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Evaluation of anti cataractogenic activity of of *Biophytum Reinwardtii* on olanzapine induced cataract on isolated goat lens

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Abstract:

Aim of Study: Evaluation of anti cataractogenic activity of of *Biophytum Reinwardtii*(HEMBR) on olanzapine induced cataract on isolated goat lens

Materials and Methods: Goat eye lenses were divided into 4 groups; Group I served as Navie control,Group II as toxic vehicle control, Group III HEMBR(250µg/ml) and Group IV HEMBR(500µg/ml) . Group II, III and IV were incubated in olanzapine(10mg) in artificial aqueous humor to induce lens opacification. Estimation of total protein, catalase, and glutathione e along with histological evaluation of lens were done to measure the lens opacification.

Results: olanzapine treated Group II lenses showed low amount of protein, decreased catalase and glutathione levels compared to navie control, while lenses treated with HEMBR (Group III and Group IV) showed significant (*$p < 0.05$) increased level of catalase, glutathione, total and decreased amount protein.

Conclusion: The present study findings suggest HEMBR exhibit anti cataract effect in olanzapine induced cataract.

Key words: *In Vitro* anticataract, lens, *Biophytum Reinwardtii*,, olanzpine

INTRODUCTION:

Cataract is the leading cause of blindness and visual impairment globally. Blindness from cataract is more common in populations with low socioeconomic status and in developing countries than in developed countries. (1) The only treatment for cataract is surgery. Phacoemulsification is the gold standard for cataract surgery in the developed world, whereas manual small incision cataract surgery is used frequently in developing countries. In general, the outcomes of surgery are good and complications, such as endophthalmitis, often can be prevented or have good ouctomes if properly managed. Femtosecond laser-assisted cataract surgery, an advanced technology, can automate several steps; initial data show no superiority of this approach over current techniques, but the results of many large clinical trials are pending. (2) The greatest challenge remains the growing 'backlog' of patients with cataract blindness in the developing world because of lack of access to affordable surgery. Efforts aimed at training additional cataract surgeons in these countries do not keep pace with the increasing demand associated with ageing population demographics. In the absence of strategy that can prevent or delay cataract formation, it is important to focus efforts and resources on developing models for efficient delivery of cataract surgical services in underserved regions.(3)

Cataract is accompanied by a decrease in the activities of protective antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH).(4-5) The high level of glucose is one of the important reasons behind progression of diabetic cataract.(6,7)Glycation of sugars and binding with amino groups on proteins to produce adducts disrupts biological properties of proteins. This results in structural changes in enzymes which ultimately causes enzyme inactivation.(8) During hyperglycemia or osmotic stress the pathway activity increases several times. Association of this condition with chronic complications in diabetes has been observed in experimental models of hyperglycemia, where significant alterations affected the opacity of lens. These changes are characterized by high sorbitol levels, alterations on the membrane permeability, low of glutathione (GSH) and a diminution of the protein synthesis.(9) By the other side, oxidative stress is associated with cataracts formation by production of hydrogen peroxide through glucose auto-oxidation.Hyperglycemia can induce oxidative stress via several mechanisms. These include glucose autoxidation, formation of advanced glycation end-products (AGE), and activation of the polyol pathway. Natural antioxidants are famous for their capacity to protect organisms and cells from damage due to oxidative stress, the latter being considered a cause of ageing and degenerative diseases. The antioxidants present in food especially vegetables, are phenolic compounds (phenolic acids and flavonoids), carotenoids, tocopherol and ascorbic acid.

Biophytum reinwardtii (Family - Oxalidaceae),
Commonly known as "Pulicenta" in south India.
The present study aims at to isolation of fractions from methanolic extract and evaluate anticataractogenic activity of in olanzapine induced cataract on isolated goat lens

MATERIALS AND METHODS

General experimental procedures

Ready-made TLC plates loaded with silica gel 60 F254 (Merck, Darmstadt, Germany) were Applied for analytical purposes.

Plant material

Biophytum reinwardtii whole plant was collected from forest area of Rampachowdavaram, East Godavari and authenticated by Dr.P.Prasanna Kumari Department of Botany, D.N.R College, Bhimavaram A voucher specimen was kept at Department of Pharmacology, Shri Vishnu College of Pharmacy.

Extraction and isolation of the fractions

Preparation of plant extract:

The Whole plant were dried under shade at room temperature The shade dried, coarsely powdered roots (500 g) was successively extracted with petroleum ether (60-80^{o}C) for 7 days to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% methanol to obtain methanolic extract. The methanolic extract was evaporated under reduced pressure at low temperature (30^{o}C) to dryness and brownish yellow colour extract of ***Biophytum reinwardtii*** was obtained.

