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RESEARCH ARTICLE

Anti-diabetic Effects of Methanol Extract of the Seeds of *Buchholzia coriacea* and its Synergistic Effects with Metformin

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ABSTRACT

Diabetes Mellitus (DM) has an estimated world prevalence of 285 million adults with high morbidity and mortality rates especially in the developing countries. The preparations of the seeds of *Buchholzia coriacea* commonly known as wonder kola is used for the management of DM in Nigerian ethnomedicine. In view of this, we studied the antidiabetic effects of the methanol extract of the seeds to scientifically ascertain the folkloric use. Alloxan induced diabetic rats were used in the study. Determination of the blood glucose concentration was done using the One Touch Ultra mini glucometer. Also acute toxicity test was performed using the Lorke's method. Result showed an oral median lethality dose (LD_{50}) greater than 5000 mg/kg, an indication of high safety profile. The extract at 100, 200, 400 mg/kg doses exhibited percentage blood glucose reduction (PBGR) of 37.73, 12.30 and 11.30 % respectively after 4 hours treatment. The combination of extract (100 mg/kg) and metformin (100 mg/kg) gave a PBGR at 4th and 7th day treatment of 73.4 and 72.2 % respectively. Therefore, the extract of the seeds of *B. coriacea* has potential hypoglycaemic effects and exhibited synergistic actions with metformin, a standard oral hypoglycaemic agent.

Keywords: *Buchholzia coriacea*, Alloxan, Diabetes Mellitus, metformin.

1. INTRODUCTION

Diabetes Mellitus (DM) has an estimated world prevalence of 285 million adults (aged 20-79 years) in 2010 and a postulated prevalence of 439 million adults worldwide in 2030 [1]. This is an indication of the growing burden of DM with high morbidity and mortality rate especially in the developing countries. Reports have it that between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries [1]. DM is one of the major causes of premature illness and death worldwide. It encompasses a group of metabolic disorders characterized by hyperglycaemia and associated abnormalities in carbohydrate, fat and protein metabolism. Diabetes may result in chronic complications including microvascular, macrovascular, and neuropathic disorders [2]. The true facts about diabetes are staggering and despite the availability of standard antidiabetic agents, the rate of diabetes is quite alarming and still on the increase. DM presently remains incurable and can only be controlled and managed with drugs [3]. However, there is growing number of cases of poorly managed or controlled diabetes which often results in untimely death especially in the developing nations of the world. Various medicinal plants that are being utilized in the management of DM in traditional medicine or ethnomedicine for better, cheap and tolerable control of the blood glucose have been reported [4, 5]. Preparations of the seeds of *Buchholzia coriacea* are used in Nigerian ethnomedicine for the management of diabetes [6].

Over the years, various plant parts especially the seed of *Buchholzia coriacea* has been used in ethnomedicine and has been shown to posssess antimicrobial, antidiabetic, antiplasmodial and anthelminthic activities, hence the name; wonder kola[7, 8, 9]. This present study was designed to evaluate the antidiabetic activity of the eastern Nigeria specie of *Buchholzia coriacea* seeds and to verify possible synergistic effects with standard agents such as metformin

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(MET). This will provide a rational basis for its use in folkloric medicine in the management of diabetes mellitus.

2. MATERIALS AND METHOD

2.1. Plant materials

Mature fruits of *Buchholzia coriacea* were collected in the month of October, 2010, from Nsukka in Enugu State, Nigeria and authenticated by Mr. A. Ozioko a taxonomist, with the International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The seeds were separated from the fruits, cut into bits and dried under shade. The dried seeds were pulverized and extracted with methanol.

