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Full Length Research Paper

The Protective Effect of *Boswellia dalzielii* Hutch Extract on Ocular Markers of Oxidative Stress in Selenite-Induced Cataractous Pups: A Randomized Controlled Trial

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The effect of *Boswellia dalzielii* Hutch extract on ocular markers of oxidative stress of selenite-induced cataractous pups was investigated in wistar rat pups. Nine groups of 5 pups each were used for the pharmacological studies. Group 1 received normal saline, groups 2 to 9 received 30 µmol/kg BW of sodium selenite. Eight days post administration of selenite, the opacity were determined with an ophthalmoscope and from these, the presence of cataract was established after which the treatment commenced and lasted for 28 days. The extract was administered at doses of 300 mg/kg body weight, 400 mg/kg 500 mg/kg, 600 mg/kg and 700 mg/kg to groups 5 to 9 respectively; groups 3 and 4 received 50mg/kg each of vitamins C and A respectively; while groups 1 and 2 received water in place of the extract. Ocular antioxidative enzymes activities (Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)) and reduced glutathione, ascorbic acid, malondialdehyde and total protein levels were estimated using standard methods. The extract in a dose dependent manner significantly ($p<0.05$) lowered ocular catalase activity. The extract improved the ocular concentration of reduced glutathione and total protein. Also obtained were non-significant decrease in ocular activities of glutathione peroxidase, superoxide dismutase, and levels of ascorbic acid. The overall experimental results indicate that *Boswellia dalzielii* Hutch leaf extract is a potential anti-oxidant modulator for the development of anti-cataract formulation.

Keywords: *Boswellia dalzielii,* Hutch, sodium selenite, cataract, ophthalmoscope, ocular anti-oxidative enzymes

INTRODUCTION

Oxidative stress has been postulated as the main mechanism in cataract formation and development (Yagci *et al.,* 2006). Reports from researchers have shown scientific proofs that the process of oxidative stress-induced lens opacities is initiated by a surge inthe oxidation of proteins, non-enzymic glycosylation,

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cross linking and accumulation of ocular proteins, lens membrane lipid peroxidation and epithelial cell death of the ocular lens (Spector, 1995). The intensity of opacification of lens can be reduced by the antioxidants which scavenge the production of free radicals (Kilie et al., 2007). There are previous studies that confirmed that diet rich in vitamins, carotenoids and flavonoids may reduce cataract intensity (Bunce *et al.,* 1990). For the elimination of free radical-induced injury, normal reference ranges of the anti-oxidant's defense mechanism are usually not sufficient. Therefore, the consumption of antioxidants from a plant source may have a promising role. *Boswellia dalzielii* Hutch is a tree plant of the savannah forest (Abdulazeez *et al.,* 2013). The phytochemical studies on *B. dalzielii* revealed potent antioxidant constituents such as catechin, quercetin, kaempferol, myricetin, epicatechin, luteolin, alpha-, beta-pinene and others (Onoriose, 2012). Sequel to this, the present study was designed to investigate the moderating effect of *B. dalzielii* hutch on ocular markers of oxidative stress in selenite-induced cataractous pups.

METHODS AND MATERIALS

Collection and identification of plant leaves

The leaves of *Boswellia dalzielii* Hutch were collected with the aid of a matchet from Maitunku hill, Bambam, Dadiya District in Gombe state. 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; were it was given the voucher number UPH/V/1247.

Extraction of plant

Collected leaves were air dried, pulverized and 600 grams of the pulverized plant samples was soaked in 6 L of methanol, and allowed to stand overnight for 24 h. Thereafter it was sieved, concentrated using rotary evaporator, weighed and stored in an air tight plastic can.

Experimental animals

Neonatal Wistar albino rat pups which initially weighed 10–18 g on the seventh day of age 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±1 °C.

Experimental design

A total of 45 neonatal Wistar albino rat pups, initially weighed 10–18 g on the seventh day of age, kept along with their dams in wired cages, were used. They 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 3.1). Group 1 received normal saline, groups 2 to 9 received 30 µmol/kg BW of sodium selenite. The 30 µmol/kg body weight sodium selenite was adopted from Mohammed (Mohammed, 2012). Eight days post administration of selenite, the treatment commenced and lasted for 28days. The extract was administered at doses of 300 mg/kg body weight, 400 mg/kg 500 mg/kg, 600 mg/kg and 700 mg/kg to groups 5 to 9 respectively; groups 3 and 4 received 50 mg/kg each of vitamins C and A respectively; while groups 1 and 2 received water in place of the extract. At the end of the treatment period, the animals were anaesthetized with chloroform and sacrificed by cutting through their jugular vein. The eye lenses were 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 a plain labelled tube analysis.

