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HYPOGLYCAEMIC AND SECONDARY COMPLICATION AMELIORATING EFFECTS OF SOLVENT FRACTIONS OF BAUHINIA THONNINGII IN EXPERIMENTAL DIABETIC RATS

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ABSTRACT

Background: ***Bauhinia thonningii*** **is a flowering plant of African origin with scientifically proved medicinal values of the crude extracts. This work was designed to study the effects of solvent fractions of** ***Bauhinia thonningii*** **leaves on fasting glucose level and blood lipid parameters in alloxan-induced diabetic rats; towards isolation and characterization of the pure active compound.**

Design: **Hundred milligram per kilogram body weight (100 mg/kg bw) of four different solvent fractions of the plant (n-hexane, chloroform, methanol, ethyl acetate) were orally administered to randomly allotted diabetic rats (≥200mg/dl) for 12 days. Blood samples were collected for estimation of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Triglycerides and Coronary Risk Index (CRI).**

Results: **Results showed that chloroform and ethyl acetate fractions reduced fasting blood glucose to a level comparable with reference drug (glibenclamide). Coronary Risk Index, which is a measure of the tendency of a cardiovascular disorder, was also improved by ethyl acetate fractions of this plant. These results suggest that ethyl acetate and chloroform fractions of** ***Bauhinia thonningii*** **have a potent hypoglycaemic ability and are capable of reversing hyperglycaemic secondary complications. Also, results showed that the bioactive molecules are cations.**

Conclusion: **Findings from the study revealed that the active compound(s) are located in these fractions paving way for isolation and characterization of the active compounds.**

INTRODUCTION

Diabetes mellitus is a major metabolic disorder for which no suitable permanent treatment has been found in spite of the advancement in medical research. The myriad of the probable aetiology further complicates the condition and casts a 'gloom' on the search for a cure. This terminal ailment presents with secondary complications that heighten the morbidity / mortality and further worsen the prognosis (1). The morbidity, ensuing complications and death are closely linked with hyperglycaemia which, mostly, is the first clinically noticeable symptom. Diabetes-induced hyperglycaemia is closely followed by, sometimes goes together with, other derangements in protein and fat metabolism (2). The cascade of events culminates in disease conditions affecting vital systems/organs in the body including the cardiovascular system. Hence a holistic approach to managing diabetes will not only include initiating steps to reduce blood glucose level but also prevent the aftermath of the deranged metabolism or ameliorate the resulting conditions if already on course (3).

The increase in the prevalence of diabetes has been a challenge in the area of drug development (4). Though there are approved drugs in use, adverse drug reactions arising from long-term administration sometimes aggravate the situation. Furthermore, no single agent has been sufficient to manage this metabolic disorder and the myriads of complications. This often necessitates polypharmacy with its attendant patient compliance with the drugs. Research efforts are therefore targeted at solving the compound problem with a single agent. This no doubt will encourage compliance and improve prognosis. In the light of this, drug development from medicinal plants has been on the increase. In recent times, however, only a few of such plants have been scientifically validated through preclinical studies or clinical trials. A number of them are available as food supplements .This notwithstanding, natural remedies have continued to gain recognition over and against allopathic therapy probably due to belief in natural-is-safer. Another reason for this trend could be because of the flexibility it affords in terms of administration (5).

Bauhinia thonningii is a flowering plant belonging to the family of legumes commonly found growing in Africa and Madagascar. Aqueous crude leaf extract of the plant has been shown to possess hypoglycaemic and hypolipidaemic effects (6). The present study aimed at investigating the beneficial effects of fractions of the plant. This includes hypoglycaemic and Coronary Risk Index (CRI) effects. CRI is a measure of capacity to alleviate cardiovascular disorder, a complication of diabetes. This is a milestone towards isolation and characterization of the active pure compound(s).

Hyperlipidaemia has been documented as a major complication of diabetes following hyperglycaemia, a major hallmark of the metabolic disorder. Empirically, medicinal plants have been used to ameliorate hyperglycaemia and hyperlipidaemia. Hypoglycaaemic effect of the plants has been attributed to three mechanism- increase in insulin through restoration of pancreatic function (secretagogues/mimetics), inhibition of intestinal glucose absorption and enhancement of metabolites in insulin dependent process. These actions have been documented to be linked to the presence of phytochemicals such as flavonoids, glyciosides, tannin, terpenoids and a host of others (7). In laying credence to drug development of antidiabetics from medicinal

plants, Patel *et al.* (8) opined that, comparatively, they are safer alternatives.

MATERIALS AND METHODS

Plant Sample Preparation and Fractionation: The plant was sourced from the Botanical garden of the University of Ibadan, Nigeria. Authentication and voucher deposition was carried out at the Forestry Research Institute of Nigeria (FHI 108865). Three hundred (300) grams of air-dried pulverised leaf of *Bauhinia thonningii* was serially extracted for 72 hrs each with n-hexane, chloroform, ethyl acetate and methanol, respectively; in order of polarity of the solvents. The yields were concentrated using Rotary evaporator and labelled as n-hexane, chloroform, ethylacetate and methanol fractions. The Percentage yield of each fraction was calculated.

