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Evaluation of the effect of *Andrographis paniculata* on the lipid profile of alloxan induced diabetic albino rats.

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ABSTRACT

Diabetes mellitus is one of the largest global health problems demanding preventive and new therapeutic interventions. Currently, there is a need for safe, effective, and less costly antidiabetic medications, and investigating medicinal plants for antidiabetic medication is imperative. Thus, this study was done to evaluate the effect of *Andrographis paniculata* on the lipid profile of alloxan induced diabetic albino rats. In both single dose and repeated daily dose treated diabetic animal model, rats were randomly divided into six groups (5 groups of diabetic rats and 1 additional group of normal rats, 6 rats per group).Group 1(diabetic control) was treated with 10ml/kg DW; Group II, III, and IV (diabetic test groups) were treated with 100mg/kg, 250mg/kg, and 500mg/kg plant extract, respectively; GroupV (diabetic positive control group) was treated with 5mg/kg glibenclamide, whereas GroupVI (normal control) was treated with 10ml/kg DW. The effect of the extract on diabetic dyslipidemia was also studied on alloxan induced diabetic rats. Results of acute toxicity test showed that the Lethal dose of *Andrographis paniculata* leaf extract was>5000mg/kg. The result of effect of extract on diabetic dislipidemia significantly reduced the level of LDL and TG and significantly increased HDL. This study revealed that the ethanolic extract of *Andrographis Paniculata* leaves possesses significant antilipedemic activity justifying the traditional use of plant for diabetes.

Keywords:*Andrographis paniculata,* lipid profile, alloxan, and diabetic rats

INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macro vascular complications that results in significant mortality [1,2,3,4].The increase in number of ageing population,consumption of calory rich diet,obesity and sedentary life style have led to increase inthe number of diabetics worldwide[5,6,7,8,9]. The current treatment, although provide a good glycemic control but do a little in preventing complications [10,11,12,13].There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents [14,15]. The World Health Organization (WHO) has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs [16].The pharmaceutical drugs are either too expensive or have undesirable side effects.Treatment with sulphonylureas and biguanides are also associated with side effects [17].Medicinal plant is an integral part of human life to combat the sufferings from the dawn of civilization. It is estimated that more than 80,000 of total plant species have been identified and used as medicinal plants around the world [18]. Among these plants, more than 1300 plant species have been used traditionally in Malaysia where the knowledge is being passed down from generation to generation. The indigenous medicinal plants and plant-derived drugs are the potential source of alternative medicine and are extensively used to treat various ailments [19,20]. Use of the medicinal plants is a core component at primary health care level 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 [21,22].Therefore, people are encouraging indigenous production and processing of these medicinal plants to use in different cultures and religion for the treatment of various diseases. *Andrographis paniculata* (AP) belongs to the family Acanthaceae. AP is used as a traditional herbal medicine in Bangladesh, China, Hong Kong, India, Pakistan, Philippines, Malaysia, Indonesia, and Thailand and is ethnobotanically used for the treatment of snake bite, bug bite, diabetes, dysentery, fever, and malaria [23,24]. In recent times, commercial preparations of this plant extracts are also used in certain countries. However, the preparations yet need to be standardized for their better efficacy. The aerial part of AP is most commonly used; its extracts contain diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides. Whole plant leaves and roots are also used as a folklore remedy for different diseases in Asia and Europe [25].

AP has been reported to have a broad range of pharmacological effects including anticancer [26], antidiarrhea, antihepatitis,anti-HIV,antihyperglycemic [27], anti-inflammatory, antimicrobial, antimalarial,antioxidant, cardiovascular, cytotoxic,hepatoprotective, immunostimulatory, and sexual dysfunctions. Since the AP is used for the treatment of many diseases in traditional medicinal systems, its intended benefits need to be evaluated critically especially in the eastern part of Nigeria where the knowledge of the plant is rare [28,29,30]. Diabetes mellitus can directly affect serum lipid levels causing diabetic dyslipidemia which is one of its complications. Diabetic dyslipidemia is mainly characterized by higher serum levels of triglyceride (TG), lower high density lipoprotein (HDL) and high small dense LDL levels [31,32,33,34]. Induction of oxidative stress is a key process in the pathogenesis of Diabetes Mellitus and its complications, and the role of antioxidants in treating diabetes and its complications has been evaluated [35,36,37,38]. Due to increasing cases of metabolic diseases, there is a renewed interest in understanding the anti-diabetic benefits of Andrographis paniculata in the eastern part of Nigeria where there is little or no knowledge of its ethnobotanical usage.

