



Effect of Methanolic Leaf Extract of *Boswellia dalzielii* Hutch on Ocular ATPase Profiles of Selenite-induced Cataract in Wistar Pups

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Authors' contributions

This work was carried out in collaboration between all authors. Author JCI designed the study. Author DAO managed the laboratory animals, performed laboratory investigations, wrote the protocol, literature review, manage the analysis of the results and wrote the first draft of the manuscript. Authors ENO and JCI managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The effect of methanolic leaf extract of *Boswellia dalzielii* Hutch on ocular ATPase of selenite-induced cataract was investigated in Wistar rat pups. Nine groups of 5 pups each were used for the study. Cataract was induced by intra-peritoneal administration of 30 $\mu\text{mol}\cdot\text{kg}^{-1}$ body weight of sodium selenite, to groups 2 to 9, while group 1 received normal saline. The administration of *Boswellia dalzielii* Hutch extract commenced eight days post administration of selenite, and lasted for 28 days. The extract was orally administered to groups 5 to 9, at doses of 300, 400, 500, 600 and 700 $\text{mg}\cdot\text{kg}^{-1}$ body weight respectively; groups 3 and 4 received 50 $\text{mg}\cdot\text{kg}^{-1}$ body weight each of vitamins C and A respectively; while groups 1 and 2 received water in place of the extract. Ocular activities of Na^+, K^+ -ATPase and Mg^{2+} ATPase were estimated by measuring the amount of inorganic phosphate released by them, using ammonium molybdate reagent. The extract treated pups showed elevated ocular Na^+, K^+ -ATPase activity and decreased ocular Mg^{2+} ATPase activity. This study showed that methanol extract from the leaves of *B. dalzielii* Hutch modulated the activities of ocular Na^+, K^+ - and Mg^{2+} ATPases, thus highlighting its possible potential for restoring the osmotic equilibrium of cataractous lenses.

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1. INTRODUCTION

Sodium selenite induces oxidative stress in the lens, compromises ocular antioxidant status, and triggers up lenticular opacity [1]. According to Li et al. [2] lens opacity is associated with damaged epithelial cell and a compromised active transport, since these are the essential site responsible for active transport. This is because the activity of Na^+, K^+ -ATPase is usually high in lens epithelial cells and lens fibre cells, which usually show a high level of Na^+, K^+ -ATPase α -1 but low enzyme activity [3]. ATPases are membrane bound enzymes which play key role in the maintenance of osmotic equilibrium and ionic gradient of the cell [4]. They catalyse the hydrolysis of ATP to adenosine diphosphate (ADP), thereby supplying the energy required for many biological processes [5]. Sodium potassium (Na^+, K^+ -ATPase) and magnesium (Mg^{2+} -ATPase) ATPases are transport protein complexes synthesized in the epithelial cells [6], which reside in the epithelial and superficial cortical region of the plasma membrane [3,7] and play a key role in the active transport and regulation of sodium (Na^+) and potassium (K^+) ions across the cell membrane [5,8]. Due to the significance of Na^+, K^+ -ATPase in the maintenance of the ionic equilibrium in the lens, its malfunction or deficiency results in the retention of Na^+ and loss of K^+ alongside hydration and swelling of the fibres of the lens, leading to the derangement of lenticular osmotic equilibrium, and subsequent development of cataract [6,9]. In cataract, there is a marked alteration in the lenticular electrolytes balance, which is an indication of a compromised Na^+, K^+ -ATPase activity [3,10]. Cataract studies in experimental animals and in human cataract reveal alterations (especially reductions) in the activity of Na^+, K^+ -ATPase although no clear pattern was documented [11,12]. Magnesium ATPase regulates trans-membrane movements of magnesium ions in and out of the cell [13]. It has been implicated in the maintenance of membrane integrity [14], and is inhibited by calcium in excess of ATP [15].

