RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2014.172.176

Evaluation of *in vitro* Anticataract Activity and Aldose Reductase Potential of *Barleria lupulina* Lindl.

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ABSTRACT

Background: *Barleria lupulina* is well recognized traditional medicine in the treatment of diabetes mellitus but no scientific data has been reported to its ethnomedical use in eye related long term complications of diabetes. Objective of the present study is to evaluate the anti-cataract activity of leaves of *Barleria lupulina* Lindl. along with its aldose reductase potential. **Materials and Methods:** Methanol extract of *Barleria lupulina* Lindl. (MEBL) was successively fractionate with hexane (HFBL), ethyl acetate (EFBL) and the aqueous fraction (AFBL). All the fractions and methanol extract were subjected to qualitative and quantitative phytochemical screening. *In vitro* antioxidant activity of the same was assessed by free radical scavenging activity using DPPH and hydrogen peroxide assay. The active ethylacetate fraction (EFBL) was further subjected to *in vitro* anti cataract evaluation against glucose induced cataractogenesis by using goat lenses. **Results:** The lenses incubated with EFBL at 200 and 400 μg mL^{-1} concentration seemed to retard the progression of lens opacification and showed a significant restoration of glutathione, SOD level ($p<0.01$) and reduced the level of TBARS ($p<0.01$) when compared with positive control group (glucose 55 mM). EFBL showed promising percentage inhibition of aldose reductase activity with lower IC_{50} value (50.118 μg mL^{-1}). **Conclusion:** Hence, this study demonstrated that the EFBL possess significant anticataract, antioxidant and aldose reductase inhibition properties.

Key words: *Barleria lupulina*, cataractogenesis, aldose reductase inhibition

Pharmacologia 5 (5): 172-176, 2014

INTRODUCTION

Cataract is the opacification or optical dysfunction of the crystalline lens, associated with the breakdown of the eye lens microarchitecture, which interferes with transmission of light onto the retina and it is the major cause of blindness in both developed and developing countries (Umamaheswari *et al*., 2012). A major complication of diabetes mellitus is the early development of cataract of lens and hence opacity is due increased rate of sorbitol formation, caused by hyperglycemia (Parmar *et al*., 2012). Herbal drugs are widely prescribed to treat diabetes and its complications because of their effectiveness, fewer side effects and relatively low cost (Mazumder *et al*., 2012a). *Barleria lupulina* (Acanthaceae) is a small herb, distributed in the South Asia region. It has been traditionally used for mental tension, diabetes, rheumatoid arthritis and snake bite (Chopra *et al*., 1968). Various phytoconstituents have been isolated from aerial parts of the plant (Mazumder *et al*., 2012b). *Barleria lupulina* is well recognized in traditional medicine as having an antidiabetic activity but no scientific data has been published related to its ethnomedical use in eye related long term complications of diabetes. Hence, the present study focuses on preventive role of *Barleria lupulina* Lindl. on glucose induced oxidative damage in the goat lens culture and understanding the action of herb on polyol pathway.

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MATERIALS AND METHODS

Plant collection and extraction: The leaves of *Barleria lupulina* Lindl. were collected from the Institute of forest productivity, Ranchi, Jharkhand in the month of July-August 2012 and was authenticated by Dr. Karthikeyan, Botanical survey of India (BSI), Kolkata. The leaves were carefully dried in shade for 15 days. The defatted powdered leaves were subjected to extraction with methanol for 48 h by soxhlet extraction. The solvent were evaporated to obtain a semisolid mass and vacuum dried to yield solid residues of methanol extract of *Barleria lupulina* Lindl. (MEBL). The obtained extract (25 g) was dissolved in distilled water and successive fractionation with hexane (HFBL), ethyl acetate (EFBL) and the remaining residue as aqueous fraction (AFBL) and concentrated to dryness to obtained respective fractions.

Phytochemical investigation and antioxidant activity: Preliminary phytochemical screening of MEBL and its fractions was performed according to the method described earlier (Brain and Turner, 1975). The total flavonoid and phenolic content was determined according to the earlier reported methods (Kumar *et al*., 2012). The *in vitro* antioxidant activity of MEBL and its fractions was estimated by DPPH free radical scavenging activity and hydrogen peroxide assay (Paramaguru *et al*., 2013). The fraction showing the highest activity was selected to carry out the further experiments.