Determination of ocular reduced glutathione (GSH) level

Ocular reduced glutathione concentration was determined spectrophotometrically according to the method of Sedlak and Lindsay (Sedlak and Lindsay, 1968). The principle is based on the development of a relatively stable yellow colour when 5,5-dithiobis-2-nitrobenzoic acid is added to sulfhydryl compounds. The resultant chromophoric product (2-nitro-5-thiobenzoic acid) possess a molar absorption at 412 nm.

Assay for glutathione peroxidase

Ocular glutathione peroxidase was determined according to the method of Rotrucket al (Rotruck *et al.,* 1973). The principle is based on the measurement of the residual glutathione remaining after the action of glutathione peroxidase at 412 nm. The enzymatic activity was expressed as µg GSH consumed/min/mg protein.

Determination of ocular ascorbic acid concentration

Ocular ascorbic acid was determined by iodine titration method of Ikewuchi and Ikewuchi (Ikewuchi and Ikewuchi, 2011). The principle is based on the fact that when iodine is added to starch solution, it reacts to form a purple colour. In the presence of vitamin C, iodine is

Table 1. Effect of *B. dalzielii* Hutchmethanol leaf extract on antioxidant parameters in selenite-induced cataractous pups

Treatment groups	Glutathione peroxidase activity (µg/min/mg protein)	Glutathione level (µg/min/mg protein)	Catalase activity x10^{-4} (units/mg protein)	Superoxide dismutase activity (units/mg protein)
Normal control	47.90±18.50^{a}	07.63±05.28^{a}	04.15±02.00^{a}	02.93±01.21^{a}
Toxic control	61.50±32.90^{a,b}	04.99±03.27^{a,b}	42.65±39.11^{c}	18.45±10.83^{ab}
Vitamin C treated	50.90±22.03^{a,b}	12.04±13.14^{a,b}	20.00±29.20^{a,c}	27.37±37.03^{c}
Vitamin A treated	46.70±06.90^{a,b}	05.50±03.60^{a,b}	10.13±08.72^{a,b}	09.89±05.20^{ac}
300 mg/kg extract	52.40±13.00^{a,b}	09.32±08.53^{a,b}	09.18±05.05^{a,b}	07.76±02.27^{ac}
400 mg/kg extract	57.30±26.30^{a,b}	05.06±02.99^{a,b}	12.34±04.91^{a,b}	09.42±04.34^{ac}
500 mg/kg extract	45.90±17.90^{a,b}	07.88±02.06^{a,b}	06.27±03.81^{a,b}	04.52±01.14^{ab}
600 mg/kg extract	39.90±21.32^{a,b}	07.74±05.31^{a,b}	10.28±05.82^{a,b}	23.58±28.76^{ac}
700 mg/kg extract	35.70±14.50^{a,b}	04.61±01.18^{a,b}	21.17±23.87^{a,c}	08.16±04.89^{ac}

Data was represented as mean ± standard deviation of n=5. Values within the same column with different superscripts (a, b and c) are significantly different at $p<0.05$

neutralized, preventing the formation of purple colour. The end point is the appearance of blue-iodine colour.

Determination of ocular catalase activity

Ocular catalase was determined according to the method of Beers and Sizer (Beers and Sizer, 1952). The principle is based on the fact that catalase catalyzes the breakdown of hydrogen peroxide to water and molecular oxygen. The residual hydrogen peroxide is quantified after incubation with the enzyme at 420 nm since it has an absorption maximum at this wavelength using a UV spectrophotometer.

Ocular total protein determination

Ocular total protein level was determined by the Biuret method using Randox kit (by Randox Laboratory Ltd, Crumlin, England, United Kingdom). The principle is based on the fact that cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex. The intensity of the final coloured complex measured at 546 nm in a spectrophotometer is proportional to the total protein concentration in the lens homogenate.