2.2. Experimental Animals

Adult albino rats (115 – 235 g) were obtained from the Animal house facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. They were kept in standard laboratory conditions, to acclimatize with rodent feed (Guinea Feeds Nig. Ltd.) and water *ad libitum*. The animals were starved for 12 hours prior to the commencement of the experiment. All animal experiments were conducted in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals (Publication No. 85 – 23, revised 1985) and approval of the University Ethical Committee on the use of laboratory animals

2.3. Extraction of the plant materials

The powdered plant material (800 g) was macerated in methanol for 48 hrs with intermittent shaking. The resulting filtrate was concentrated by evaporation to dryness using a rotary evaporator, under reduced pressure at a temperature of 40 ^{0}C. The crude extract obtained, *Bulchhozia coriacea* seed extract (BCE), was stored in airtight container in a refrigerator for screening studies.

2.4. Acute Toxicity Study

The estimated lethal dose (LD_{50}) of the plant extract was ascertained by the method described by Lorke [10]. In the first phase, 9 mice were divided into 3 groups of 3 mice per group, and treated with the BCE at the doses of 10, 100 and 1000 mg/kg respectively. The animals were observed for 24 hrs for signs of toxicity. The second phase 4 mice treated with BCE doses of 1600, 2800 and 5000 mg/kg, while the fourth mouse was the control. The animals were observed for 24 hrs.

2.5. Phytochemical Analysis

Phytochemical analysis was carried out on the BCE using the standard procedures outlined by Harborne [11] and Trease and Evans [12]. Briefly; frothing test for saponins, Salkowski test for terpenoids, Liebermann-Burchard tests for steroids, ferric chloride test for tannins, Keller-Killiani test for cardiac glycosides, Dragendorff's and Mayer's test for alkaloids, Fehling's test for reducing sugars, xanthoproteic test for proteins, iodine test for carbohydrates or starch and ammonia test for detection of flavonoids were performed for qualitative identification of the phytoconstituents present.

2.6 Induction of diabetes

Alloxan monohydrate (75 mg/kg) (Sigma Chemical CO, USA) dissolved in normal saline was injected intravenously through the tail vein to induce diabetes in the rats. Dextrose (5 %) was administered to overcome the early phase of hypoglycaemia [13]. Blood samples were collected by tail tipping and determination of blood glucose concentration was carried out using the One Touch Ultra mini glucometer (Life Scan Inc; Miliptal California, USA). The animal with fasting blood glucose (FBG) levels greater than 250 mg/dl were considered diabetic and selected for the study.

2.7. Acute anti-diabetic study

The alloxan induced diabetic rats were segregated randomly in six groups (A-F) of five animals per group. Group A, the diabetic control group received 0.4 ml of 10 % Tween 80 solution. Groups B, C and D received 100, 200 and 400 mg/kg of BCE (p.o.) respectively. Group E received 100 mg/kg of BCE + 100 mg/kg of metformin (p.o.) while Group F, the positive control, received 100 mg/kg of Metformin (p.o.). The treatments were done once as a single dose and the blood glucose concentration was monitored after 1, 2, 3 and 4 h of treatments.

2.8 Prolonged anti-diabetic study

The *Buchholzia coriacea* seed extract (BCE) was reconstituted in 10 % Tween 80 solutions prior to administration. The vehicle, standard agent and BCE solutions were administered orally every 24 hours (daily) over a period of 7 days for the prolonged treatment. Blood glucose concentration was monitored at the end of days 1, 4 and 7.

The percentage blood glucose reduction (PBGR) was calculated for the various times and days using the formula;

$PBGR = (BGL_0 - BGL_T / BGL_0) X 100$. Where;

PBGR = Percentage blood glucose reduction,

BGL_0 = Blood glucose level at zero hour (for acute study) or day zero (for prolonged study)

BGL_T = Blood glucose level at a particular hour (for acute study) or particular day (for prolonged study).

2.8. Statistical Analysis

Data obtained were analysed by SPSS (Version 14) using One Way Analysis of Variance (ANOVA) with Dunnet test for multiple comparisms with the control. Values are in mean ± SEM and were considered significant at $p < 0.05$.

3. RESULTS

3.1. Phytochemical Analysis

Phytochemical screening of the methanol extract of *Buchholzia coriacea* seeds revealed the presence of tannins, carbohydrates, saponins, flavonoids, saponins, resins, terpenoids and alkaloids.