Boswellia dalzielii Hutch mostly called the "Frankincense tree" [16], belongs to the "Burseraceae" family [17]. It is a savannah tree plant which grows up to 13 m high [18], with a characteristic rough, light-brown coloured bark and bears white fragrant flowers [19]. Onoriose [20] reported that *Boswellia dalzielii* Hutch leaves

phytoconstituents include protocatechuric acid, catechin, quercetin, gamma terpinene, trans-sabinene hydrate, alpha campholenal, boswellic acid, 3-acetyl beta boswellic acid, 11-keto beta boswellic acid and 3-acetyl-11-keto beta boswellic acid.

The plant's stem bark is used in alleviating gastrointestinal disorders [21]. Onoriose et al. [22] reported the use of the leaves in alleviating liver damage induced by carbon tetrachloride, while Abdulazeez et al. [23] reported the use of the leaves as an antimicrobial agent. However, the effect of *B. dalzielii* Hutch leaf on ocular ATPase activities in cataract is yet to be reported. Thus, this study, investigated the effect of *B. dalzielii* Hutch on ocular ATPase profiles of selenite-induced cataractous Wistar pups.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The leaves of *Boswellia dalzielii* Hutch were collected from Maitunku hill, Bambam, Dadiya District in Gombe state. They were identified by Mr. Thlama Daniel Mshelbwala, Forestry Technology Department, Federal College of Forestry, Jos and confirmed by Dr. Ekeke Chimezie, University of Port Harcourt Reference Herbarium for Research and Germplasm Conservation, Department of Plant Sciences and Biotechnology, University of Port Harcourt, Nigeria. They were documented with the voucher number UPH/V/1247.

2.2 Preparation of the Extract

The leaves were air dried and pulverized. The pulverized sample (600 g) was soaked in 6 L of methanol, and allowed to stand for 24 h. The resultant mixture was sieved, and the filtrate was concentrated using a rotary evaporator. The residue (extract) was weighed and stored in an air tight plastic can, for subsequent use.

2.3 Animals

All experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised in 1996). Neonatal Wistar rat pups which initially weighed 10–18 g on the seventh day of age, were obtained together with their dams from Mr. Kinsley Nwuzi, Biochemistry Department,

University of Port Harcourt. The rat pups were kept along with their dams in wired cages, at $27\pm 1^{\circ}\text{C}$.

2.4 Experimental Design

The pups were divided into nine groups comprising of five pups each, such that the difference in weight between one rat group and another was ± 1 g (Table 1). Group 1 received normal saline, groups 2 to 9 received $30\ \mu\text{mol/kg}$ body weight (bw) of sodium selenite. The $30\ \mu\text{mol/kg}$ bw sodium selenite was adopted from Mohammed [24]. Eight days post administration of selenite, cataract was confirmed with an ophthalmoscope by Dr. Mbah Kingsley Chikezie, Senior Registrar, Department of Ophthalmology, University of Port Harcourt Teaching Hospital. Treatment commenced and lasted for 28 days. The extract was administered at doses of $300\ \text{mg}\cdot\text{kg}^{-1}$ body weight, $400\ \text{mg}\cdot\text{kg}^{-1}$, $500\ \text{mg}\cdot\text{kg}^{-1}$, $600\ \text{mg}\cdot\text{kg}^{-1}$ and $700\ \text{mg}\cdot\text{kg}^{-1}$ to groups 5 to 9 respectively; the reference drugs (vitamins A and C) were administered at $50\ \text{mg}\cdot\text{kg}^{-1}$, to groups 3 and 4, respectively; while water was administered to groups 1 and 2, in place of the extract. At the end of the treatment period, the animals were anaesthetized with chloroform and sacrificed. Their eye lenses were immediately excised posteriorly. One of the lenses was homogenize in 2 mL of distilled water, centrifuged at 1200 g for 5 min, and the clear supernatant separated into plain labelled tubes, and used for the analysis.

