UJMR, Vol. 7 Number 1, June, 2022, pp 61-65 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2271.010

Received: 26th Apr, 2022 **Accepted:** 16th Mat, 2022

Antidiabetic Properties of Aqueous Leaf and Stem Extracts of *Andrographis paniculata* on Alloxan Induced Diabetes in Wistar Rats

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Abstract

Diabetes mellitus is a disorder in which the body does not produce enough or respond normally to insulin, causing blood sugar (glucose) levels to be abnormally high. The aim of the study was to assess the effectiveness of *Andrographis paniculata* leaf and stem bark extracts in management of Alloxan induced diabetes Wistar rats. Fifty (50) Wistar rats were divided into eight groups, namely; group A (normal control), B, C, D, E, F, G and H (treatment groups). The study was conducted for four (4) weeks by measuring blood glucose levels in first four weeks. The result showed that the diabetic rats administered with 500mg/kg of aqueous leaf extract and 250mg/kg of stem bark extract were significantly lower than those of untreated control groups. However, the combined dose of 250mg/kg aqueous leaf and 250mg/kg stem extracts were not significantly different from untreated control group. Findings of the study have demonstrated that leaves and stem extract of *A. paniculata* exhibited significant blood sugar lowering property in Alloxan induced diabetic Wistar rats.

Keywords: *Andrographis paniculata*; Blood sugar; Diabetes mellitus; Wistar rat.

INTRODUCTION

Diabetes mellitus is a chronic disorder of metabolism caused by a relative or absolute lack of insulin in the body (Dahiru *et al.*, 2016). Its primary characteristic is a high level of sugar in the body, in the fasting or post meal stages (Akbar *et al.*, 2011). According to the International Diabetes Federation (IDF, 2019), an estimated 463 million adults aged 20-79 years are currently living with diabetes, representing 9.3% of the world's population in this age group. The total number is predicted to rise to 578 million (10.2%) by 2030 and to 700 million (10.9%) by 2045. Africa accounts for 3.9 % (19.4 million cases) of the global incidence of diabetes and the number is expected to rise by 143 percent by 2045 (Dahiru *et al.*, 2016; IDF, 2019). There are two types of diabetes mellitus, viz. Type I and Type II.

Medicinal plants and plant-base drugs are the potential sources of alternative medicines and are extensively used to treat various health diseases (Kavishankar *et al.*, 2011). Use of these medicinal plants is a major component at primary health care due to availability, acceptability, compatibility, and affordability. Dependency on these medicinal plants varies from country to country. It is estimated that about 75-80% of people of developing countries and about 25% of people of developed countries depend either directly or indirectly on medicinal plants for the first line of treatment (Burkill*et al.*, 1966; Hajighaee *et al.*, 2012).

Andrographis paniculata(Burm. f.) Wall. ex Nees (AP) is an important medicinal plant and widely used around the world. It belongs to the family *Acanthaceae* (Akbar *et al.*, 2011; Kabir *et al.*, 2014). It is an annual and branched plant with lanceolate green leaves and attains heights of 60-70 cm (Mishra *et al.*, 2007). The plant is also known as the 'king of bitters' (Shahid*et al.*, 2011) because every part of the plant body is extremely bitter in taste. The aerial parts (leaves and stems) of the plants are most commonly used to extract the active phytochemicals (Shahid*et al.*, 2011). The aim of this study is to assess the effectiveness of *Andrographis paniculata* leaf and stem extracts in controlling induced type I diabetes in Wistar rats.

MATERIALS AND METHODS

Plant Sample Collection

The leaf and stem of *A. paniculata* were obtained from a garden at Nigerian Air Force Base Kaduna. The samples were transported in a sterile polythene bag to Department of Biological Sciences, Nigerian Defence Academy (NDA) for further identification and authentication with the herbarium number of ZYH001.

Selection and management of experimental animals

Fifty (50) male and female Wistar albino rats, weighing 100 to 150 g, were obtained from the experimental animal research unit of National Institute of Trypanosomiasis Research (NITR) Ungwan Rimi, Kaduna, Nigeria for the experiment. The animals were kept and maintained under laboratory conditions with standard temperature, humidity and day/night regime in the Department of Biological Sciences department. The rats were fed with pelletized animal grower feed (Vital Feed Ltd, Nigeria) and given water *ad libitum*.