Experimental Design: The care and handling of experimental animals were as prescribed by the Institution Review Board (DEL/FBMS/0902/6102. Experimental diabetes was induced in Wistar albino rats by single sterile intra-peritoneal administration of 130mg/kg alloxan monohydrate. Rats with blood glucose ≥200mg/dl were taken as diabetic rats used in the study. Diabetic rats were randomly allotted into groups (5 animals per group). Aside from the non-diabetic and diabetic control groups, rats in other groups were administered 100mg/kg of each fraction once daily for 12 days. Glibenclamide (10mg/kg) was the reference hypoglycaemic agent, administered to another group, once daily. Fasting blood glucose level was checked two days apart throughout the period of the experiment. Twenty-four hour after the last administration, blood samples were obtained from the rats and assayed for some clinical chemical parameters.

Biochemical Analyses: Total cholesterol was estimated by the method of Allain *et al* (9). High-density lipoprotein cholesterol was assayed as described by Warnick and Albers (10). Serum triglycerides were estimated by the method of Fossati and Prencipe (11). Low-density lipoprotein cholesterol was calculated from serum lipid parameters above using Friedewald *et al* (12) method.

RESULTS

Results of percentage solvent fractions yield are presented in Table 1. Methanol solvent produced the highest yield, while others were within a close range below 2%.

Effects of administration of fractions of the plant on fasting blood glucose of diabetic rats are presented in Table 2. Ethyl acetate fraction caused 270% reduction in fasting blood glucose over a period of 12 days while chloroform fraction caused 210% reduction in fasting blood glucose. The effects of ethyl acetate and chloroform fractions were higher compared to the standard drug, glibenclamide, which produced 146 % reduction (see Table 2).

Table 1

Percentage yield of solvent fractions of Bauhinia thonningii

Solvent fraction	Weight (g)	Percentage yield
Methanol	16.08	69.91
Ethylacetate	1.75	7.61
n-hexane	1.10	4.78
Chloroform	1.92	8.34

Table 2

Effect of fractions of Bauhinia thonningii on fasting blood glucose level in hyperglycemic rats

Group/Treatment	Glucose Level Day 0 (mg/dl)	Glucose Level Day 3 (mg/dl)	Glucose Level Day 6 (mg/dl)	Glucose Level Day 9 (mg/dl)	Glucose Level Day 12(mg/dl)	Change in glucose level (day 0 to day 12)	% Change day 0-day 12
Non-Diabetic	81.60±12.38	81.33±15.01	94.34±11.59	92.67±9.29	90.80±15.06	9.20	10.13
Diabetic not treated	330.67±55.37	368.43±29.6 2	397.45±45.25	400.34±77.23	479.33±98.84	148.66	31.01
Diabetic treated with Glibenclamide 10mg/kg	437.50±39.30	417.67±56.21	314.33±81.40	294.43±61.04	177.75±28.99	-259.75	-146.13
Diabetic treated with 100mg/kg ethyl acetate fraction	512.33±48.21	325.34±77.06	217.67±41.90	203.52±84.11	138.33±11.05	-374.00	-270.37
Diabetic treated with 100mg/kg methanol fraction	311.33±97.25	372.34±55.34	317.56±62.68	370.67±75.04	310.46±59.68	-0.87	-0.28
Diabetic treated with 100mg/kg n-hexane fraction	423.67±72.47	439.89±89.74	405.43±53.47	398.33±82.65	472.67±46.37	49.00	10.37
Diabetic treated with 100mg/kg chloroform fraction	532.67±90.19	370.67±69.50	313.33±33.54	205.43±70.64	171.33±98.46	-361.34	-210.90

Table 3 shows the lipid profile and Coronary Risk Index (CRI) in rats administered the fractions of *B. thonningii*. The CRI of rats administered ethyl acetate (2.12) was

comparably similar to the rats administered the reference drug, glibenclamide (1.70).

