with 250mg/kg body weight of chloroform extract (E3)
Group VI: diabetic, administered with 250mg/kg body weight of ethyl acetate extract (E4)
Group VII: diabetic, administered with 250mg/kg body weight of methanol extract (E5)

After two weeks of extract administration, the rats were euthanized and blood samples was collected for analyses liver and kidney functions.

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 [11].

3. RESULTS AND DISCUSSION

3.1 Results

The result for LD_{50} determination is presented in table 1. In the initial phase of no mortality and toxic symptoms were observed in all the extract. Although some rats exhibited symptoms of weakness in the second phase, no mortality was observed (Table 1.1b)

Table 1.1a: phase I of LD_{50} determination

Doses (mg/kg)	n-Hexane fraction	Chloroform	Ethyl acetate	Methanol
10	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3

Table 1.1b: Phase II of LD_{50} determination

Doses (mg/kg)	n-Hexane fraction	Chloroform	Ethyl acetate	Methanol
2500	0/1	0/1	0/1	0/1
3500	0/1	0/1	0/1	0/1
5000	0/1	0/1	0/1	0/1

The effect of solvents extract on fasting blood glucose concentrations in diabetic rats was shown in table 2. The blood glucose levels of group II (diabetic control group) and test groups (groups III –VII) increase significantly ($p<0.05$) compared to normal control (group I) after STZ administration. Thus, confirming induction of diabetes in the diabetic control group and test groups. Following extracts administration, a significant ($p<0.05$) fall in fasting blood glucose was observed in all group except for group IV that were administered with hexane extract. Group VI (i.e Ethyl acetate extract) was shown to possess the highest anti hyperglycemic activity compared to the remaining extract.

Table 2: Fasting blood glucose of rats administered with solvents extracts of *Adansonia digitata* for two weeks measured at an interval of 3 days

Groups	Before STZ	48 hours after STZ	Day 3	Day 6	Day 9	Day 12	Day 15
G I	83.60 ± 2.60	84.23 ± 4.09	83.34 ± 2.44	86.00 ± 2.68	85.33 ± 2.51	85.55 ± 1.51	87.23 ± 2.51
G II	98.33 ± 13.56[a]	183.33 ± 13.96[a]	214.56 ± 11.53	285.00 ± 10.17	287.34 ± 12.32	330.45 ± 12.36	323.6 ± 20.66
G III	89.44 ± 12.67[a]	214.10 ± 12.58[a,b,c,d]	160.66 ± 13.95	146.67 ± 2.30	120.56 ± 11.91[b]	116.70 ± 13.24[c]	102.7 ± 11.45[d]
G IV	88.42 ± 13.56[a]	187.56 ± 11.28[a]	250.56 ± 13.51	276.45 ± 11.38	253.00 ± 12.60	232.50 ± 13.30	200.23 ± 11.01
G V	96.43 ± 11.90[a]	183.44 ± 11.13[a,b]	228.88 ± 12.75	176.34 ± 12.63	157.75 ± 12.54	139.99 ± 12.01[b]	135.45 ± 12.49[c]
G VI	87.44 ± 10.50[a]	200.46 ± 12.01[a,b,c,d,e]	146.10 ± 10.30	135.00 ± 12.49[b]	126.56 ± 11.96[c]	104.64 ± 12.70[d]	92.45 ± 11.58[e]
G VII	102.34 ± 6.50[a]	204.77 ± 10.58[a,b,c,d,e]	178.34 ± 11.23	157.56 ± 12.54[b]	146.67 ± 13.95[c]	120.45 ± 11.91[d]	116.66 ± 13.24[e]

Results are expressed as mean ±SD, n=5. Values in the same row bearing the same superscript are significantly different at $P<0.05$.

Table 3 presents Liver function indices (AST, ALT, ALP, DB, TB, TP and ALB) of rats administered with solvents extracts. There was a significant ($p<0.05$) increase in serum AST, ALT, ALP in diabetic control group

and hexane extract administered groups compared to the normal control. A decrease ($p<0.05$) in serum AST, ALT, ALP level was observed in standard drug and ethyl acetate administered groups compared with diabetic control group.