Toxicity Test

Nine (9) albino rats weighing 250 g to 300 g were used for the acute toxicity test. The minimum lethal dose (LD_{50}) of leaf and stem of *A. paniculata* methanolic extract was determined according to the modified method of Lorke (2003).

The animals (not induced) were fasted for 12 hr. and randomly divided into three (3) groups (A, B and C) of three (3) animals each. Group A served as control group. Each of the rats in group A was treated with normal saline (2 ml/kg) only, while group B and C were treated with 500 ml/body weight (bwt) of leaf and stem, 1000 ml/bwt of leaf and stem aqueous extracts. They were all allowed free access to food and drinking water during which they were closely monitored for mortality and or signs of acute toxicity for a period of 72 hr. The number of deaths within that period was recorded. Log-dose plots were constructed for each of the plant extracts; from which the median lethal doses (LD_{50}) of the aqueous extracts were determined (Ojewole, 2003).

Induction of Diabetes Mellitus

The chemical, Alloxan Monohydrate (0.9 g mixed into 30 ml of normal saline buffer) using insulin injection intraperitoneally (Etuck and Mohamed, 2010).

Administration of Plant Extracts

The leaf and stem extracts of *Andrographis paniculata* was administered orally as shown below:

A: was normal control not induced with Alloxan and was given normal saline (2ml/100g body weight).

Group B: was the diabetic control, induced with Alloxan and administered normal saline (2ml/100g body weight).

Group C: was the positive control, induced with Alloxan and was administered with Insulin (Pfizer Plc) at a dosage of 60 IU.

Group D and E: were induced with Alloxan and administered with 250 and 500 mg/kg of *Andrographis paniculata* leaf extract, respectively.

Group F and G: were induced with Alloxan and administered with 250 and 500 mg/kg, respectively, of stem extract of the plant.

Group H: was administered with a mixture of 250 mg/kg of leaf extract and 250 mg/kg of stem extract.

Determination of Blood Glucose Level

Continuous measurement of the blood glucose level was carried out weekly for a period of four weeks, with glucometer test strips using a modified method.

Statistical analysis

The results of the findings were analyzed by one-way analysis of variance (ANOVA) and Tukey comparison of individual groups, in Graphpad Prism 4 statistical software. Difference of means was considered significant at $p< 0.05$. The data are presented in form of table.

RESULTS

Results of the acute toxicity of the aqueous extracts of the stem and leaves of *Andrographis paniculata* were shown in (Table 1). For both the leaf and stem extracts no mortality was recorded at the highest dose of 1000 mg/kg.

Table 1: Acute toxicity of the aqueous extracts of the stem and leaves of *Andrographis paniculata*

Dose (mg/kg)	No. of animals	No. of mortality	
		Leaf extract	Stem extract
10	3	0	0
100	3	0	0
1000	3	0	0

Table 2 present the fasting glucose concentration in Wistar rats at baseline (prior to induction of type 1 diabetes) and weekly over a period of four weeks The table below shows the fasting glucose concentration of Wistar rats, in which the fasting blood glucose concentration did not differ significantly over the course of five weeks.

The fasting blood glucose of group B was significantly lower than the values recorded after induction over the period of four weeks. Furthermore, group E and F did not differ significantly from group A over the period of four weeks.

Table 2: Blood glucose level (mg/dl) before and after four weeks of treatment

Treatment groups	Baseline	72 hours post induction	Week 1	Week 2	Week 3	Week 4
A	5.25±0.49^{a}	4.96±0.25^{a}	4.85±0.64^{a}	3.70±0.85^{a}	5.00±0.14^{a}	4.85±0.21^{a}
B	5.00±0.28^{a}	23.1±0.64^{b}	24.9±0.92^{cd}	28.8±0.64^{e}	29.9±0.21^{d}	30.9±0.21^{d}
C	4.80±0.28^{a}	24.8±0.02^{b}	26.3±1.41^{d}	27.3±1.34^{de}	28.5±0.85^{d}	27.3±1.34^{d}
D	5.35±0.35^{a}	22.9±3.11^{b}	23.7±1.20^{c}	22.0±0.14^{c}	23.9±0.99^{c}	23.5±4.59^{c}
E	5.35±0.21^{a}	24.5±1.43^{b}	8.45±1.77^{b}	8.40±3.54^{b}	6.70±0.85^{b}	6.60±0.42^{b}
F	4.55±0.21^{a}	23.9±1.46^{b}	9.75±2.05^{b}	9.50±4.53^{b}	10.8±5.66^{b}	9.45±4.31^{b}
G	5.20±0.42^{a}	24.5±0.28^{b}	28.5±2.97^{e}	21.8±2.62^{c}	23.3±1.91^{c}	29.8±4.67^{d}
H	5.50±0.14^{a}	21.9±3.49^{b}	22.3±0.64^{c}	24.3±0.85^{cd}	28.6±1.27^{d}	29.9±0.99^{d}
p value	0.226	<0.001	<0.001	<0.001	<0.001	<0.001

