Original Research Article

Hepatotoxicity Assessment of Stem Back Extract of *Acacia nilotica* in Alloxan Induced Diabetic Rats

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ABSTRACT

Aims: This research work aimed to determine the effect of methanolic Acacia nolitica stem bark extract on liver functions in alloxan induced diabetic rats**.**
Methodology: Thirty (30) young albino rats were grouped into six (6), comprising of five (5) rats per group. Diabetes was induced by single intraperitoneal injection of freshly prepared alloxan monohydrate and blood glucose level was determined forty eight hours (48 hrs) after injection. After induction of diabetes, Metformin and selected doses of Acacia nolitica extract were orally administered for 4 weeks after which serum biochemical markers were determined.
Results: Highest hypoglycemic effect was observed at 600mg/kg dose. Total Protein and Albumin were slightly elevated in extract treated groups compared to diabetic untreated group while serum bilirubin decreased. There was decrease in the serum level of ALT, ALP and AST in a dose dependent manner.
Conclusion: Convincingly, the plant extract may exert not only hypoglycemic effect but also hepatic protective effect.

Keywords: Acacia nilotic; Alloxan; Diabetic rat; Hypoglycemic; Biochemical markers; Metformin

1. INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat, and protein metabolism characterized by increased fasting and postprandial blood sugar levels. Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction [1]. The world health organization (WHO) estimated that diabetes would be the seventh leading cause of death by the year 2030 and suggested that a healthy lifestyle, right medication and regular screening can prevent the consequences of diabetes.
For many decades, medicinal plants have been beneficial resources for the treatment of several diseases, including diabetes [2, 3, 4, 5]. Some well-known drugs such as metformin drug derived from the *Galega officinalis* are currently used for the treatment of diabetes. Plants containing phytochemicals such as carotenoids, flavonoids, terpenoids, alkaloids, and glycosides exert anti-diabetic effects by improving the performance of pancreatic tissue, which is done by increasing insulin secretions or reducing the intestinal absorption of glucose [6].
Acacia nilotica pods and tender leaves are considered very beneficial in folk medicine to treat diabetes mellitus. It contains variety of bioactive components such as phenolic acids, alkaloids, terpenes, tannins and flavonoids which are responsible for numerous biological and pharmacological properties like hypoglycemic, anti-inflammatory, anti-bacterial, anti-platelet aggregatory, anti-hypertensive, analgesic, anticancer, and anti-atherosclerotic due to their strong antioxidant and free radical scavenging activities [7]. It has also been reported to have ant-mutagenic, cytotoxic, antifungal and antiviral activity [8]**.**

Though various studies indicate the benefits of medicinal plants in therapeutic development, there is still problem associated with these herbal therapies which is lack of standardization [9]. So, there is a need for scientific validation of herbal therapies to evaluate their safety and efficacy as well as the side effects associated with them. Therefore, the aim of this research work is to investigate the effect of methanolic *A. nilotica* stem bark extract on liver functions in alloxan induced diabetic albino rats.

2. MATERIAL AND METHODS

2.1 Plant Collection and Authentication

The plant sample was collected in Sokoto State Metropolis. It was authenticated in the botany unit, Biology department of Sokoto State University. The collected sample was cut into small pieces and dried under shade at room temperature for four weeks, and then ground into fine powder by a mechanical grinder, followed by sieving through a 40 mesh sieve. The grounded sample was packed in clean dry plastic air tight bag.

One hundred grams (100 g) powder of the plant sample was later extracted in 1 liter of distilled water at 60^{o}C in a metallic shaker for 6 hours. The extract was decanted into clean dry conical flasks and then filtered through Whatman filter paper number using buchner funnel. The filtrates were stored in a refrigerator at 4^{o}C. Freeze drying was done in 200ml portions in a Modulyo freeze dryer for 48 hours and the freeze dried materials were stored in a freezer at -20^{o}C until the time it was used.

2.2 Experimental Design and Induction of Experimental Diabetes

Thirty (30) healthy young albino rats were used in the study. The animals were allowed to acclimatize for a period of two weeks in the animal house at the department of Biochemistry, Sokoto State University, Sokoto. The rats were housed in polypropylene cages, maintained under standard laboratory conditions while fed with standard mice pellets. The total of thirty (30) albino rats were grouped into six (6), comprising of five (5) rats per group (n=5 rats in each group). Group I assigned as Control, Group 2 as Diabetic control on Alloxan monohydrate, Group 3 as Positive control on Metformin, Group 4 as Treated I (methanolic extract of *A. nilotica* 300 mg/kg), Group 5 as Treated II (methanolic extract of *A. nilotica* 600 mg/kg) and Group 6 as Treated III (methanolic extract of *A. nilotica* 1200 mg/kg).