Aim of the Study

The aim of this research was to evaluate the effect of Andrographis paniculata on the lipid profile of alloxan induced diabetic albino rats.

MATERIALS AND METHODS

PLANT MATERIALS: Fresh leaves of Andrographis paniculata plant that was used in this study was procured from a garden in Uli, ihiala Anambra State, and identified by Dr Ogbuozobe. A, a Taxonomist in Botany Department Nnamdi Azikiwe University Awka with a Voucher no NAUH/65A for future reference.

Preparation of Plant Material Extract. The leaves of the plant was first thoroughly washed with distilled water and allowed to dry under shade with optimal ventilation. The dried leaves were then chopped to coarse powder. The coarse powdered plant material was soaked in 80% ethanol for 72 hours and then the extract was filtered using whatman filter paper. Then, the residue was remacerated two times with fresh solvent, each for 72 hours, and the filtrates obtained from the successive maceration were dried in a hot air oven at 40 degree centigrade. The dried extract was then kept in a desiccators to maintain dryness till used in the experiment.

ANIMAL MATERIAL: Male Wistar rats weighing between (25-30g and age of 8-12 weeks) were used for the study. The rats were obtained from an animal House of pharmacognosy Department,

faculty of pharmaceutical sciences Agulu. The rats were fed with rat pellets and water ad libitum. The normal rat feed used in this study was grower's mash of top feed, produced by premier mill company, Calabar, Nigeria. The animals were acclimatized to the laboratory conditions for I week before the initiation of the experiment.

Grouping and Dosing of Animals

After acclimatization, the rats were randomly distributed into six groups of five rats each as follows:

Groups	Treatment
A	Treated with 10ml/kg distilled water (Diabetic Negative Control)
B	Treated with 100mg/kg plant extract (Diabetic test group)
C	Treated with 250mg/kg plant extract (Diabetic test group)
D	Treated with 500mg/kg plant extract (Diabetic test group)
E	Treated with 5mg/kg glibenclamide(Diabetic Positive Control group)
F	Treated with10ml/kg distilled water (Normal Control)

Glibenclamide was selected as a standard drug based on reports of previous studies. The three doses of the plant extract were determined based on the acute oral toxicity study result. Oral route of administration was used in the study because people traditionally use the plant material orally. All the doses were given using an oral gavage after dissolving the plant extract in distilled water at a volume not exceeding 10ml/kg weight of the rat.

ACUTE TOXICITY STUDY ACCORDING TO THE METHOD OF [24]

Procedure:

On the first day of the test, the rats were fasted for 3 hours before administering the plant extract. This was carried out in two phases which are phase 1 and 2 respectively.

Phase l

This requires nine animals. The nine animals were divided into three groups of three animals each. Each group of animals was administered different doses (10mg/kg, 100mg/kg and 1000mg/kg) of the plant extract. Then the rats were kept under strict observation for physical or behavioral changes for 24 h, with special attention during the first 4 hours to check mortality as well.

Phase 2

This requires four animals. The four animals were divided into four groups of one animal each. Each group of animals was administered different doses (2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg) of the plant extract. Then the rats were kept under strict observation for physical or behavioral changes for 24 h, with special attention during the first 4 hours to check mortality as well. The observation continued for a total of 14 days for any sign of toxicity and mortality.

INDUCTION OF EXPERIMENTAL DIABETES.

Procedure

Alloxan was first dissolved in 0.1M cold citrate buffer (pH=4.5). Rats were fasted overnight for 16 hours prior to Alloxan administration .Then the freshly prepared solution was given intraperitonially to the rats at a dose of 150mg/kg. The animals were allowed food and water thirty minutes after the administration of Alloxan. Six hours after Alloxan administration, animals were allowed to drink 5% glucose solution for the next 24 hours to prevent hypoglycemic shock and death. Four days after Alloxan induction, the animals were screened for diabetes.

