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Short Communication Research Article

IN VIVO AMELIORATIVE EFFECT OF METHANOLIC EXTRACT OF *BOSWELLIA DALZIELLI HUTCH* (MEBDH) STEM BARK ON TRITON X-100 INDUCED HYPERLIPIDAEMIA

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ABSTRACT
Hyperlipidemia is a major risk factor for coronary heart diseases and ischemia that leading to high rates of mortalities. Conventionally, hyperlipidemia is managed using agents that facilitate the clearance of total cholesterol (TC), triacylglycerides (TAG) and low density lipoprotein (LDL) from the body. Despite the use of these drugs, the disease still remained a global burden that affects the quality of life. The current study was done to investigate hypolipidemic properties of the methanolic extract of *Boswellia dalzielli* hutch (MEBDH) stem bark in rodents. The phytochemicals of the MEBDH were screened and determined qualitatively before performing acute toxicity study to determine the LD_{50} of the extract. Twenty five male albino rats 2-3 months old (150-210) g were distributed randomly into five groups [Group 1: normal control received 200 µL normal saline daily for 3 weeks, Group 2: hyperlipidemic control induced by a single dose of Triton X-100 (150 mg/kg body weight) subcutaneously, followed by oral administration of 200 µL normal saline daily for 3 weeks. Group 2 and 4: hyperlipidemic rats treated orally with MEBDH (200 and 400 mg/kg body weight) respectively for 21 days. Group 5: hyperlipidemic rats treated with Simvastatin (5 mg/kg body weight) daily throughout the experimental period as positive control]. Administration of the extract did not cause any mortality regardless of the dose. However, the extract caused significant ($p<0.05$) decrease in TC, TAG and LDL-cholesterol levels in the treated rats. The decrease observed is significantly lower than that of untreated rats. In contrast, the level of HDL increased significantly ($p<0.05$) after treating the rats with the MEBDH stem bark. The extract of MEBDH possessed hypolipidemic agents and could be the potential substitute hyperlipidemic agents with side effect.

Keywords: Albino Rats, Atherosclerosis, *Boswellia dalzielli hutch,* Coronary Heart Disease, Hyperlipidemia, Triton X100.

INTRODUCTION
Cholesterol, a class of lipid plays major role in the assembly of biological membranes, brain development in children and other fundamental biological functions in animals (Khera *et al.,* 2011). Despite the integral role lipids play to sustain cellular functions, high amounts of lipid is catastrophic and leads to the medical condition known as hyperlipidemia which is a metabolic disorder characterized by increased concentrations of total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and low concentrations of high density lipoprotein (HDL) (Chapman *et al.,* 2011). Hyperlipidemia may equally induce other abnormalities like oxidation of free fatty acid that leads to the formation of ketone bodies (Maruthappan and Shree, 2010; Mishra *et al*., 2011), insulin resistance and consequently diabetes (Tang *et al.,* 2011; Bako *et al.,* 2014). It is a major risk factor for coronary heart diseases and ischemia that leading to high rates of mortalities. Clinically, statin, one of the inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase and most effective class of serum LDL cholesterol lowering drugs is associated with persistent side effects that includes diarrhea, nausea, gastric irritation and hyperuricemia (Baigent *et al.,* 2005). The goal of this work is to look for alternative treatment methods from natural sources that can circumvent the undue side effects caused by the conventional drugs.
Natural products from plants contain numerous phytochemicals that are therapeutically potent (Bulus *et al*., 2011; Abdul *et al*., 2014). For instance, plants from the *Boswella* species have been used locally for the treatment of hyperlipidemia (Mishra and Allan, 2010). Specifically, the extract of the leaves, bark and roots of *Boswella serrate*, *cartarri* and *papirifera* has been administered to hyperlipidemic patients (Ben-Yehoshua *et al*., 2012). However, the extract of *B. dalzielli* hutch species is yet to be investigated for antihyperlipidemic activities even though it contains diverse phytochemicals that may be therapeutically useful.
In the current study, we evaluated the ability of the methanolic extract of *B. dalziella* to decrease the total cholesterol,

triacylglycerides, low density lipoprotein and high density lipoprotein concentrations of triton-X 100 induced hyperlipedemic rats.

In this study, we carried out various experiments to investigate the acute toxicity and antihyperlipidemic activities of methanolic extract of *B. dalzielli* stem bark on Triton X-100 induced hyperlipidemia in rats which is considered one of the most effective *in vivo* model for antihyperlipidemic properties evaluation (Sudha *et al.,* 2011; Ja'afaru *et al.,* 2016).

MATERIALS AND METHODS

Collection of plant materials

Fresh stem bark of *B. dalzielli hutch* was purchased from a local market at Kawo, Kaduna, Nigeria and identified by a certified botanist at the Department of Biological Sciences, Ahmadu Bello University (ABU) Zaria. The stem bark was chopped into small pieces and shade dried for 3 weeks, grinded to fine particles and then subjected to extraction.