The animals were fasted for 8 hours, but allowed free access to water. Diabetes was induced experimentally by single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg) in groups 2, 3, 4, 5 and 6. Forty eight hours after injection, blood glucose was determined using glucose analyzer model with glucometer strips. Rat with blood glucose level above 2000 mg/L (>11.1 mmol/L), were considered diabetic and suitable for use in the study. After induction of diabetes, Metformin and selected doses of *A. nilotica* extract were orally administered to groups 3, 4, 5 and 6 respectively for 4 weeks.

2.3 Estimation of serum biochemical markers

Using corresponding commercially available diagnostic kits, spectrophotometric estimations of Blood glucose (BG), Total Bilirubin (TB), Direct Bilirubin (DB), Total Protein (TP), Albumin (ALB), Alanine aminotransferase (ALT), Akaline Phosphatase (ALP) and Aspartate aminotransferase (AST) were performed as primary markers of diabetic hepatic injury, hyperlipidemia and diabetic nephrotoxicity.

3. RESULTS AND DISCUSSION

The data in table (1) shows a decrease in blood glucose concentration in *A. nilotica* treated diabetic rats compared with diabetic control. It is worth mentioning that the higher hypoglycemic effect was observed at 600mg/kg dose (table 1). Other researchers also indicated decrease in hyperglycemic conditions with medicinal plants based therapies [10]. This decrease in hyperglycemia could be associated either with enhancement in the insulin level because of positive impact of flavonoids present in formulation on the β-cells of pancreas or improvement in the transport of glucose to the peripheral tissues [11].

Table 1. Biochemical parameters of the serum of different experimental groups

Parameters	Control	Diabetic control	Positive control	Treated I (300mg/kg)	Treated II (600mg/kg)	Treated III (1200mg/kg)
BG(mMol/L)	4.31±0.69	8.92±0.74	7.36±1.35	6.16±0.78	4.66±0.67	6.38± 0.59
TP(g/L)	67.00±1.54	52.00±3.32	68.60±2.88	68.40±2.56	71.20±7.19	71.40±2.51
ALB(g/L)	41.20±2.30	29.80±2.50	36.40±3.44	34.40±2.07	37.80±2.70	36.40±2.58
DB(mg/dL)	0.16±0.09	0.46±0.11	0.26±0.09	0.24±0.09	0.20±0.07	0.28±0.08
TB(mg/dL)	0.44±0.11	0.80±0.16	0.68±0.29	0.56±0.17	0.82±0.19	0.78±0.28

Values are expressed as mean ± SE with different letters within a row differ significantly from each other (P≤0.05).

In the current study, serum total protein and albumin level slightly reduced in diabetic rats, but increase in positive and treated group (table 1). It is also important to note that, the increase in albumin in treated groups is dose dependent. The findings of our study also showed that the experimental induction of diabetes noticeably elevated the level of serum DB and TB. The increment in serum bilirubin may occur due to reduction of liver uptake, increase of bilirubin formation or conjugation [10]. However, metformin and *A. nilotica* induced a massive decrease in both serum bilirubin levels. The reduction in TB was also dose dependent (table 1).

Metabolic disorders such as diabetes can cause damage of hepatocytes. The injury to hepatic cells is responsible for the release of intracellular elements into systemic circulation. Measurement of serum concentrations of hepatic enzymes provides a valuable mean for scientific diagnosis of hepatic damage [10]. Induction of diabetes mellitus with alloxan directed a noteworthy increase in the level of serum AST, ALT and ALP in the diabetic rats in comparison to the control group (table 2).

Table 2. Enzymatic parameters of the serum of different experimental groups

Parameters	Control	Diabetic control	Positive control	Treated I (300mg/kg)	Treated II (600mg/kg)	Treated III (1200mg/kg)
ALT(U/L)	5.00 ±1.87	8.00 ±1.39	7.60±1.19	7.40±2.70	7.80±2.39	6.00±1.54
ALP (U/L)	73.00±11.19	85.60±1.15	73.40±1.32	61.40±1.93	77.40±8.65	76.80±1.07
AST (U/L)	4.60±1.52	11.00±1.58	8.00± 1.00	8.80±1.92	8.00±2.65	8.20±1.64

Values are expressed as mean ± SE with different letters within a row differ significantly from each other (P≤0.05).

The Upsurge in the levels of hepatic enzymes in alloxan induced diabetes might be due to outflow of enzymes from the hepatic tissue into the plasma. The extract reduced the level of ALT, ALP and AST. The reduction in hepatic enzyme levels was dose dependent. The results of current study are in agreement with previous research studies [12, 13].

4. CONCLUSION

Current study has revealed the hypoglycemic potential of *A. nilotica* stem bark extract. The extract reduced the alloxan monohydrate induced hyperglycemia and improved the biochemical parameters including serum glucose, TP, ALB, DB, TB, ALT, AST and ALP.

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