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* stem back 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 long-term condition of the metabolism of carbohydrates, fats, and proteins marked by elevated fasting and postprandial blood sugar levels. Diabetes mellitus is a complicated metabolic condition caused by insulin deficiency or malfunction" [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- 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].
In traditional medicine, the seeds and succulent leaves of *Acacia nilotica* are highly effective in treating diabetes mellitus. It contains a variety of bioactive substances, including phenolic acids, alkaloids, terpenes, tannins, and flavonoids, which are responsible for many biological and pharmacological properties, including hypoglycemia, hypoglycemia, anti-inflammatory, antibacterial, anti-platelet aggregation, anti-hypertension, analgesia, anti-cancer, and anti-atherosclerosis [7,8]. It has also been reported to have ant-mutagenic, cytotoxic, antifungal and antiviral activity [9]**.**

"Despite numerous studies indicating the advantages of medicinal plants in therapeutic applications, there is still a problem associated with these herbal therapies, which is an inadequate standardization" [10]. As a result, there is a requirement for scientific validation of herbal medicines in order to evaluate their efficacy, safety, and side effects. This study aims to investigate the effects of methanolic A. nilotica stem bark extract on the liver enzymes of 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 for four weeks in the shade at room temperature. A mechanical grinder was used to grind the dried sample into fine powder, which was then sieved through a 40 mesh sieve. The ground sample was stored in a clean, dry, airtight plastic 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 before being filtered using a Buchner funnel and Whatman filter paper number 1. The filtrates were kept chilled, at a temperature of 4 ℃ In a Modulyo freeze drier, freeze drying was carried out in 200ml portions over the course of 48 hours. The materials were then stored at −20 ° c until they were needed.

2.2 Experimental Design and Induction of Experimental Diabetes

The study included thirty (30) healthy young albino rats. The animals were permitted to acclimate at the animal house at Sokoto State University's Department of Biochemistry for two weeks. The rats were kept in polypropylene cages and fed normal mice pellets under standard laboratory settings. The total of thirty (30) albino rats were grouped into six (6), comprising of five (5) rats per group (n=5). 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 group 2 - 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 - 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.

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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). Our result is in agreement with that of [11] who reported hypolipidemic and hypoglycemic effect of *A. nilotica* leaves extract in alloxan induced diabetic rats. Other studies have found that medicinal plant-based therapies can reduce hyperglycemia [12]. This reduction in hyperglycemia could be attributed to either an increase in insulin levels due to the positive effect of flavonoids in the formulation on pancreatic -cells or an improvement in glucose transport to peripheral tissues [13].

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” [12]. However, metformin and *A. nilotica* induced a massive decrease in both serum bilirubin levels. The reduction in TB was also dose dependent (table 1).

Diabetes and other metabolic disorders can harm hepatocytes. The flow of intracellular elements into the blood circulation is caused by hepatic cell injury. Serum hepatic enzyme concentrations provide a valuable means for scientific diagnosis of hepatic damage [12]. Diabetes mellitus induction with alloxan resulted in a significant increase in serum AST, ALT, and ALP levels in diabetic rats compared to the control group (table 2).

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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 increase in hepatic enzyme levels in alloxan-induced diabetes may be due to enzyme outflow from hepatic tissue into plasma. The extract decreased the levels of ALT, ALP, and AST in a dose-dependent manner. The current study's findings are consistent with previous research studies"[14, 15].

4. CONCLUSION

Current study has revealed the hypoglycemic potential of *A. nilotica* stem bark extract. It also enhanced the biochemical markers, including blood glucose, TP, ALB, DB, TB, ALT, AST, and ALP, in alloxan monohydrate-induced hyperglycemia.

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