Determination of ocular superoxide dismutase (S.O.D) activity

Ocular superoxide dismutase was determined by the method reported by Misra and Fridovich (Misra and Fridovich, 1972). The principle is based on rapid auto-oxidation of adrenalin in aqueous solution to adrenochrome, which depend on superoxide anions availability. Superoxide dismutase inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anions. The degree of inhibition is a function of the activity of S.O.D determined at 520 nm.

Determination of ocular malondialdehyde (MDA)

Ocular malondialdehyde level was determined spectrophotometrically according to the method of Hunter et al (Hunter et al., 1963) as modified by Gutteridge and Wilkins (Gutteridge and Wilkins, 1982). The principle is based on heating MDA with 2-thiobarbituric acid under acidic conditions, MDA forms a pink coloured MDA-TBA2 complex which has a maximum absorbance at 532 nm.

Statistical analysis of data

Data was expressed as mean ± standard deviation (SD). 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.

RESULT

Table 1 shows the effect of *Boswellia dalzielii* Hutch methanol leaf extract on antioxidant parameters. The ocular reduced glutathione concentration of 300 mg/kg, 400 mg/kg, 500 mg/kg and 600 mg/kg extract and vitamins A and C treated groups showed improvement when compared to the toxic control group. The ocular reduced glutathione concentration of 700 mg/kg extract treated group showed reduction in ocular reduced glutathione levels when compared to the toxic control group. These changes in glutathione levels were not significant ($p<0.05$). The activity of the ocular glutathione

Table 2. Effect of *B. dalzielii* Hutch methanol leaf extract on ascorbic acid and total protein in selenite-induced cataractous pups

Treatment groups	Ascorbic acid level (mmol/mg protein)	Total protein (mg/mL)	Malondialdehyde x10^{-2} (nmol/mg protein)
Normal control	03.21±01.19^{a}	19.97±07.25^{a}	01.92±00.01^{a}
Toxic control	09.34±04.73^{a,b}	06.41±03.53^{b}	16.04±00.14^{ab}
Vitamin C treated	16.82±21.52^{c}	12.30±12.36^{a,b}	I7.06±00.21^{ab}
Vitamin A treated	05.58±03.18^{a,c}	12.12±08.96^{a,b}	06.71±00.04^{ab}
300 mg/kg extract	03.47±01.14^{ab}	12.12±02.75^{a,b}	05.01±00.02^{ab}
400 mg/kg extract	04.76±02.62^{ac}	09.36±06.16^{b}	06.62±00.05^{ab}
500 mg/kg extract	03.63±01.41^{ac}	13.81±01.65^{a,b}	03.16±01.89^{ab}
600 mg/kg extract	15.93±21.68^{ac}	09.90±09.25^{b}	20.26±00.28^{b}
700 mg/kg extract	06.59±02.45^{ac}	08.47±04.52^{b}	09.52±00.10^{ab}

Data was represented as mean ± standard deviation of n=5. Values within the same column with different superscripts (a, b and c) are significantly different at p<0.05

peroxidase of the vitamins A and C, and 300 mg/kg, 400 mg/kg, 500 mg, 600 mg/kg and 700 mg/kg extract treated groups showed reduction in ocular glutathione peroxidase activity when compared to the toxic control group. These changes in ocular glutathione peroxidase activity were not significant at (p<0.05). The ocular catalase activity of the 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg extract and vitamin A treated pups showed significant (p<0.05) reduction while the vitamin C and 700 mg/kg extract treated pups showed non-significant (p<0.05) reduction in ocular catalase activity when compared to the toxic control group. The ocular superoxide dismutase activity of the 300 mg/kg, 400 mg/kg, 500 mg/kg and 700 mg/kg extract and vitamin A treated pups showed reduction, while the 600 mg/kg extract treated pups showed a rise, although these alterations were not significantly different from the toxic control and reference. The vitamin C treated pups show significant (p<0.05) rise in ocular superoxide dismutase activity when compared to the toxic control pups. The ocular ascorbic acid concentration of the extract treated (300 mg/kg, 400 mg/kg, 500 mg/kg and 700 mg/kg) pups show significant (p<0.05) decrease, while the 600 mg/kg extract treated pups show a non-significant (p<0.05) when compared to the toxic control group. The ocular ascorbic acid concentration of the vitamin C treated pups showed a significant (p<0.05) rise when compared to the toxic control pups.

