Asian Journal of Biochemistry, Genetics and Molecular Biology

6(3): 51-55, 2020; Article no.AJBGMB.63342
ISSN: 2582-3698

Effect of Methanolic Extract of African Ebony (*Diospyros mespiliformis)* Stem Bark on Liver and Kidney Function Biomarkers in Alloxan Induced Diabetic Albino Rats

N. B. Muhammad[1*], R. S. U. Wasagu[2] and B. Sani[3]

[1]Department of Biochemistry, Sokoto State University, Sokoto, Nigeria.
[2]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.
[3]Chemical Pathology Laboratory, Usmanu Danfodio University Teaching Hospital, Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author NBM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RSUW and BS managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v6i330156
Editor(s):
(1) Dr. Arulselvan Palanisamy, Muthayammal Centre for Advanced Research (MCAR), India.
Reviewers:
(1) Lluvia Itzel López López, Universidad Autónoma de San Luis Potosí, Mexico.
(2) Jeneffer Soares dos Santos Mamede, Federal University of Mato Grosso (UFMT), Brazil.
(3) Nikolaos Andreas Chrysanthakopoulos, University of Athens, Greece.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63342

Original Research Article

Received 06 October 2020
Accepted 11 December 2020
Published 29 December 2020

ABSTRACT

The effect of methanolic extract of African Ebony *(Diospyros mespiliformis*) on glucose level, liver and kidney function biomarkers in alloxan induced diabetic rat was investigated. The rats were grouped into four of ten (10) rats each. The rats in Group B, C and D were induced with 0.4mL of alloxan. Group C was treated with 0.3 mL of methanol stem back of *Diospyros mespiliformis*, Group D was treated with 1.3 mL of metformin; Group A non-diabetic and Group B diabetic untreated. There was a significance increase (P<0.05) in serum glucose level (23.95±1.04mmol/L) of diabetic untreated rat when compared with the normal rat (5.59±0.22mmol/L) Group A. There was also a significant difference P<0.05 in glucose level of the Groups treated with extract (15.90±0.29mmol/L) and Group treated with metformin (17.25±0.28mmol/L) when compared with diabetic untreated rat

**Corresponding author: E-mail: muhammad.nurabello@ssu.edu.ng, nurambello@gmail.com;*

(23.95±1.04mmol/L). The effect of extract on Liver marker enzymes AST shows a significant (P<0.05) decrease in the Group treated with extract (11.00±0.40U/L) and metformin (11.04±1.08U/L) when compared with untreated (12.88±0.53g/L). ALT showed significant (P<0.05) decrease in the Groups treated with extract (11.05±1.07U/L) and metformin (11.02±1.11U/L) when compared with untreated Group (14.58±0.76g/L). There was a significance P<0.05 increase of Total protein in untreated rats (66.00±2.26g/L) when compared with extract (11.25±1.15g/L) and treated with metformin (11.75±1.11g/L). There was a significance P<0.05 decrease in ALB in diabetic untreated (41.25±0.25g/L) when compared with that treated with extract (57.25±4.37g/L) and metformin (60.20±1.88g/L) and untreated rats. The values of renal function, Urea (6.74±1.76) mMol/l for Group A, (6.50±1.56) mMol/l for Group B, (8.87±1.10)mMol/l for Group C and (8.44±1.21) mMol/l for Group D. Creatinine values (0.44±0.01) mMol/l for Group A, (4.02±0.36) mMol/l for Group B, (3.16±0.10) mMol/l for Group C and (0.46±0.10) mMol/l. The stem bark extracts possesses hypoglycemic effect and also has a positive effect on liver and kidney function biomarker.

Keywords: Alanine transaminase (ALT); aspartate transaminase (AST); Alloxan.

1. INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The effects of diabetes mellitus include long– term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death [2]. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [3].