Preparation of extract

The methanolic extract of *Boswella dalzielli* hutch (MEBDH) stem bark was prepared according to the method described by Ja'afaru *et al.,* (2016). Briefly, 300g of the powdered sample was soaked in 1.25 L of 95% methanol (analytical grade) at room temperature for 72 h and filtered successively using muslin cloth and Whatman filter (GE healthcare Pte Ltd, Singapore). The extract was evaporated to dryness in a rotary evaporator at 45^{o}C and the residue was collected and stored at 4^{o}C.

Phytochemical analysis

The MEBDH stem bark was screened for the presence of saponins, tannins, flavonoids, glucosides, terpenoids, steroids, boswellic acids and alkaloids according to reagent-based phytochemical analyses described by Evans (2009).

Experimental animals and induction of hyperlipidemia

Thirty male albino rats 2 to 3 months old (150 – 210 g) were purchased from the animal unit of Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITR), Kaduna, Nigeria and used for the experiment. The rats were acclimatized in the animal house, Kaduna State University (KASU) according to the international guidelines for animal handling, and they were allowed free access to standard animal diet and water *ad libitum* throughout the experimental period. Hyperlipidemia was induced by single subcutaneous administration of Triton X-100 (150 mg/kg body weight) to overnight fasted rats. The rats with elevated serum lipid profile parameters three days post-induction were considered hyperlipidemic and used for the experiment.

Acute toxicity study

Short term toxic effect of the working extract was investigated by oral administration of the extract up to five times in multiple proportion (ranges from 250-4000 mg/kg body weight) to three rats in the acute toxicity treatment group at stipulated time interval. On the other hand, 3 rats in normal control groups received 200 µL normal saline solution (containing 9.0 g/L Sodium Chloride with an osmolarity of 308 mOsmol/L, and 154 mEq/L Sodium and Chloride at 4.5 to 7.0 pH range). The rats were kept under frequent observation for the period of 24 hours to monitor certain behavioral changes and abnormal signs including tediousness, sedation, ruffled hair, clumping together, itching, restlessness and dose dependent mortality as reported by Muhammad *et al.,* (2011) and Udem *et al.,* (2011).

Experimental design

The animals were divided into five groups that comprised five rats each. Group one was considered normal control and were administered normal saline only. Group two comprised hyperlipidemic rats administered normal saline. Groups three and four are the test groups (hyperlipidemic rats) treated with 200 mg/kg and 400 mg/kg body weight respectively. Group five was regarded as standard and the rats were administered with the standard drug, Simvastatin, at a dose of 5 mg/kg body weight, and all the treatments were carried out orally. After daily treatment for 21 days, rats were fasted overnight and blood samples were collected through cardiac puncture prior to sacrifice upon cervical dislocation. The whole blood was centrifuged, the serum were collected in a separate falcon tubes and were stored at 4ºC for further biochemical analysis.

Lipid profile assay

Serum total cholesterol (TC) and triglycerides (TGR) were evaluated according to the method described by Hyman *et al.,* (2000) and Bako *et al.,* (2014) respectively. Meanwhile, low density lipoprotein (LDL) and high density lipoprotein (HDL) were estimated in line with the procedures outlined by Baskol *et al.,* (2007).

Statistical analysis: One way analysis of variance (ANOVA) was carried out on the extracted data using SPSS statistical software version 21 (IBM Inc.), followed by Student-Newman-Keuls multiple comparison test. The result was expressed as mean ± SD and differences at $p<0.05$ were considered statistically significant.

RESULTS

Phytochemical analysis

Table 1 shows some of the major phytochemicals present in the stem bark of *B. dalzielli*. The phytochemicals include boswellic acid, saponins, tannins, flavonoids, alkaloids, terpenoids, steroids and cardiac glycosides.

Acute Toxicity test

The methanolic extract of the stem bark of *B. dalziella* was not lethal to the rats even at higher doses of about 4000 mg/kg body weight. No significant physical and behavioral changes after administration of the extract in any of the group were observed.

Table 1: Summary of phytochemical constituent of MEBDH detected qualitatively

Constituents	Indication
Steroids	+
Cardiac glycosides	+
Flavonoids	+
Boswellic acid	+
Saponins	+
Alkaloids	-
Tannins	+
Terpenoids	-

(+) stands for presence, whereas (–) stands for absence

Lipid profile assay

The serum lipid profile of the hyperlipidemic rats treated with MEBDH stem bark is shown in Table 2. The result shows that treatment of hyperlipedemic rats with MEBDH significantly decreased the concentrations of TC, TAG, LDL and VLDL in a dose dependent manner. Also, the decrease observed in the treated group is significantly higher ($p<0.05$) than that of the untreated group (control group) administered with normal saline. Similarly, the standard drug, Simvastatin, reversed hyperlipidemia in treated rats by decreasing the levels of TC, TAG, LDL and VDL and increased the level of HDL significantly ($p<0.05$) (Table 2).