3.2 Acute toxicity study (LD_{50})

After the first and second phases, no visible sign of toxicity was observed after 48 hrs of monitoring. This therefore, indicated an LD_{50} greater than 5000 mg/kg.

3.3 Acute anti-diabetic study

Results of the evaluation of acute anti-diabetic activity of rats treated with BCE showed a continuous percentage blood glucose reduction (PBGR) which peaked at the second hour of treatment and lasted till the fourth hour for the Groups that received only BCE or Metformin. While the Group E (100 mg/kg of BCE + MET) exhibited PBGR from the first hour and lasted till the fourth hour and exhibited the highest reduction of 73.4%. However the PBGR was dose related for BCE as 100 mg/kg dose exhibited the highest PBGR of 51.02 and 37.73% at the second and fourth hour respectively, while the 400 mg/kg was the least with 20.5 and 11.3% at the second and fourth hour of monitoring respectively. Metformin, a standard agent, exhibited a PBGR of 35% and 19.8% at the second and fourth hour respectively after administration.

Treatment	Fasting Blood Glucose Concentration (mg/dl)				
	0 h	1 h	2 h	3 h	4 h
Control	286.67±33.20 (0.0)	326.33±70.00 (-13.8)	346.00±21.03 (-20.7)	327.67±13.74 (-14.3)	327.33±15.07 (-14.2)
BCE 100	256.50±11.60 (0.0)	181.60±12.45 (45.1)	124.75±33.09* (51.02)	106.20±14.14 (42.1)	103.65±23.83 (37.73)
BCE 200	446.80±28.20 (0.0)	376.40±18.18 (12.2)	298.65±28.04 (20.1)	263.00±11.08 (15.0)	254.20±13.5 (12.3)
BCE 400	389.20±40.69 (0.0)	365.00±20.40 (3.5)	325.00±10.5 (20.5)	298.20±21.30 (17.8)	296.00±1.15 (11.3)
BCE 100 + MET 100	512.45±1.76 (0.0)	315.00±23.18 (47.4)	176.22±31.31 (52.1)	86.00±14.51 (70.2)	74.51±1.37 (73.4)
MET 100	452.00±2.83 (0.0)	307.50±2.76 (25.3)	204.20±21.08 (35.0)	156.30±8.84 (32.4)	129.42±2.26 (19.8)

Table 1: Effect of *Buchholzia coriacea* seed extract on alloxanized diabetic rats.

Values are expressed as the mean ± SEM using ANOVA, Dunnet as Post hoc, and significant at *p < 0.05 compared with the control; n=5. Values in parenthesis are the PBGR.

Also the control exhibited continuous rise in the blood glucose during the hours of study shown by the negative values of their PBGR (Table 1, Fig. 1).

3.4. Prolonged anti-diabetic study

After 7 days of treatment the BCE and the MET exhibited anti-diabetic activity at all the doses tested. The combined effects of BCE and MET gave a significant ($p < 0.05$) PBGR of 60% and 72% at day 4 and 7 respectively. Metformin (MET) alone showed a PBGR of 56.4 and 50.5% at the day 4 and 7 respectively, whereas BCE gave a PBGR alone of 50.7 and 40.2% at 100 mg/kg dose on day 4 and 7 respectively. However, the hypoglycaemic effect of all the doses of BCE and MET peaked at day 4 and lasted till the seventh day (Table 2, Fig. 2).