***In vitro* anticataract activity of EFBL on isolated goat eye lens**

Preparation of lens culture: Goat eye balls were obtained from the slaughter house, Ranchi. The lenses were removed from goat eye balls by extracapsular extraction and total of 24 clear lenses were incubated in artificial humor (sodium chloride 140 mM), potassium chloride (5 mM), magnesium chloride (2 mM), sodium bicarbonate (0.5 mM), sodium diphosphate (0.5 mM), calcium chloride (0.4 mM), glucose (55 mM), at room temperature and pH 7.8 for 72 h. Penicillin 32 mg and streptomycin 250 mg were added to culture media to prevent contamination. Glucose in a concentration of 55 mM was used to induce cataract (Chandorkar *et al*., 1981). Lenses were divided into following groups (n = 4 in each group):

Group 1: Normal control group consisting of artificial aqueous humor solution
Group 2: Cataract control group consisting of artificial aqueous humor solution with 55 mM glucose
Group 3: Test group-I consisting of artificial aqueous humor solution with 55 mM glucose and EFBL 200 μg mL^{-1}
Group 4: Test group-II consisting of artificial aqueous humor solution with 55 mM glucose and EFBL 400 μg mL^{-1}

After incubation for 72 h, lenses were processed for the estimation of biochemical parameters and aldose reductase inhibition.

Biochemical parameters: The estimation of degree of oxidative stress and different antioxidant enzyme activity was assessed by measuring levels of Gluthathione (GSH) (Moron *et al*., 1979), malondialdehyde (MDA) (Slater and Sawyer, 1971) and Superoxide dismutase (SOD) (Misra and Fridovich, 1972).

***In vitro* aldose reductase activity:** A sample cuvette containing 0.7 mL of phosphate buffer (0.067 M), 0.1 mL of NADPH (25×10^{-5} M), 0.1 mL of lens supernatant, 0.1 mL of DL-glyceraldehyde (5×10^{-4} M) as substrate to a final volume of 1 mL and the final pH of the reaction mixture was 6.2 was read against a reference cuvette containing all the components except the substrate, DL-glyceraldehyde. 0.1 mL of each concentration (25, 50, 75, 100, 200 and 300 μg mL^{-1}) of EFBL was prepared in phosphate buffered saline and was added to both the reference and standard cuvettes. The enzymatic reaction was started by the addition of the substrate and the absorbance (OD) was recorded at 340 nm for 3 min at 30 sec interval. AR activity expressed as OD min^{-1} mg^{-1} protein (Suryanarayana *et al*., 2004). Quercetin was used as the reference standard.

Statistical analysis: Statistical analysis was carried out by using one-way Analysis of Variance (ANOVA) followed by Dunnett's test. Results were expressed as Mean±SEM. Probability values of $^{a}p<0.01$ were compared with normal control. Probability values of $^{b}p<0.01$ were compared with disease control.

RESULTS

Preliminary phytochemical investigation: Preliminary phytochemical screening of methanol extract MEBL revealed the presence of flavonoid, sterol, alkaloid and phenolic compounds. Among fractions, n-hexane fraction HFBL did not showed presence of any phytoconstituents other than glycosides, ethylacetate fraction EFBL showed the presence of flavonoid, sterol and phenolic compounds and aqueous fraction AFBL showed the presence of alkaloids, reducing sugars and phenolic compounds.

Total phenolic content analysis: Total Phenolic content (TPC) was estimated by using folin-Ciocalteu reagent and expressed as mg g^{-1} gallic acid equivalent (GAE). Ethyl acetate fraction EFBL showed highest value of TPC about 174.50 GAE, followed by, extract MEBL of about 97.01 GAE and AFBL of about 36.25 GAE also showed significant values.

Total flavonoid content analysis: Total flavonoid content (TFC) was estimated and expressed as mg g^{-1} quercetin equivalent. Ethyl acetate fraction EFBL showed highest value of TFC about 153.40 mg g^{-1} quercetin equivalent, followed by, extract MEBL of about 112.17 mg g^{-1} quercetin equivalent and AFBL of about 19.84 mg g^{-1} quercetin equivalent also showed significant values.

***In vitro* antioxidant activity:** The free radical scavenging activity of extracts by DPPH method was studied for its ability to reduce the nitrogen centered free radical DPPH. Ethylacetate fraction EFBL showed

lowest IC_{50} value 104.6 μg mL^{-1} followed by the extract MEBL 132.72 μg mL^{-1}, aqueous fraction AFBL 186.0 μg mL^{-1} and n-hexane fraction HFBL showed IC_{50} value of about 264.0 μg mL^{-1} (Table 1). In free radical scavenging activity by hydrogen peroxide assay ethylacetate fraction EFBL showed lowest IC_{50} value 25.18 μg mL^{-1} followed by the extract MEBL 31.45 μg mL^{-1}, aqueous fraction AFBL 39.81 μg mL^{-1} and n-hexane fraction HFBL showed IC_{50} value of about 63.09 μg mL^{-1} (Table 1).