2.5 Determination of ATPase Activity

The activities of Na^+, K^+ - and Mg^{2+} ATPases was determined spectrophotometrically according to

the method reported by Hesketh et al. [25]. Inorganic phosphate concentration was determined by the method of Fiske and Subbarow [26]; while the protein content of the lens homogenate was determined by Lowry method [27].

2.6 Statistical Analysis of Data

Data were expressed as mean \pm standard deviation. The results were analyzed statistically by one way analysis of variance (ANOVA), followed by multiple comparison test of least significant difference (LSD). Significance was accepted at a p -value of 0.05.

3. RESULTS

The effect of *B. dalzielii* methanol leaf extract on ocular sodium potassium ATPase (Na^+, K^+ ATPase) and magnesium ATPase (Mg^{2+} ATPase) in selenite-induced cataractous pups, is presented in Table 1. The ocular ATPase activity of the toxic control group was significantly ($p < 0.05$) lower than that of the normal control group; not significantly ($p > 0.05$) lower than those of the vitamins A and C, and $300\ \text{mg}\cdot\text{kg}^{-1}$, $400\ \text{mg}\cdot\text{kg}^{-1}$ and $500\ \text{mg}\cdot\text{kg}^{-1}$ extract treated groups; and not significantly ($p > 0.05$) higher than those of the $600\ \text{mg}\cdot\text{kg}^{-1}$ and $700\ \text{mg}\cdot\text{kg}^{-1}$ extract treated groups. The ocular Mg^{2+} ATPase activity of toxic control group was significantly ($p < 0.05$) higher than that of the normal control group, but not significantly ($p > 0.05$) higher than those of the vitamins A and C, and $300\ \text{mg}\cdot\text{kg}^{-1}$, $400\ \text{mg}\cdot\text{kg}^{-1}$, $500\ \text{mg}\cdot\text{kg}^{-1}$, $600\ \text{mg}\cdot\text{kg}^{-1}$ and $700\ \text{mg}\cdot\text{kg}^{-1}$ extract treated groups.

Table 1. Effect of *B. dalzielii* methanol leaf extract on ocular sodium potassium ATPase (Na^+, K^+ ATPase) and magnesium ATPase (Mg^{2+} ATPase) activities in selenite-induced cataractous pups

Treatment groups	Activities ($\mu\text{mol Pi}$ liberated/ mg protein/min)	
	Na^+, K^+ ATPase	Mg^{2+} ATPase
Normal control	0.887 ± 0.383^a	0.158 ± 0.095^a
Toxic control	$0.318\pm 0.180^{b,c,d}$	12.848 ± 24.736^b
Vitamin C treatment	$0.582\pm 0.242^{a,b,d}$	$5.040\pm 5.292^{a,b}$
Vitamin A treatment	$0.510\pm 0.206^{b,c,d}$	$0.736\pm 0.626^{a,b}$
$300\ \text{mg}\cdot\text{kg}^{-1}$ extract treatment	$0.323\pm 0.192^{b,c,d}$	$0.536\pm 0.526^{a,b}$
$400\ \text{mg}\cdot\text{kg}^{-1}$ extract treatment	$0.598\pm 0.132^{a,d}$	$4.512\pm 4.042^{a,b}$
$500\ \text{mg}\cdot\text{kg}^{-1}$ extract treatment	$0.658\pm 0.449^{a,b}$	$3.899\pm 4.412^{a,b}$
$600\ \text{mg}\cdot\text{kg}^{-1}$ extract treatment	0.186 ± 0.139^c	$0.353\pm 0.319^{a,b}$
$700\ \text{mg}\cdot\text{kg}^{-1}$ extract treatment	$0.280\pm 0.393^{c,d}$	$0.459\pm 0.236^{a,b}$

Data were represented as mean \pm standard deviation of $n=5$

Values in the same column with different superscript letters (a, b, c and d) are significantly different at $p < 0.05$

4. DISCUSSION

The activity of ATPases can be influenced either by changing the fluidity of the membrane/lipid bilayer, or by direct interaction between the ATPase molecule and the modifying agent [28]. In this study, the Na⁺,K⁺-ATPase activity of the toxic control group was lower than those of the normal control. This is in line with the report by Kinoshita [12], that Na⁺,K⁺-ATPase activities are decreased in cataract. The decrease in the activity of Na⁺,K⁺-ATPase could be due to disorganisation of the membranes of the epithelial and fibre cells, which may have resulted to the decreased clarity observed in the cataractous lens. Generally, the activity of Na⁺,K⁺-ATPase is inhibited by or via any of the following.