TREATMENT (mg/kg)	Fasting Blood Glucose Concentration (mg/dl)		
	Day 0	Day 4	Day 7
Control	256.56±5.21 (0.00)	261.83±12.62 (-2.05)	258.92±11.12 (-0.9)
BCE 100	267.50±11.60 (0.00)	132.00±11.15 (50.7)	160.00±35.09 (40.2)
BCE 200	476.20±45.30 (0.00)	246.20±62.75 (48.3)	257.40±46.88 (45.9)
BCE 400	303.60±37.81 (0.00)	189.00±13.32 (37.8)	245.40±53.40 (19.2)
BCE 100 + MET 100	447.25±46.49 (0.00)	175.00±62.99 (60.0)	125.25±27.57* (72.0)
MET 100	518.00±16.04 (0.00)	226.00±9.85* (56.4)	156.33±1.22 (50.5)

Table 2: Effect of *Buchholzia coriacea* seed extract on alloxanized diabetic rats

Values are expressed as the mean ± SEM using ANOVA, Dunnet as Post hoc, and significant at *P < 0.05 compared with the control; n=5. Values in parenthesis are the PBGR.

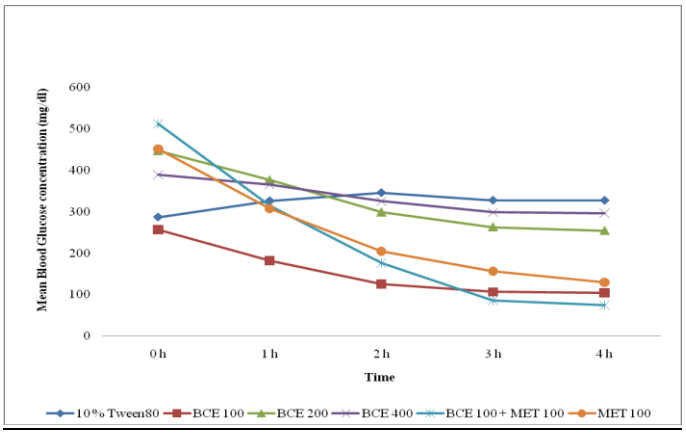

Fig. 1: Graph of blood glucose concentration (mg/dl) versus time (hours) after single dose of BCE.

The control group that did not receive BCE manifested increase in the blood glucose shown by the negative sign of the PBGR (Table 2). Remarkably the effect of the various doses of BCE, for the single and multiple administrations, showed an inverse dose related activity where the PBGR of the doses in mg/kg is the order; 100 > 200 > 400.

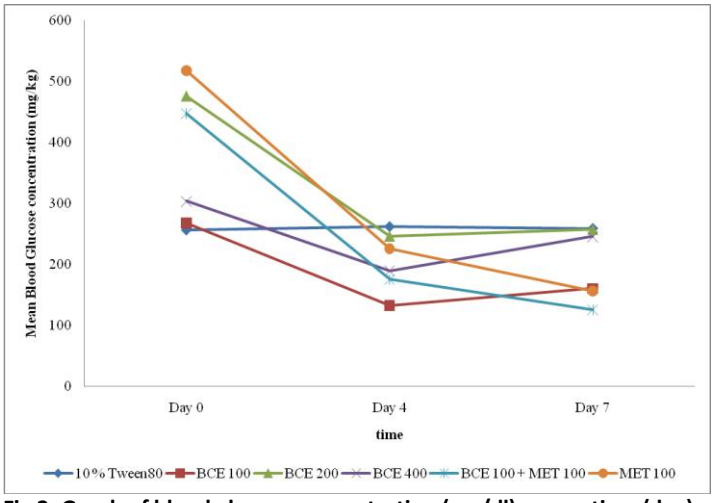

Fig 2: Graph of blood glucose concentration (mg/dl) versus time (day) after prolonged treatment

4. DISCUSSION

The extract of the seeds of *Buchholzia coriacea* has exhibited potent hypoglycaemic effects and synergistically offered better hypoglycemic activity when combined with metformin a standard agent.

In this study, diabetes mellitus (DM) was induced using intravenous alloxan, a known diabetogen, and it has been indicated that alloxan induces beta cell damage which is mediated through the generation of cytotoxic oxygen free radicals such as intracellular thiols like glutathione [3, 14]. In a separate documented study, ethanolic extract of the seeds of *B. coriacea* was found to exhibit hypoglycaemic effect on streptozotocin-induced diabetes in rodents [6]. Although chemical induction of diabetes with streptozotocin is the most widely used, alloxan-induced diabetes model seem to be the best known drug induced diabetes as it appears to be the easiest, reliable and most practicable method of inducing diabetes [15, 3].