Studies examining data trends within Africa point to evidence of a dramatic increase in prevalence in both rural and urban setting, and affecting both gender proportionally [3]. According to the World Fact book report in 2008, in Africa the prevalence of diabetes mellitus was 3.2%, and 40,895 persons (2.0%) was in Ethiopia [4]. Although T2DM is widely diagnosed in adults, its frequency has markedly increased in the pediatric age group over the past two decades. Depending on the population studied, T2DM now represents 8-45% of all new cases of diabetes reported among children and adolescent [5]. The prevalence of T2DM in the pediatric population is higher among girls than boys, just as it is higher among women than men [6]. The mean age of onset of T2DM is 12-16 years; this period coincides with puberty, when a physiologic state of insulin resistance develops. In this physiologic state, T2DM develops only if inadequate beta-cell function is associated with other risk factors (e.g. obesity) [3]. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries, where the majority of patients are aged between 45 and 64 years. The greatest increase in prevalence's, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 [7].

Diospyros Mespiliformis belongs to the family Ebenaceae and is extremely wide spread in African countries such as Senegal east, Ethiopia, Kenya, south to Namibia, northern South Africa and Swaziland, and Nigeria (Oyibani et al., 2017). It's commonly known as Ebony tree, Jackal berry or jackal Bessie [8] and in Northern part of Nigeria it is commonly called Kanya (Hausa), Nelbi (Fulani) [9]. *D. mespiliformis* is a large deciduous tree found mostly in the savannahs, reaching about 10 meters in height (Oyibani et al., 2017). Mature trees have dark gray fissured bark and adult tree reaches an average of 4 to 6 metres in height, though occasionally some trees reach can reach up to 25 metres. The foliage is dense and dark green with elliptical leaves, which are often eaten by grazing animals such as elephants and buffalo. The tree flowers in the rainy season; the flowers

are imperfect, with genders on separate trees, and are cream-colored. In some parts of Africa, ripe fruits of *D. mespiliformis* are consumed as food because of its high nutrition value while gum for binding loose pages and pasting papers on walls were extracted from the unripe fruits [10]. The leaves, bark and root of *D. mespiliformis* have been reported to be medicinal containing some bioactive compounds including tannin, which are used as a styptic to staunch bleeding [10].

2. MATERIALS AND METHODS

2.1 Preparation of Extract

The plant sample was collected and identified by a qualified botanists. The plant material of *Diospyros Mespiliformis* stem bark was rinsed with tap water and dried in room temperature for 7 days and then crushed into powdered form with mortar and pestle. 200 g of the powdered stem bark was dissolved in 900 ml of methanol for 72 hours with occasional mixing. The extract was sieved using a muslin cloth and filtered using a whattman NO. 1 filter paper and the filtrate was evaporated using water bath at 45°C to constant weight.

Adult (male and female) albino rats weighing 120-200g were used for this research. The animals were wired housed in rubber cages under standard conditions. They were maintained on standard pallets and water *ad libitum.* The animals were acclimatized for 4 weeks before commencement of the experiment.

2.2 Animals Grouping

The animals were grouped into four groups of ten (10) rats, each for the determination of the effect of the plant extract on glucose and some liver and kidney function biomarkers.

Group 1: Non Diabetic (Negative control)
Group 2: Diabetic untreated (Positive control)
Group 3: Diabetic rat treated with plant extract
Group 4: Diabetic treated with metformin

A day prior to the induction, the rats were allowed to fast overnight (6pm-8am) and the following day, the weight of the rats were measured and recorded. Diabetes was induced by a single subcutaneous administration of alloxan monohydrate (150 mg/kg BW.). The rats with blood glucose level greater than 150 mg/dl, two days after induction, were considered diabetic [8].

2.3 Sample Collection and Processing

Blood was collected from the tail of the rats after every four days to assay for the glucose level. On the last day of treatment, the animals were anaesthesized with chloroform and 5 ml of blood sample was collected via cardiac puncture [11]. The blood samples obtained were collected in plain bottles and centrifuged at 5000 rpm for 10 minutes. The serum samples obtained were used for the analysis

2.4 Biochemical Analysis

Determination of Glucose (glucose oxidase method)
Determination of AST and ALT (Reitman and Frankel method)
Determination of Total protein (Biuret method)
Determination of Albumin (Bromocresol Green method)
Determination of Urea (Urease method)
Determination of Creatinine (Jaffe's method)
Determination of Sodium (Na) and Potassium (K) (Flame photometry method)