Table 3: Liver function indices of rats administered with Solvent extracts of *Adansonia digitata* for two weeks

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TBIL (mg/dl)	DBIL (mg/dl)	ALB (g/dl)	TP (g/dl)
GI	24.34 ± 1.41[a]	19.23 ± 1.78[a]	106.00 ± 3.28[a]	0.35 ± 0.04	0.30 ± 0.10	3.78 ± 0.29	4.47 ± 0.51
GII	45.22 ± 3.67[a,b,c,d]	59.44 ± 1.78[a,b,c,d]	188.12 ± 5.28[a,,b,c,d]	0.32 ± 0.68	0.30 ± 0.09	3.84 ± 0.35	5.22 ± 0.47
GIII	26.65 ± 4.51[b]	20.00 ± 3.67[b]	129.05 ± 7.09[b]	0.34 ± 0.05	0.24 ± 0.07	3.02 ± 0.27	4.58 ± 0.38
GIV	46.00 ± 5.51	41.23 ± 6.67	145.67 ± 1.09	0.42 ± 0.07	0.34 ± 0.09	3.10 ± 0.27	3.86 ± 0.26
GV	41.23 ± 4.52[c]	37.34 ± 2.51[c]	127.12 ± 4.50[c]	0.31 ± 0.10	0.46 ± 0.15	3.88 ± 0.25	3.70 ± 0.40
GVI	28.14 ± 2.40[d]	19.33 ± 4.78[d]	116.88 ± 5.28[d]	0.33 ± 0.08	0.34 ± 0.05	3.02 ± 0.36	4.02 ± 0.58
GVII	31.33 ± 3.52	27.42 ± 3.51[e]	122.10 ± 9.30[e]	0.31 ± 0.11	0.40 ± 0.23	3.01 ± 0.65	3.80 ± 0.30

Values in the same column bearing the same superscript are significantly different at $P<0.05$.
Results are expressed as mean ±SD, n=5
Key: AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP= Alkaline phosphatase, TBIL= Total bilirubin, DBIL= Direct bilirubin, ALB= Albumin, TP= Total protein.

Kidney function indices of diabetic rats administered with solvents extract was presented in table 4. No significant changes was observed in all parameters between groups.

Table 4: Kidney function indices of diabetic rats administered with solvents extract of *Adansonia digitata*

Group	Na^+ (mMol/L)	K^+ (mMol/L)	HCO_3^- (mMol/L)	Cl^-(mmol/L)	Urea (mMol/L)	Creatinine (mMol/L)
GI	144.20±0.83	5.72±0.25	25.80±1.09	96.00±2.00	17.00±1.22	0.34±0.08
GII	144.00±0.70	5.92±0.27	26.00±0.07	96.80±1.41	17.45±0.83	0.36±0.08
GIII	145.20±1.64	6.18±0.23	25.20±1.30	98.00±1.41	17.40±0.54	0.40±0.10
GIV	145.80±1.09	5.96±0.15	26.00±1.00	98.80±1.09	18.54±0.54	0.42±0.10
GV	142.00±0.57	6.73±0.37	26.33±1.15	98.66±1.15	21.66±2.51	0.40±1.36
GVI	145.20±1.65	5.88±0.31	26.40±0.89	97.60±1.67	18.40±0.54	0.36±0.08
GVII	140.00±5.00	5.80±0.87	27.33±0.55	99.66±2.15	22.66±2.50	0.38±0.66

3.2 Discussion

Acute toxicity is an adverse or undesirable effect that is manifested within a relatively short period of time ranging from almost immediately to within few days following exposure to a foreign compound [12]. The current study shows the oral LD_{50} of all solvents extracts to be greater than 5000 mg/kg body weight which is interpreted as practically nontoxic according to scale of classification [13]. Muhammad et al reported similar results when establishing the oral LD_{50} of aqueous fruit pulp extract of *Adansonia digitata* [5].

Successful induction of diabetes was achieved by administration of 40mg/Kg body weight of STZ, which selectively destroy β- cells of pancreas. This causes an insulin dependent diabetes mellitus to the animals, which is characteristically similar to Type 1 diabetes in humans. These finding is in accordance with several studies that reported successful induction of diabetes with STZ [14, 15]

Administration of the extracts for two week shows a considerable hypoglycaemic activities in all solvents extract with ethyl acetate extract having the highest activity in decreasing glucose levels among all test groups, however both methanol and chloroform extract produce substantial decrease in blood glucose level. This is in accordance with the findings of Muhammad et al [5] who reported "that aqueous fruit extract of *Adansonia digitata* exhibit strong anti-hyperglycemic properties in alloxan induced diabetic rats. Gwarzo et al [16] also reported methanolic fruit pulp extract of *Adansonia digitata* to possess antidiabetic effect on alloxan induced diabetic rats".