- Free radicals and reactive oxygen species [29,30,31,32,33,34].
- Decrease in membrane fluidity [28,35,36]
- Modification of the lipid environment or mode of interaction with lipids, such as dissociation from polar lipids, lipoperoxidation, etc. [31,37,38].

The above may have accounted for the lowered Na⁺,K⁺-ATPase activity of the toxic control compared to the normal control. The treatment with the extract and vitamins A and C improved but not significantly, ocular Na⁺,K⁺-ATPase activities, while lowering but not significantly, ocular Mg²⁺ ATPase activity, compared to the toxic control group. The observed improvement of Na⁺,K⁺-ATPase activity by vitamin C (ascorbic acid) treatment is an affirmation of the report by Kumar et al. [34] that ascorbic acid treatment reversed/prevented oxidative stress induced reduction of Na⁺,K⁺-ATPase activity.

There was a dose dependent increase in the activity of Na⁺,K⁺-ATPase in the 300 mg·kg⁻¹, 400 mg·kg⁻¹ and 500 mg·kg⁻¹ extract treated groups, with the 500 mg·kg⁻¹ treatment being the effective dose. This increase in the activities of Na⁺,K⁺-ATPase in *B. dalzielii* extract treated group is indicative of the ability of the extract to restore the ionic equilibrium in the lens. It therefore, follows that the improved lenticular Na⁺,K⁺-ATPase activity observed in the extract treated groups could have been effected through any of two mechanisms. Firstly, it may be due to the direct interaction between the enzyme and the phytoconstituents in the extract, which may have resulted in changes

in the enzyme's structure, and subsequent changes in its activity. Secondly, it may have been due to the impact of the phytoconstituents on the lipids of the lenticular plasma membrane, which in turn resulted to changes in the fluidity, and consequently, to altered enzyme activity.

The ability of the extract to increase Na⁺,K⁺-ATPase activity, may be due to the presence of any or both of resveratrol and epicatechin, earlier reported in it by Onoriose [20]. Resveratrol and epicatechin have been reported to improve oxidative stress induced reduction in Na⁺,K⁺-ATPase activity [33,34]. The decreased activity of Na⁺,K⁺-ATPase in the 600 mg·kg⁻¹ and 700 mg·kg⁻¹ *B. dalzielii* extract treated group could infer toxicity of the extract as a result of high dosage of the extract.

In the case of Mg²⁺ ATPase, its activity is enhanced by decreased fluidity of the membrane/lipid bilayer because, according to Riordan [39], there is an inverse correlation between Mg²⁺ ATPase activity and bilayer fluidity. Its activity is also enhanced by separation from phospholipids [39]. So, the reduction in Mg²⁺ ATPase activity observed in the treated rats may have been due to the increased membrane fluidity, earlier mentioned (while discussing Na⁺,K⁺-ATPase); and corroborates the improved Na⁺,K⁺-ATPase activity.

5. CONCLUSION

Therefore, it is possible to conclude that the extract acted by modifying the micro-viscosity of the lenticular membrane; and that the resultant increased fluidity caused the improvement in Na⁺,K⁺-ATPase activity, and reduction in Mg²⁺ ATPase activity. This study revealed that *B. dalzielii* Hutch leaf extract improved the activities of lenticular ATPase and so maybe useful for the management of lenticular osmotic or electrolyte imbalances. This therefore, is an indication of its potential as a resource for the management of cataract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-

23, revised 1985) were followed, as well as specific national laws where applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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