Key: A - normal control; B - negative control; C – positive (insulin) control; D – 250 mg/kg leaf extract; E – 500 mg/kg leaf extract; F – 250 mg/kg stem extract; G – 500 mg/kg stem extract; H – combined 250 mg/kg leaf and stem extracts.

DISCUSSION

The acute toxicity results from this study showed that administration of 1000 mg/kg dose the aqueous leaf or stem extracts of *Andrographis paniculata* do not cause any obvert physiological or behavioural alterations in rats, neither were there any record of mortality. These observations agree favourably with those of Adedapo *et al*. (2014), Borgohain and Kakoti (2019), Etti *et al*. (2019), and Worasuttayangkurn *et al*. (2019). Worasuttayangkurn *et al*. (2019) reported that *A. paniculata* had no significant acute toxicological effect in mice at 5000 mg/kg. Adedapo *et al*. (2014) also reported that no mortality and apparent behavioral changes were observed in mice treated with up to 1600 mg/kg.

In all animal groups that received Alloxan, it was observed that there were significant elevations in fasting blood glucose concentrations after 72 hours of administration of Alloxan. However, in the normal control group, the FBG concentration did not vary significantly throughout the duration of the study. These observations are consistent with reports of the diabetogenic properties of alloxan in animal models (Oshkondali *et al*., 2019; Sari *et al*., 2015; Victor *et al*., 201). In the diabetic control that did not receive any drug or extract treatment, it was noted that the fasting blood glucose concentration was persistently elevated throughout the duration of the study. Alloxan usually induces stable hyperglycemia in animal models resulting from insulin deficiency. The lack or insufficiency of insulin is reported to result from the destruction of the beta cells of the pancreas which secrete endogenous insulin in healthy subjects. Sari *et al*. (2015) reported that in animals administered Alloxan monohydrate, there was significant decline in the number of functional islet cells; tissue histology also revealed that the pancreatic beta cells underwent changes in size and appeared shrunk in Alloxan diabetic rats.

In this group of rats, it was observed that the Fasting Blood Glucose (FBG) concentrations were comparable to the untreated control group. This apparent lack of glucose lowering effect of exogenous insulin may be associated with the mode of administration of insulin. Sari *et al*. (2015) demonstrated that continuous infusion of insulin via pumps achieves optimum glucose control in diabetic rats than injections as injectable insulin control glucose concentrations for only limited duration. Furthermore, they also showed that even long acting insulin analogs may require multiple administrations per day to achieve optimal glycemic control in diabetic subjects. In the present study, insulin was administered once daily, while the experimental subjects feed almost all day though the injected insulin has a limited time for optimum activity, its glucose lowering effects may have been dissipated at the same time when daily glucose measurements were read. The elevated fasting blood glucose levels in the insulin treated group may be related to the dose of insulin administered.

Significant glucose lowering effects were observed in groups that received daily administration of 500 mg/kg of the aqueous leaf extract of *A. paniculata*. Similar glucose lowering activity was also observed in rats

treated with 250 mg/kg of the aqueous stem extract. There are reports on the hypoglycemic properties of *Andrographis paniculata*. Chowdhury and Biswas (2011) showed that oral administration of ethanol and hot water extracts of *Andrographis paniculata* exhibited glucose lowering properties in diabetic animal models.

CONCLUSION

Findings of the study have demonstrated that leaves and stem extract of *A. paniculata* exhibited significant blood sugar lowering property in Alloxan induced diabetic Wistar rats.

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