



SHORT COMMUNICATION

PHYTOCHEMICAL SCREENING AND HYPOGLYCEMIC ACTIVITY OF *Nauclea latifolia* and *Spondiamombin* EXTRACTS IN ALLOXAN-INDUCED DIABETIC RATS.

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Abstract

Medicinal plants have been used since time immemorial for treatment and management of diseases. This work sought to investigate the phytochemical present and hypoglycemic ability of *Nauclea latifolia* and *Spondia mombin* extracts in alloxan-induced diabetes. Diabetic was induced by single intraperitoneal injection of alloxan monohydrate (100mg/kg). Normal and diabetic rats were divided into seven groups to receive different doses of *Nauclea latifolia* and *Spondia mombin* extracts for twenty-eight days. The hypoglycemic effects were monitored on blood glucose and oral glucose tolerance test (OGTT). Phytochemical screening of the extracts showed the presence of tannins, steroid, resin, phenol, glycoside, flavonoid and cardiac glycoside. The extracts were able to significantly ($p < 0.05$) lower the blood glucose level and improved the OGTT of the diabetic rats. Therefore, it could be concluded that extracts of *Nauclea latifolia* and *Spondia mombin* possess antihyperglycemic agent which could be used in the management of diabetes mellitus.

Keywords: Phytochemicals; *Nauclea latifolia*; *Spondia mombin*; Diabetes

Introduction

Diabetes mellitus (DM), a metabolic disorder characterized by persistent hyperglycaemia is affecting people in both developed and developing countries. If it is not effectively controlled, it can cause blindness, kidney failure or nerve damage. It is a major public health problem and has become a menace globally. According to International diabetic federation (IDF), 425 million people are currently suffering from diabetes mellitus and the number is estimated to rise to 642 million by the year 2040 and it was also estimated to cause the mortality of a person every 6 s in year 2015

(IDF 2017). Even with considerable progress in the treatment of diabetes by oral hypoglycemic, the overall toxicity of insulin therapy and other oral hypoglycemic agents have necessitated the search for more effective and safer antidiabetic drugs (Rao *et al.*, 1997). Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely in used, and have considerable importance in international trade (Ahmed *et al.*, 2006).

Nauclea latifolia (NL) is a member of the Rubiaceae family commonly known as pin cushion tree is a straggling shrub or

small tree native to tropical Africa and Asia. Parts of the plant are commonly prescribed traditionally as a remedy for diabetes mellitus (Okwori *et al.*, 2008; Ameh *et al.*, 2009).

Spondia mombin Linn (SM) is a member of Anacardiaceae family. Its leaves and fruits are mostly used in treating various diseases including diarrhea, allergies and microbial infectious (Okolie *et al.*, 2008) and also in brain disorder (Ayoka *et al.*, 2005, Ayoka *et al.*, 2006). The volatile and non-volatile plant extracts have also been reported to have antioxidant, cytotoxic and antidiabetic activities (Iweala *et al.*, 2011, Oladimeji *et al.*, 2016). With the high rate of diabetes mellitus in the society, there is need to scientifically justify the use of some medicinal plants to gain global recognition. Hence, the study is designed with the aim to evaluate the phytochemical components and hypoglycemic property of NL and SM in Alloxan-induced diabetic rats.

Materials and Methods

The leaves of *Spondia mombin* and root of *Nauclea latifolia* were gotten from Ilorin west Local Government, Kwara State. The plant was identified and authenticated in the Department of Plant biology, University of Ilorin where a Voucher specimen (Unilorin Voucher No 106132) was deposited at the Herbarium of the University.

Preparation of extracts

The leaves of *Spondia mombin* and chopped roots of *Nauclea latifolia* were air dried for two weeks and pulverized using waring blender. 500g of each ground samples were soaked with 3L of absolute methanol. The mixtures were allowed to stay for 3 days and filtered using Whatman No 2 filter paper, the filtrates were concentrated under reduced pressure using rotary evaporator. The concentrated extracts were used for the research.