Acute and chronic treatment on alloxan-induced diabetic rats with the methanol extract of *Buchholzia coriacea* seeds (BCE), showed a non dose dependent hypoglycaemic activity. The combination of BCE (100 mg/kg) and metformin (100 mg/kg) showed a maximum percentage blood glucose reduction (PBGR) of 72% on the 7th day of treatment with a significant ($p < 0.05$) reduction in the fasting blood glucose concentration. Metformin alone offered PBGR of 50.5% on the same day. However, on the single oral administration, the combination of the BCE (100 mg/kg) and metformin (100 mg/kg) also showed a better hypoglycaemic effect than metformin (100 mg/kg) with PBGR of 73.4% and 19.8% respectively at the 4th hour. The results of both acute and prolonged treatment on alloxan-induced diabetic rats showed a synergistic reduction in blood glucose concentration on combination of the BCE, (100 mg/kg) and metformin (100 mg/kg). However, the combined effects of BCE and metformin correlated with the synergistic hypoglycaemic effects of metformin and extract of the leaves of *Vernonia amygdalina*, found in Nigeria, which is often consumed locally as vegetable in soup [16, 15]. In addition, the results of the combined treatment with BCE and Metformin may cause a reduction in the daily dose of metformin for those patients who could be taking the seeds. This could is a possible therapeutic advantage in enhancing the compliance of the standard agent as diabetes is a chronic disease. Therefore, rural dwellers that are known diabetics and could have assess and availability of the seeds of *B. coriacea* should be encouraged towards increased consumption, at the same time clinical monitoring should be necessary for any potential adverse effects of the BCE although, the LD_{50} was estimated to be greater than 5000 mg/kg. This could also be an indication that BCE could be used in combination with other standard agents for better and effective blood glucose control. Result of this study is therefore, in consonance with that reported for the ethanol extract and butanol fraction of *Buchholzia coriacea* in normal and streptozotocin diabetic rats [6]. Metformin which is not an insulin secretagogue and therefore, not a hypoglycaemic agent, exhibits its mechanism of action by decreasing hepatic glucose production and increasing the mobilization of glucose in skeletal muscles and adipose tissues [17]. The BCE synergistically enhances the effects of metformin hence the extract may possibly be exhibiting its effects through any of these mechanisms ascribable to metformin, though the specific mechanism of action of BCE could not be ascertained at this point of the study. Moreover since alloxan is a known diabetogenic agent used to induce Type II diabetes in animals by causing necrosis of the pancreatic beta cells through the generation of free radicals [3] and the fact that BCE exhibited activity against this pathological phenomenon, one can simply extrapolate the possible antioxidant effects of the BCE. It is evident that the PBGR of the various doses of BCE which was inversely related could be due to desensitization.

Phytochemical analysis of the methanol extract of *Buchholzia coriacea* seeds revealed the presence of alkaloid, flavonoids and saponins, terpenoids, terpenes,

acidic compounds and carbohydrates. However, the hypoglycaemic effects of alkaloids and flavonoids have been reported [18]. There is the possibility that both or either of these constituents could be responsible for the claimed oral hypoglycaemic effects of *Buchholzia coriacea* seeds extract. The results of the acute toxicity test gave an oral lethality dose (LD_{50}) greater than 5000 mg/kg body weight. The lack of associated lethality with this high dose indicates a high safety profile of the extract of *Buchholzia coriacea* seeds as reported by Lorke [10].

5. CONCLUSION

The results of the study showed that the extract of *Buchholzia coriacea* seeds has potential hypoglycaemic effects and that it may be used synergistically with metformin, a standard antidiabetic agent for a better potent activity.

6. ACKNOWLEDGEMENT

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Conflict of Interest: None Declared