3. RESULTS

The result of the effect of methanolic extract of *Diospyrus mespliformis*(African Ebony) in alloxan induced diabetic rats was presented below:

4. DISCUSSION

The administration of the methanolic extract of stem bark *Diospyros mespiliformis* caused a reduction in the blood glucose concentration in diabetic rats (as seen in Table 1 above). This result agrees with the findings of other researchers who reported a significant hypoglycemic effect on rats using *D. mespiliformis* Mohammed et al. [12]. The hypoglycemic activity of these plant may be as a result of several phytochemical compounds present in the plant such as flavonoid, sterols, terpenoids, alkaloids, saponins, and phenolics which are reported to be as bioactive compounds [13].

The result of glucose level obtained shows a significant difference $P<0.05$ between glucose level of diabetic rats untreated when compared with the Groups treated with stem bark extract and that treated with metformin.

Table 1. Effects of stem bark methanolic extract of *Diospyros Mespiliformis* on glucose level in alloxan induced diabetic albino rats

Parameter (mmol/L)	Group A	Group B	Group C	Group D
GLU0	5.45±0.396	5.85±0.17	5.13±0.16	5.29±0.14
GLU4	5.62±0.153	21.02±0.80**	19.49±0.48*	19.48±0.82*
GLU8	5.83±0.24	19.78±0.77*	19.06±0.72*	18.85±0.45*
GLU12	5.77±0.22	21.01±0.86**	18.44±0.56*	18.00±0.28*
GLU16	5.79±0.10	23.06±1.02**	17.39±0.44*	17.25±0.28*
GLU21	5.59±0.22	23.95±1.04**	15.90±0.29*	17.25±0.28*

*GLU0=Initial Glucose before induction, GLU4=Glucose at day 4 after induction, GLU8=Glucose at day 8 after treatment, GLU12=Glucose at day 12 after treatment, GLU16=Glucose at day 16 after treatment, GLU21=Glucose at day 21after sacrifice. Values are expressed in terms of mean and standard error of mean, and has a significance of **P<0.0001, *p<0.01*

Table 2. Effect of stem bark extract on some liver biomarkers in alloxan induced diabetic rats

Parameters	Group A	Group B	Group C	Group D
ALT(U/L)	9.75±1.11	14.58±0.76*	11.05±1.07*	11.02±1.11*
AST(U/L)	9.00±1.47	12.88±0.53*	11.00±0.40*	11.04±1.08*
TP(g/L)	12.50±0.99	66.00±2.26**	11.25±1.15*	11.75±1.11*
Albumin(g/L)	63.05±2.44	41.25±0.25*	57.25±4.37*	60.20±1.88*

*ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, TP=Total Protein, Albumin Values are expressed in terms of mean and standard error of mean (SEM), n=4 and has a *p<0.05 which signifies level of significances*

Table 3. Effect of stem bark methanolic extract of *Diospyros Mespiliformis* on kidney functoin biomarkers in alloxan induce diabetic rats

Parameter (mMol/l)	Group A	Group B	Group C	Group D
Urea (mmol/l)	6.74±1.76	6.50±1.56*	8.87±1.10	8.44±1.21*
Creatinine (mmol/l)	0.44±0.01	4.02±0.36*	3.16±0.10	0.46±0.10*
Sodium (mmol/l)	17.11±0.65*	19.23±1.90*	19.58±1.79	21.61±1.45
Potassium (mmol/l)	4.65±046*	4.02±0.47	3.88±0.74	3.32±0.46

Values are expressed in terms of the standard error of the mean (SEM) n=4 in each group p <0.05 and p <0.01.Creatinine, Urea, Sodium and Potassium

The result obtained from the liver function biomarkers indicated the effect of stem bark methanolic extract of *Diospyros Mespiliformis* on the concentration of enzymes The untreated diabetic rats shows significant elevation ($p<0.05$) of both serum ALT and AST compared to other groups, treated with plant extract and metformin. This finding is in line with the work of Iraniloye et al.; [14].

The value obtained in Total protein and Albumin revealed that there is decrease in the concentration in the untreated diabetic rats compared with the groups treated with plant extract and metformin**.** The decrease in total protein and albumin levels is an indication of liver damage or nutritional deficiency. The result obtain is in coincide with the work of Jigam et al., 1995.

