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

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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 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 Boswellia species have been used locally for the treatment of hyperlipidemia (Mishra and Allan, 2010). Specifically, the extract of the leaves, bark and roots of Boswellia serrate, cartari and papinera has been administered to hyperlipidemic patients (Ben-Yehoshua et al., 2012). However, the extract of B. dalziella hatch 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 hyperlipidemic 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 *Boswellia 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°C and the residue was collected and stored at 4°C.

**Phytochemical analysis**

The MEBDH stem bark was screened for the presence of saponins, tannins, flavonoids, gluco sides, 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 Oncocerciasis 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 osmolality 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 4°C 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. dalzielli* 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

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Boswellic acid</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) 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 hyperlipidemic 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 VLDL 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).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment Dose (mg/kg)</th>
<th>Lipid Profile Parameters (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TC</td>
</tr>
<tr>
<td>n-Control</td>
<td>n-Saline</td>
<td>47.0±5.3</td>
</tr>
<tr>
<td>hi-Control</td>
<td>n-Saline</td>
<td>64.7±2.1</td>
</tr>
<tr>
<td>MEBDH</td>
<td>200</td>
<td>40.3±4.1</td>
</tr>
<tr>
<td>MEBDH</td>
<td>400</td>
<td>28.9±3</td>
</tr>
<tr>
<td>SVT</td>
<td>5</td>
<td>38.5±6.9</td>
</tr>
</tbody>
</table>

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 (hi-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 (Attaawi 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 hyperlipidemic 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 LDL greater than 4000 mg/kg, revealing certain level of safety of the extract. However, acute toxicity examination does not guarantee 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.

Acknowledgement
We thank Dr. Timothy Bulus, who is currently the Head of Biochemistry Department, Kaduna State University, for generously providing us with Triton X-100 used for the induction of hyperlipidemia. We also thank the entire laboratory staff in the Department for their tireless assistance in various ways.

REFERENCES

In Vivo Ameliorative Effect Of Methanolic Extract Of Boswellia Dalzielli Hutch (Mebdh) Stem Bark On Triton X-100 Induced Hyperlipidaemia
participants in 14 randomised trials of statins. The Lancet, 366(9493), 1267.


Pandey, R. S., Singh, B. K., & Tripathi, Y. B. (2005). Extract of gum resins of Boswellia serrata L inhibits lipopolysaccharide induced nitric oxide production in rat macrophages along with hypolipidemic property.


Tang, J. J., Li, J. G., Qi, W., Qiu, W. W., Li, P. S., Li, B. L., ... & Song, B. L. (2011). Inhibition of SREBP by a small molecule, betulin, improves hyperlipidemia and insulin resistance and reduces atherosclerotic plaques. Cell metabolism, 13(1), 44-56.