The MDA levels of vitamin A treated and 300 mg/kg, 400 mg/kg, 500 mg/kg and 700 mg/kg extract treated pups showed reduction, while 600 mg/kg extract and vitamin C treated pups showed rise when compared to the toxic control pups. However, these alterations were not significantly different from the toxic control pups. Ocular total protein levels of vitamins A and C, and 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg and 700 mg/kg extract treated pups showed increase when compared to the toxic control pups. This increase in total protein was not significantly different from the toxic control group.

DISCUSSION

Compared to the toxic control, treatment with extract improved, though not significantly, ocular glutathione levels and significantly lowered ocular catalase activities, while non-significantly lowering ocular glutathione peroxidase and superoxide dismutase activities and ascorbic acid levels of the treated animals. This means that the management of Wistar pups with *B. dalzielii* Hutch extract subsequent to sodium selenite administration reduced the opacity of ocular lens which suggests that the phytoconstituents of the extract may have an antioxidative effect on the cataract. This is evidenced in the reduction in the activities of ocular catalase, superoxide dismutase and glutathione peroxidase and level of ascorbic acid, and rise in the concentration of glutathione relative to the toxic control group (Table 1). This result is at variance with the report of earlier studies. Seham *et al.* (Seham *et al.,* 2013) reported that broccoli reduces selenite induced cataract formation by elevating the ocular activity of catalase, glutathione peroxidase and superoxide dismutase.

The decrease in the activities of ocular catalase, superoxide dismutase and glutathione peroxidase despite the increase in the concentration of reduced glutathione is suggestive of the antioxidative potency of the extract which may have led to the regeneration of the lens epithelium. The rise in the level of reduced glutathione observed in this study is indicative of a reversal of the redox potential in the epithelial cells of the lens from an oxidizing state to a potent reducing state as a result of

the extract treatment. According to Seham *et al* (Seham *et al.,* 2013) reduced glutathione preserves thio groups of protein in the reduced state and prevent inter-linking of soluble crystalline. This way, GSH and ascorbic acid can stop the formation of free radical induced reaction thereby obstructing the autocatalytic reactions of lipid peroxidation.

Furthermore, the fall in the level ascorbic acid despite the rise in the concentration of reduced glutathione observed in *B. dalzielii* extract treated groups also infer decreases in glycation of lens proteins and oxidative stress. In the lens glycation of proteins and formation of superoxide anions is enhanced by oxidized vitamin C or dehydroascorbate (Linetsky *et al.,* 1999; Linetsky *et al.,* 2008). Hence, the phytoconstituents of *B. dalzielii* leaves showed tendency to alleviate cataract induced by selenite.The treatment improved, though not significantly, the cataract induced lowering of ocular total protein content. Again, similar results were reported by Nair et al (Nair *et al.,* 2010) and Seham *et al* (Seham *et al.,* 2013) who respectively, observed significant increase in ocular total protein concentration of *Embelica officinalis* and broccoli treated selenite-induced cataractous rats.

The administration of the extract produced lower (though not significant) levels of ocular MDA in the treated animals, compared to the toxic control group. This is similar to the findings of Nair *et al* (Nair *et al.,* 2010) and Seham *et al* (Seham *et al.,* 2013) who reported significant decreases as a result of treatments with *Embelica officinalis* aqueous extract and broccoli, respectively. Malondialdehyde is a lipid peroxidation product used mostly as an index of lipid peroxidation (Micelli-Ferrari *et al.,* 1996). Therefore, the decreased concentrations of MDA produced in the *B. dalzielii* extract treated groups infer restoration of the structure and metabolic process in the cell membrane.

The extract induced reduction in the ocular activities of antioxidant enzymes were not accompanied by increases in lipid peroxidation (measured by MDA levels). This is indicative of the presence of a very strong antioxidant in the extract. This is in line with the earlier report by Onoriose (Onoriose, 2012) that the leaves of *B. dalzielii* have very high contents of flavonoids, which are very strong antioxidants.

The overall experimental results indicate that *B. dalzielii* Hutch leaf extract is a potential anti-oxidant modulator for the development of anti-cataract formulation.

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