Phytochemical analysis

The extracts were evaluated for the presence of alkaloid, tannins, glycoside, saponin, steroid, flavonoid, resin, phenol, carbohydrate and anthraquinones using simple qualitative methods (Sofowora 1984) and (Harborne 1993).

Test for Saponins

Extracts (0.2 g) were dissolved in 5ml distilled water. Two millilitres of the resulted solution were taken into a test tube and was shaken vigorously for a few minutes frothing which persist on warming was taken as an evidence of the present of saponins.

Test for flavonoids

Extract (0.2 g) was dissolved in (50%) NaOH. A yellow solution that turns colourless on addition of 50% dilute HCl acids indicates the presence of flavonoids.

Test for alkaloids

0.2 g of sample of methanol extract was acidified with 1% HCl for 2 minutes and was treated with few drops dragendorffs reagents in a test tube. The formation of white precipitate indicates the presence of alkaloid.

Test for tannins

Methanol extract (0.2 g) was stirred with water and filtered. A dirty green precipitate or blue black or blue green precipitate on addition of few drops of 5% Ferric Chloride (FeCl_3) to the test extract was taken as an indication of the presence of tannins.

Test for glycosides

Five millilitres of H_2SO_4 was added to 0.2 g sample of the extract, the mixture was heated in boiling water for 15 minutes. Fehling solution was then added and the resulting mixture was heated to boiling. A brick-red precipitate indicates the presence of glycosides.

Test for steroids

Extract (0.2 g) was dissolved in 2ml of chloroform. Concentrated H_2SO_4 (0.2ml) was carefully added to form a lower layer; a

reddish brown at the interface between the layers indicates the deoxy-sugar characteristic of cadenolides which indicated the presence of steroids.

Test for cardiac glycosides

Extract (0.2 g) was dissolved in 2ml glacial acetic acid containing one drop of Ferric chloride solution. This was then reacted with 1ml concentrated sulphuric acid. A brown ring at the interface indicates the presence of deoxy-sugar characteristic of cardiac glycosides.

Test for phenols

Sample (0.2 g) of test extracts was dissolved in 2ml ferric chloride solution, blue-black or brown colouration indicates the presence of phenol.

Test for anthraquinones

0.2 g sample of the test extracts were shaken with 4ml of benzene. The mixture was filtered and 2ml of 10% ammonia solution was added to filtrate. The mixture was shaken and the presence of a pink red or violet colour in ammonical solution (lower phase) indicated the presence of free anthraquinones.

Animal study

Thirty healthy male Albino rats of Wistar strain, weighing 95±5g were obtained from animal house of the Department of Biochemistry, University of Ilorin, Nigeria. They were kept in clean cages in a well-ventilated house with free access to food and water. The animals were used according to the guidelines of National Research Council Guide for the care and use of laboratory animals (National research council 2011) and in accordance with the principles of good laboratory procedure (WHO 1998). The laboratory animals were completely randomized into six groups (A-F) of five rats each and were acclimatized for a period of seven days before being made diabetic by alloxan induction.

Induction of diabetes mellitus

The animals were fasted overnight prior to the induction of diabetes. Alloxan monohydrate, freshly prepared in normal saline, was immediately injected intravenously (150 mg/kg body weight) through tail vein to induce diabetes (El-Alfy et al 2007). After 72 hours for development of diabetes, blood glucose was measured with one-touch glucometer and rats with blood glucose level of 300mg/dl and above are considered diabetic and used in this study.

Experimental design

After successful induction of diabetes, control and diabetic rats were divided into 7 groups of at least 6 animals. Group A served as control, Group B served as diabetic control, Group C-F was given 0.5ml graded dose of NL and SM extracts. Group G served as standard drug group and were given Glibenclamide. The effect of the extracts was studied for a period of twenty-eight days by monitoring the changes in their blood glucose level at seven days intervals. Blood glucose level measurements were conducted weekly during the experiment using one-touch glucometer

Statistical analysis

Data are presented as mean ± SD. The significance of the differences between the means of the samples were established by the analysis of variance (ANOVA)

Results

The result of the phytochemicals screening is presented in table 1. *Spondia mombin* showed the presence of alkaloid, tannins, glycoside, saponin, steroid, resin, carbohydrate and anthraquinones while the presence of phenol, flavonoid, tannins, resin, steroid and glycoside was confirmed in NL (Table 1). Many experimental findings have shown that phytochemical can prevent or cure many chronic diseases due to their antioxidant, anticancer, anti-inflammatory and

antimicrobial properties (Sucupira et al., 2012). One of these phytochemical components might be responsible for the hypoglycemic activity of the extracts or they acted synergistically.