***In vitro* prevention of cataract using EAFBL on isolated goat eye lens:** Incubation of lenses with glucose 55 mM showed opacification starting after 8 h at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 72 h as compared to lenses incubated in 5.5 mM glucose where transparency was maintained and squares were clearly visible. The lenses incubated with ethyl acetate fraction of *Barleria lupulina* Lindl. at 200 and 400 μg mL^{-1} concentration seems to retard the progression of lens opacification, compared with cataract control group (glucose 55 mM) (Fig. 1a-d).

Biochemical parameters: The mean GSH value in the normal lenses was 1.29±0.01 nmoles g^{-1} of tissue. A significant decrease in GSH level was observed in the presence of Glucose in the control as opposed to the normal group ($p<0.01$). On treatment with EFBL (200 and 400 μg mL^{-1}) there was a significant restoration of GSH level in the treated lenses ($p<0.01$) as opposed to the diabetic control lenses (Table 2). A significant increase in MDA level was found in the control opposed to the normal lenses (2.63±1.72 nmoles g^{-1} of tissue; $p<0.01$). Treatment with EFBL (200 and 400 μg mL^{-1}) significantly protected ($p<0.01$) the test group lenses from lipid peroxidation; the MDA content was 1.75±0.05 and 1.88±0.04 nmoles g^{-1} of tissue for the test group I and test group II (Table 2). A significant decrease in SOD level was observed in the presence of glucose in the diabetic control group when compared to that of normal group ($p<0.01$). Treatment with EFBL (200 and 400 μg mL^{-1}) showed a significant restoration of SOD level ($p<0.01$) when compared to the cataract control lenses (Table 2).

Table 1: Free radical scavenging activity of methanol extract and various fractions of *Barleria lupulina*

	IC_{50} values (μg mL^{-1})	
Extracts	DPPH	Hydroxyl radical
MEBL	132.72	31.45
HFBL	264.00	63.09
EFBL	104.60	25.18
AFBL	186.00	39.81
Ascorbic acid	26.51	19.95

MEBL: Methanol extract of *B. lupulina*, HFBL: n-hexane fraction of *B. lupulina*, EFBL: Ethylacetate fraction of *B. lupulina*, AFBL: Aqueous fraction of *B. lupulina*

***In vitro* aldose reductase activity:** The percentage inhibition of aldose reductase activity of EFBL was estimated and quercetin was used as a standard. IC_{50} values of EFBL and Quercitin were found to be 50.118 and 4.50 μg mL^{-1}, respectively.

DISCUSSION

Cataract is a major cause of blindness all over the world. It is an age related phenomenon, over and above oxidative stress also plays its role (Parmar *et al*., 2012). The qualitative and quantitative preliminary phytochemical analysis of extract and various fractions of *Barleria lupulina* clearly showed that ethyl acetate fraction EFBL possesses higher level of phenolic and flavonoid content which are responsible for antioxidant property of plants. *In vitro* antioxidant activity was determined by DPPH free radical scavenging activity and hydrogen peroxide assay. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple color fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance (Paramaguru *et al*., 2013). Hydroxyl radical is known to react with all the components of DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Paramaguru *et al*., 2013). In this study, ethyl acetate fractions EFBL showed highest DPPH and hydrogen peroxide scavenging activity with low IC_{50} values. Hence, EFBL was selected for further studies. Oxidative stresses being an important factor in cataractogenesis, the decrease in levels of TBARS and increase in the levels of glutathione and SOD in EAFBL (200 and 400 μg mL^{-1}) treated lenses when compared to positive control lenses. This clearly implicit the potential role of EAFBL in decreasing the oxidative stress and

Table 2: Estimation of different antioxidant enzyme levels in goat lens culture homogenate

Groups	Normal control	Disease control	Test group-I (EFBL 200 μg mL^{-1})	Test group-II (EFBL 400 μg mL^{-1})
GSH (nmoles g^{-1} of tissue)	1.29±0.01	0.64±0.02[a]	1.34±0.04[b]	1.36±0.01[b]
MDA (nmoles g^{-1} of tissue)	1.25±0.03	2.63±1.72[a]	1.75±0.05[b]	1.88±0.04[b]
SOD (U mL^{-1} of tissue)	1.76±0.01	1.24±0.01[a]	1.63±0.02[b]	1.69±0.01[b]

GSH: Gluthathione, MDA: Malondialdehyde and SOD: Superoxide dismutase. The results are expressed as Mean±Standard error of the mean (SEM). Probability values of [a]$p<0.01$ were compared with normal control. Probability values of [b]$p<0.01$ were compared with disease control