The hypoglycemic ability exhibited by the extract may be connected with the presence of secondary metabolites which mediate the hypoglycemic ability of the plant. Ethyl acetate with highest activity extracted more of the bioactive constituents from the plant. The exact bioactive compound(s) and its mechanism of action(s) is yet to be elucidated, however it may also acts by inhibiting α- glucosidase and α-amylase activities and/or stimulating insulin production and release from the destroyed beta cells [17].

4. CONCLUSION

The research established that solvents extracts of *A. digitata* stem bark is practically nontoxic with an LD_{50} greater than 5000 mg/kg body weight via oral administration route. Secondly, the extracts were found to possess hypoglycemic activity with ethyl acetate extract having the highest activity.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shoback, D. (2011). *Greenspan's basic & clinical endocrinology.* 9th Edn. New York: McGraw-Hill Medical. ISBN 0-07-162243-8.
2. Kitabchi, A.E., Umpierrez, G.E., Miles, J.M and Fisher, J.N. (2009). Hyperglycaemic crises in Adult patients with diabetes. *Diabetes Care* 32 (7): 1335–43.
3. Picot, J., Jones, J., Colquitt, J.L., Gospodarevskaya, E., Loveman, E., Baxter, L. and Clegg, A.J. (2009). The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation. *Health technology assessment,* 13(41): 215–357.
4. Aliyu, B.S. (2006). Common ethnological plants of the semiarid regions West Africa ISBN: 12764248 Pp 25-29.
5. Muhammad, I.U. Jarumi, I.K., Alhassan A.J., Wudil A.M. and Dangambo, M. A. (2016). Acute Toxicity and Hypoglycemic Activity of Aqueous Fruit Pulp Extract of Adansonia digitata L. (AFPEAD) on Alloxan Induced Diabetic Rats. *Journal of Advances in Medical and Pharmaceutical Sciences* 6(3): 1-6.
6. NIH. Guidelines for the care and use of laboratory animals. National Academic Press, (NIH Publication No. 85:23); 1996.
7. Zimmermann M. Ethical guidelines for investigation of experimental pain in conscious animals. Pain. 1983;19:109-110.
8. Muhammad, I. U., Alhassan, A. J., Sule, M. S., Idi, A., Mohammed, A., El- ta'alu A. B., Dangambo, M. A. and Abdulmumin, Y. (2016b). Anti-Hyperglycaemic Activity of Solvents Extract of *Khaya senegalensis* Stem Bark in Alloxan Induced Diabetic Rats. *Journal of Advances in Biology & Biotechnology,* 6(2): 1-8.
9. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch toxicol* **53** 275-287
10. Sadish, S., Kumar, Y., Khan, M.S., Anbu, J. and Sam, K.G. (2009). Acute toxicity study andantipyretic effect of the brown alga*Turbinaria conoides* (J. Agardh) Kuetz. *African Journal of Traditional medicine,* 6(3):233-240.
11. GraphPad Instat3 Software; 2000. Available: www.graphpad.com. Retrieve on 23rd February 2021.
12. Phillip L, Williams RC, Stephen MR. Principles of Toxicology: Environmental and Industrial Applications, Second Edition; 2000. ISBN 0-471-29321-0. 22.
13. Timbrell JA. Principle of biochemical toxicology 4th edition. Informa. 25; 2009.
14. Kolawole OT, Kolawole SO, Ayankunle AA, Olaniran OI. Anti-hyperglycemic effect of Khaya senegalensis aqueous stem bark in rats. European Journal of Medicinal Plants. 2012; 2(1):66-73.
15. Szudelski TS. The mechanism of alloxan and streptozotocin action in ß cells of the rat pancreas. Journal of Physiology Research. 2001;50:536-546.
16. Gwarzo MY, Bako HY. Hypoglycemic activity of methanolic fruit pulp extract of *Adansonia digitata* on blood glucose levels of alloxan induced diabetic rats. *International Journal of Animal and Veterinary Advances*. 2013;5(3):108-113.

17. Kaboré DH, Sawadogo-Lingani B, Diawara CS, Compaoré MH, Dicko, Jakobsen M. A review of baobab (Adansonia digitata) products: Effect of processing techniques, medicinal properties and uses. African Journal of Food Science. 2011;5(16):833- 84