The result of the effect of SM and NL on the blood glucose level of diabetic rats is presented in table 2. The induction of

experimental diabetes led to significant ($p < 0.05$) increment in the blood glucose level of diabetic rats when compared with the control. However, the oral administration of extracts after 28 days was able to significantly ($p < 0.05$) lower the elevated blood glucose level of diabetic rats.

Table 1: The phytochemical composition of SM and NL

	SM	NL
Alkaloid	+	-
Tannins	+	+
Saponins	+	-
Steroid	+	+
Flavonoid	-	-
Resin	+	+
Phenol	-	+
Anthraquinones	+	-
Glycoside	+	+
Cardiac glycoside	-	-

+ = presence, - = absence. SM= *Spondia mombin*; NL= *Nauclea latifolia*

Table 2: Effect of Methanol extracts of SM and NL on blood glucose level in alloxan-induced diabetic rats

	Day 0	Day 7	Day 14	Day 28
Control	87.0 ± 2.50	80.0 ± 3.10	89.4 ± 3.05	82.0 ± 4.80
Diabetic control	306.0 ± 12.20 ^a	312.1 ± 8.80 ^a	341.3 ± 10.4 ^a	332.1 ± 9.20 ^a
SM (200mg/kg)	339.6 ± 13.80 ^a	213.3 ± 5.40 ^{ab}	211.3 ± 4.30 ^{ab}	145.3 ± 3.40 ^{ab}
SM (400mg/kg)	509.3 ± 15.50 ^{ab}	316.0 ± 10.40 ^a	257.2 ± 7.80 ^{ab}	146.8 ± 4.00 ^{ab}
NL(200mg/kg)	307.0 ± 10.10 ^a	329.8 ± 9.80 ^a	256.4 ± 5.50 ^{ab}	275.3 ± 3.89 ^{ab}
NL(400mg/kg)	503.3 ± 17.50 ^{ab}	292.0 ± 6.90 ^a	256.3 ± 3.20 ^{ab}	225.2 ± 4.30 ^{ab}
Glibenclamide	323 ± 11.10 ^a	380.7 ± 6.60 ^a	185.8 ± 3.70 ^{ab}	110.3 ± 3.90 ^{ab}

Values are expressed as Mean ± SD. SM= *Spondia mombin*; NL= *Nauclea latifolia*. a $p < 0.05$ when experimental groups were compared with control group, b $p < 0.05$ when experimental groups were compared with diabetic control group

Alloxan monohydrate administration has been shown to cause pancreatic islet-cells damage in different experimental animal models, thus leading to decreasing the

secretion of insulin and hyperglycemia (Szkudelski 2001), hence, its choice in the present study. The action of this cytotoxic

agent is mediated by reactive oxygen species on pancreas. In this present work, oral administration of NL and SM extracts were able to reduce the elevated blood glucose level of the alloxan induced diabetic rats. The reduction in the elevated blood may be due to the glucose lowering mechanism of the extracts which might be through stimulation of either surviving β cells or regenerated β cells of islets of Langerhans to release more insulin from the pancreas (Olaiya and Karigidi 2016). This stimulatory effect on the remnant β cell might be due to the vast presence of phytochemicals in the extracts as many studies had proven phytochemicals as hypoglycemic agents (El Barky et al 2017; Sarian et al 2017).

Conclusion

It can be concluded that methanol extracts of NL and SM possess hypoglycemic active compound which could be used in the management of diabetes. In addition, *Nauclea latifolia* showed better activity than *Spondia mombin*. Further research is suggested to determine the mechanism of action of the extracts.

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