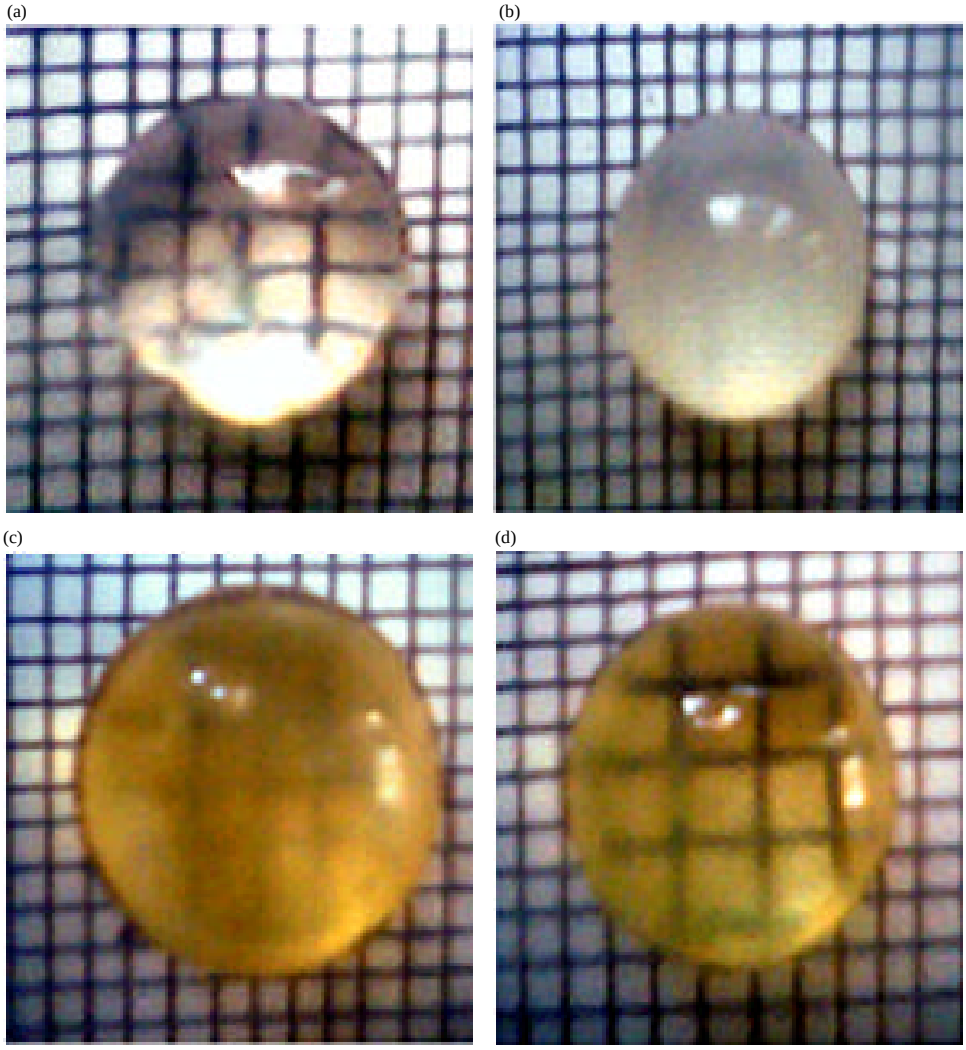

Fig. 1(a-d): Photographs of lenses in normal and experimental groups, (a) Normal lens after 72 h of incubation (Transparency maintained, more squares clearly visible), (b) Complete cataractogenesis after 72 h of incubation in Glucose 55 mM (Complete loss of transparency, no squares visible through lens), (c) After 72 h of incubation in Glucose 55 mM+EFBL 200 μg mL^{-1}, lens appears slightly hazy (less No. of squares slightly clearly visible), (d) After 72 h of incubation in Glucose 55 mM+EFBL 400 μg mL^{-1}, lens appears slightly hazy (more No. of squares clearly visible)

hence, opacity of lens treated with EAFBL were less developed compared with the control lenses. Aldose reductase is one of the key enzyme in polyol pathway and this enzyme induced/mediated changes being major insults in the development of diabetic complications such as cataract, retinopathy, neuropathy and nephropathy (Williamson *et al*., 1992). The inhibition of aldose reductase is one of the potential pharmacological approach that has been proposed to treat or ameliorate secondary complications of diabetes including cataract (Moghaddam *et al*., 2005). EAFBL showed promising percentage inhibition of aldose reductase activity with lower IC_{50} value.

CONCLUSION

Hence, the above study demonstrated that EAFBL possess significant anticataract, antioxidant and aldose reductase inhibition properties. The present work, for the first time, revealed the anticataract and aldose reductase inhibitory potential of leaves of

Barleria lupulina Lindl. and this could be the initiative for the further pharmacological studies related to diabetic complications on this plant.

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