Table 3

Effects of fractions of B. thonningii Lipid profile and Coronary Risk Index of diabetic rats

Group/Treatment	Total Cholesterol(TC) (mg/dl)	LDL (mg/dl)	High Density Lipoprotein (HDL) (mg/dl)	Triglycerides	LDL/HDL	CRI (TC/HDL)
Non-Diabetic	100.91±12.44	145.46±19.01	87.19±8.01	63.17±0.19	1.67	1.16
Diabetic not treated	120.51±0.88	137.36±18.58	25.46±11.74	109.02±2.05*	5.40*	4.73*
Diabetic treated with Glibenclamide	109.12±4.76	98.15±4.03	64.78±11.46	65.81±1.07	1.51	1.68
Diabetic treated with ethylacetate fraction	127.57±23.12	123.15±13.40	60.68±17.64	80.77±3.05*	2.03	2.10
Diabetic treated with methanol fraction	125.94±14.72	143.29±31.30	27.56±12.29	74.05±2.65*	5.60*	4.57*
Diabetic treated n-hexane fraction	121.78±7.56	74.15±9.15	24.85±0.91	75.09±1.39*	2.98	4.90*
Diabetic treated with chloroform fraction	127.29±9.36	133.85±7.39	40.53±7.79	65.27±4.51	3.30	3.14*

Results are presented as mean ± standard deviation (n=5). *Significantly different compared with the non-diabetic rats at $p<0.05$

DISCUSSION

The hallmark of diabetes is hyperglycaemia. Poor management or late intervention may cascade to dysfunction and derangement of a network of metabolic processes in the body. Since numerous metabolic processes take place in the body simultaneous, it is not surprising that vital organs and systems are affected. Valuable therapeutic plan in the management of diabetes, therefore, involve controlling post-prandial hyperglycaemia on one hand and reducing hyperlipidaemia. The results obtained in this study showed the possibility of this treatment strategy with the hypoglycaemic and hypolipidemic effects of some of the fractions in the diabetic state.

The beneficial effects of medicinal plants have been attributed to the presence of secondary metabolites (13). It was opined that secondary metabolites (natural products) found in medicinal plants have been a core

part of ancient traditional medicine systems (14). In this study, we assessed the study the effects of solvent fractions (ethyl acetate, methanol, hexane and chloroform) of *Bauhinia thonningii* leaves on fasting glucose level and lipid profile in alloxan-induced diabetic rats. Different solvents have extraction efficiencies (15) and produced different levels of biological activities. Methanol produced the highest yield compared to other solvents used in the study; this substantiated the preference of alcohol in ethnomedicinal use of many plants.

Dyslipidemia is a metabolic outcome of diabetes mellitus characterized by abnormalities in lipoprotein concentrations with high levels of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C) and low levels of high-density lipoprotein-cholesterol (HDL-C) (16) which was noticed in alloxan-induced diabetes in this study. Alloxan diabetic effect on lipid homeostasis is substantiated by several other studies (17,18,19). These abnormalities are responsible for cardiovascular-related morbidity and mortalities with increasing fold in diabetes (20). Regulation of dyslipidemia by ethyl acetate and chloroform fractions of *B. thonningii* could minimize progression to cardiovascular disorder in a diabetic state. Variation in the effect of these fractions on dyslipidemia in the diabetic rats could be ascribed to the partitioning and distributions of associated phytochemicals in different solvents. Different plant extracts have been reported in several studies to produce varying hypoglycaemic or antidiabetic properties (6, 21).

Isolation of bioactive molecules in plants is pivotal in drug discovery from medicinal plants. This paves way for the synthesis of drugs in the laboratory and serves as a template for synthesis of bioactive compounds. It has been documented that solvent play an important role in extraction of bioactive molecules (22). This may not be unconnected with the polarity of the bioactive molecules influencing solubility of the molecule in liquid phase solvents.

The polarity of any solvent is a determining factor on the type of compounds it can dissolve. It also determines its miscibility with other solvents. Taken together, polar solvents will dissolve polar molecules and will be miscible with other polar solvents. An exception to the rule is chloroform, a non-polar solvent miscible with water (polar solvent). Furthermore, aprotic solvents will solvate cations while protics will solvate anions (23). The results obtained in this study are in agreement with the findings of Annapandian and Sundaram (24). They concluded that the non-polar extracts of the plant under study showed higher antidiabetic activity than the polar extracts. It may be inferred from the foregoing, as it relates to our study, that the active bioactive molecules with hypoglycaemic effect dissolved in solvents with mid-polarity (ethyl acetate and chloroform) while the bioactive molecule with CRI lowering /hypolipidaemic effects solvate in a solvent with mid polarity (chloroform) and that is miscible with water. Furthermore, it could be inferred that the bioactive molecules are cations.

Studies have shown that phytochemical such as phenols and flavonoids linked with hypoglycaemic and hypolipidaemic effects solvate in solvents with mid-polarity (25, 26). Based on this, it may be inferred that phenols phytochemicals in the plant under study in our work are phenols and flavonoids. This is so since they solvate in mid-polar solvents and showed hypoglycaemic and hypolipidaemic effects.

CONCLUSION

In conclusion, fractions of *Bauhinia thonningii* exhibited hypoglycemic effect with the noteworthy effect seen in ethyl acetate and chloroform fractions. The bioactive molecules could be adjudged to be cations. This revelation paves way for further isolation, purification and characterization of the bioactive molecules. Furthermore, this could be a lead in the optimization process of drug discovery for this metabolic disorder, from this plant.

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