LIPID PROFILE

Serum triglyceride, HDL Cholesterol and LDL Cholesterol were determined by the methods of [3,5].

Statistical Analysis

All the data were expressed as mean ± standard error of the mean (SEM). Between and within group analysis were carried out using one-way ANOVA. The results were considered to be significant when the P-value was less than 0.05. SPSS Version 20 software was used for data processing and analysis.

RESULTS

Table 1 Result of acute toxicity study of *Andrographis paniculata* Ethanolic leaf extract.

PHASE1 Dosage (mg/kg)	No of death	PHASE2 Dosage (mg/kg)	No of death
10	0/3	2000	0/1
100	0/3	3000	0/1
1000	0/3	4000	0/1
		5000	0/1

The acute toxicity study of *Andrographis paniculata* leaf extract did not show mortality in the rats at the limit dose of 5000mg/kg during the observation period. Thus, the median lethal dose (LD50) of the leaf extract is greater than 5000mg/kg. Besides, the toxicity study of *Andrographis paniculata* did not show any sign of toxicity: Behavioral, neurological or physical changes. This project result shows that with the lethal dose of the plant greater than 5000mg/kg, it has a wide margin of safety.

Table 2 Effect of daily dose of *Andrographis paniculata* Ethanolic leaf extract on serum lipid level of alloxan induced diabetic rats.

Group	LDL mg/kg	HDL mg/kg	TG mg/kg
Diabetic control	146.2±5.87	29.6±2.54	208.1±5.89
AP 100mg/kg	129.5±6.87*	34.13±2.66	193.2±4.91
AP 250mg/kg	124.3±4.13*	37.86±2.11*	184.2±3.33*
AP 500mg/kg	119.8±3.86*	41.2±1.26*	167.8±10.1*
GLC 5mg/kg	112.7±3.34*	39.6±3.44*	88.1±7.86*
Normal control	102.6±2.34*	43.2±4.26*	72.4±8.11*

Each value represents mean± SEM; n=6 for each treatment. AP=*Andrographis paniculata,* DW= distilled water, GLC= glibenclamide, *stands for significant (P<0.05). The result above on lipid profile indicated that administration of all the three doses (100mg/kg, 250mg/kg, and 500mg/kg) of *Andrographis paniculata* for 14days significantly reduced the level of LDL and TG when compared with the control group. There was also a significant increase in the HDL level when compared with the control group.

DISCUSSION

Diabetes is one of the largest global health emergencies of the 21st century [8]. There is need for safer, more effective, and less costly treatment as currently available drug of diabetes mellitus have limitations. Long –term safety should be targeted for patients with coexisting diabetes and dyslipidemia.

Acute toxicity test (LD50) of *Andrographis paniculata*

The result on the research study Table 1 revealed that the median lethal dose (LD50) of the plant extract is greater than 5000mg/kg showing a wide margin of safety. This was in line with the report by [24]. Based on the result, the plant derived compounds for Diabetes Mellitus are believed to be safe and this is in line with [32] although the plant is not easily accessible in the eastern part of the country.

Lipid profile test result

The result of the lipid profile in Table 2 indicated that the alloxan induced diabetic rats manifested significant ($p<0.05$), decrease in the level of triglycerides, total cholesterol, low density lipoprotein and significant decrease in high density lipoprotein when compared to diabetic control. The results showed that diabetic control was146.2±5.87, 29.6±2.54, 208.1±5.89, for LDL, HDL and TG respectively while the *A. paniculata* treated groups (500mg/kg) showed119.8±3.86*, 41.2±1.26*, 167.8±10* for LDL, HDL & TG respectively This was in line with (Wu *et al*, 2014) who reported that Lipid abnormality is also one of the complications of diabetes mellitus, manifested mainly by high serum TG, TC, and low HDL-C. This shows that *Andrographis paniculata* leaves extract could reduce the risk of developing cardiovascular diseases such as congenital heart disease, coronary artery disease, myocardial infarction, stroke etc.

CONCLUSION

In conclusion, the present study clearly showed that *Andrographis paniculata* crude extract had a very great potential as drug alternative in diabetic patients and related disorder.

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