Table 2: Serum lipid profile of Triton X-100 induced hyperlipidemia after 21 days of oral administration of MEBDH stem bark and Simvastatin (SVT).

Groups	Treatment Dose (mg/kg)	Lipid Profile Parameters (mg/dl)				
		TC	TAG	LDL	HDL	VLDL
n-Control	n-Saline	47.08±5.3	193.95±13.5	26.13±7.5	34.42±0.7	38.79±2.7
hl-Control	n-Saline	64.75±2.1*	423.35±8.8*	79.34±2.1*	59.41±0.8*	84.67±1.8*
MEBDH	200	40.34±3.1[a]	254.72±7.9[b]	51.11±2.3[c]	35.43±1.8[d]	53.55±1.7[e]
MEBDH	400	28.96±3.7[f]	233.44±9.6[g]	43.06±5.5[h]	25.33±1.5[i]	46.69±1.9[j]
SVT	5	38.56±9.0[k]	137.14±15.1[l]	18.78±6.9[m]	29.90±1.1[n]	27.25±3.0[p]

Results are expressed as mean ± standard deviation (SD). Values with asterisk symbol differed significantly ($p<0.050$) with normal control (n-Control) in each column. Values bearing superscripts in each column are also significantly lower ($p<0.05$) than the control (hl-Control). MEBDH: methanolic extract of *Boswellia dalzielli hutch*; SVT: simvastatin (standard drug).

DISCUSSION

The use of natural products is the mainstay of traditional medicine practiced in many developing nations of the world (Atawodi *et al*., 2011). Even though most naturally occurring secondary metabolites are therapeutically potent, the exact mechanism of action and toxic levels of many of them are yet to be ascertained. In the current study, we investigated the ameliorative effect of MEBDH stem bark on hyperlipidemia in rats and it was observed that MEBDH stem bark has anti-hyperlipidemic properties. Hyperlipidemia is a major risk factor that contributes to the development of cardiovascular diseases and is associated with diabetes, coronary heart disease and obesity amongst many others (Robinson *et al*., 2014). Clinically, hyperlipidemia is marred by increased and decreased concentrations of TC, TAG, LDL and HDL respectively (Mizuguchi et al., 2007). In our study, we observed that MEBDH significantly decreased the concentration of TC in hyperlipidemic rats in a dose dependent manner. This we presume may be due to the ability of the extract to stimulate the excretion of cholesterol. Several antihyperlipidemic drugs such as Fluvastatin, Simvastatin and Alirocumab are known to act in a similar manner. The MEBDH stem bark also caused decreased in the level of TAG and LDL in the treated rats which may have occurred via increased catabolism of TAG and LDL. The catabolism of LDL produces bile acids that is easily eliminated by the body (Pandey *et al*., 2005). This activity helps to regulate the level of LDL-cholesterol in the body.

Conversely, hyperlipidemic rats treated with MEBDH showed increased levels of HDL-cholesterol. High HDL-cholesterol level is known to protect against coronary heart diseases (Warnholtz *et al.,* 2001). It is for this reason that HDL-cholesterol is often referred to as 'good cholesterol'. The ability of the MEBDH stem bark to stimulate the biosynthesis of HDL-cholesterol in hypolipidemic rats demonstrates the therapeutic potency of the extract. Although our phytochemical analysis shows the presence of tannins, cardiac glycosides and boswellic acids amongst others. We presume that these compounds synergistically reversed hyperlipidemia in the treated rats.

Conclusion

The present study is the first of its kind to demonstrate the hypolipidemic potentials of MEBDH stem bark in rats, which brings about significant reduction in chances of developing cardiovascular complications especially atherosclerosis. Though the exact mechanism of hypolipidemic action of the extract was not established, the possible beneficial effect of phytochemicals present in the extract can be taken into consideration as they are potent antioxidants and can prevent the oxidation of Low density lipoprotein cholesterol. The study also indicated that administration of MEBDH at high dose does not acutely affect the experimental animals in all respect, with LD_{50} greater than 4000 mg/kg, revealing certain level of safety of the extract. However, acute toxicity examination does not guaranteed the safety of active principles under investigations until all the other criteria for toxicity study were fulfilled. Therefore, MEBDH stem bark showed hypolipidemic and cardio-protective properties in triton x-induced hyperlipidemic rats. The most active compounds of the extract need to be screened for potential use as a hypolipidemic agent. This study also corroborates previous findings that report the hypolipidemic properties of the species of *Boswellia*.

Recommendation

MEBDH stem bark was found to possessed potential bioactive compounds that ameliorate anomalies in hyperlipidemic condition which are yet to be studied as individuals. Therefore, we recommend that the safety and hypolipidemic potential of phytochemicals especially boswellic acid extracted from *B. dalzielli hutch* should be further explored both *in vivo* and *in vitro* in order to understand the mechanistic pathways involved in the process.

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