The results of the kidney functions parameters (Urea and Creatinine) showed that there is no any significance different ($p>0.05$) between normal (control) and normal treated groups, but there is significant difference ($p<0.05$) between diabetic untreated and diabetic treated groups due to the diabetic complications. This indicates that the kidney was not affected by the plant extract. Urea and Creatinine are waste products of protein metabolism that need to be excreted by the kidney [14]. Therefore, increase in serum urea and creatinine is an indication of functional damage to the kidneys. The kidney is the primary organ for clearance and excretion of xenobiotics including drugs and drug products from the body Muhammad et al., 2013).

The value of sodium (Na) and potassium (k) obtained indicated there is no significant difference ($p>0.05$) between group treated with extract and that treated with metformin. Whereas as untreated group revealed elevation of electrolyte which manifest the condition of electrolyte imbalance associated with diabetes mellitus.

5. CONCLUSION

The research conducted shows that the stem bark methanolic extract of *Diospyros mespiliformis* (African Ebony) is effective in the treatment of diabetes because of its hypoglycemic activities and can restore the altered levels of liver and kidney function biomarkers to almost normal level.

ETHICAL APPROVAL

The Ethical approval was obtained from the Research Ethical Committee of faculty of Vet nary medicine Usmanu Danfodiyo University, Sokoto.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Report of World Health Organization/ International Diabetes Federation (WHO/IDF). Geneva. 2016;11-52.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2016;33(Suppl.1):62-69.
3. King H, Aubert R, Herman W. Global burden of diabetes, 1995-2025, Prevalence, numerical estimates and projections. *Diabetes Care.* 1998;21:1414–31. American Diabetes Association: Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2001;24(Suppl.1):S5–S20.
4. Fadini GP, Bonora BM, Avogaro A. SGLT2 inhibitors and diabetic ketoacidosis: Data from the FDA Adverse Event Reporting System. Diabetologia. 2017;60:1385–1389.
5. Rossouw JE, Lewis B, Rifkind BM. The value of lowering cholesterol after myocardial infarction. N Engl J Med. 1990;323:1112-9.
6. Wild S, Roghi D, Green A, Sicree A, King H. Global prevalence of diabetes: estimate for the year 2000 and projection for 2030. Journal of Diabetes Care. 2004;27(5): 1045-1053.
7. Kolodziej H, Kiderlen AF. Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells. Phytochemistry. 2005;66(17):2056-2071.
8. Shagal MH, Kubmarawa D, Alim H. Preliminary phytochemical investigation and antimicrobial evaluation of roots, stem-bark and leaves extracts of *Diospyros mespiliformis*. International Research Journal of Biochemistry and Bioinformatics. 2012;2(1):011-015. (ISSN-2250-9941).
9. Oyebanji OO, Adeyemi SB, Agboola OO, Bolarinwa K. Taxonomy, ethnobotany and vegetation analysis of biodiversity in Dutse local government, Jigawa State, Nigeria. FUW Trends in Science & Technology Journal. 2017;2(2):679- 683.
10. Mohamed IE, El Nur EE, Choudhary MI, Khan SN. Bioactive natural products from two Sudanese medicinal plants Diospyros mespiliformis and Croton zambesicus. Rec. Nat. Prod. 2009;3:198-203.
11. Norhayati I, Getha K, Haffiz JM, Ilham AM, Sahira HL, Syarifah MMS, Syamil AM. *In-vitro* anti-trypanosomal activity of Malaysian plants. J. Trop. For. Sci. 2013;25:52-59.
12. Tiwari AK, Madhusudana RJ. Diabetes mellitus and multiple therapeutic approaches of Phytochemicals: present status and future prospects. Current science. 2002;83:30-38.
13. Iranloye BO, Arikawe AP, Rotimi G, Sogbade AO. Anti-diabetic and anti-oxidant effects of Zingiber officinale on alloxan-induced and insulin-resistant diabetic male rats. Nigerian journal of physiological sciences: official publication of the Physiological Society of Nigeria. 2011;26(1):89-96.
14. Vidal A. Global report on diabetes. World Health Organization. Geneva. 2003;6-86.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63342