Seperation of active compounds by coloumn chromatography:

A cylinder shaped glass column containing stationary phase (silica gel) is encountered slowly from the top with a liquid solvent (mobile phase) that flows down the column with the help gravity or external pressure applied. This technique is used for the purification of compounds from a mixture. Once the column is ready, the sample is loaded inside the top of the column. The mobile solvent is then allowed to flow down through the column. The compounds in mixture have different interactions ability with stationary phase (silica gel), and mobile phase, thereby will flow along the mobile phase at different time intervals or degrees. In this way, the separation of compounds from the mixture is achieved. The individual compounds are collected as fractions and analyzed further for structure elucidation (10)

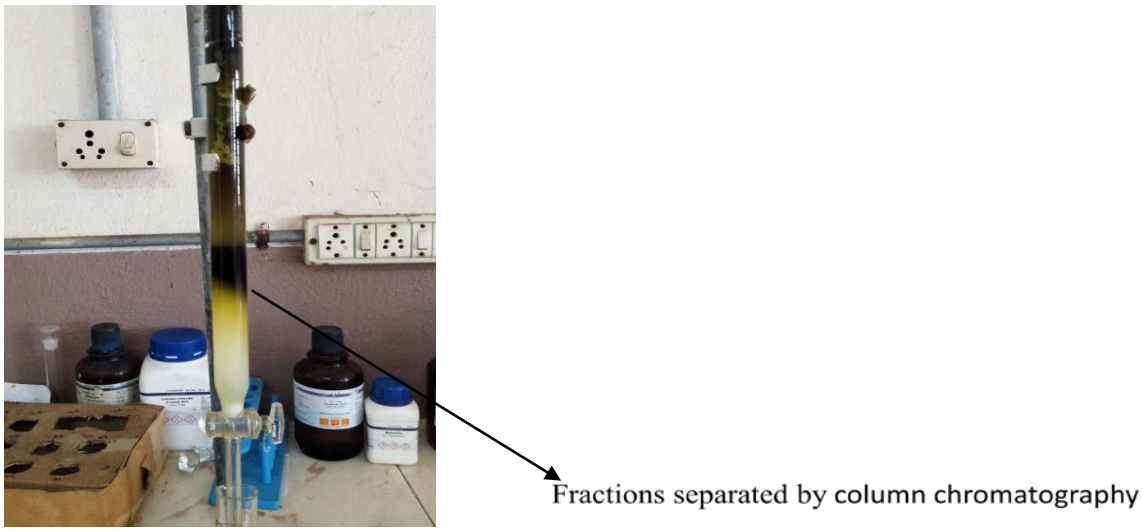


Figure: 1: Seperation of fractions by column chromatography

Table:1: Gradient solvent system used in column chromatography

Gradient solvent system used in column chromatography for the isolation of fractions in Biophytum reinwardtii whole plant		
Solvent system	**Ratio**	**Fraction**
Hexane	100%	-
Hexane : Ethyl acetate	**95 : 5**	**1**
Hexane : Ethyl acetate	70 : 30	2
Hexane : Ethyl acetate	50 :50	3
Only ethyl acetate	100%	4
Only methanol	100%	5

Preliminary Phytochemical Screening

Different fractions were subjected to preliminary phytochemical evaluation for the detection of various constituents.

Anti Cataractogenic activity

Procedure for induction of cataract by olanzapine:

olanzapine is an atypical antipsychotic which mediates its effects by serotonin-dopamine antagonism. It has been hypothesized that serotonin antagonism results in reduced glucose responsiveness of the pancreatic beta-cells (11-12). This explains olanzepine's diabetogenic effect, as was observed in patient. In diabetic patients, impaired glucose tolerance causes sorbitol accumulation in the lens via the polyol pathway, resulting in oxidative stress and cataract formation. (13)Besides that, non-enzymatic glycation of lens proteins may also contribute to formations of cataract. (14) Diabetic cataracts are characterised by diffuse subcapsular or cortical 'snowflake' opacities at the initial stage, later followed by generalized cortical cataract, as in our patient. (15) Cortical cataract has been found to be associated with diabetes mellitus regardless of glucose control. (16)

Lens culture:
Fresh goat eyeballs were obtained from slaughterhouse immediately after slaughter and transported to the laboratory at 0-4°C. The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor(NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, NaH (PO4) 2 0.5 mM, CaCl2 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Penicillin(32mg %) and streptomycin (250 mg %) were added to the culture media to prevent bacterial contamination.(17)

Experimental design
Study drugs and groups design
In the present study, a dose of 250 µg/mL and 500 µg/mL of Fraction (Hexane: Ethyl acetate) of MEBR used

A total of 24 lenses were divided into following groups (n = 6 in):
Group I : Normal control
Group II : olanzapine 10mg for 5 days
Group III : olanzapine 10mg + HEMBR (250 µg/mL) for 5 days
Group IV : olanzapine 10mg + HEMBR (500 µg/mL) for 5 days

Photographic evaluation
After 5 days of incubation, lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of squares clearly visible through the lens) was observed through the lens as a measure of opacity. The degree of opacity was graded as follows: • 0 - Absence of opacity • 1 - Slight degree of opacity • 2 - Presence of diffuse opacity • 3 - Presence of extensive thick opacity.

Preparation of lens homogenate
After 5 days of incubation, homogenate of lenses was prepared in Tris-buffer (0.23 M, pH 7.8) containing EDTA 0.25×10^{-3} M, and homogenate was adjusted to 10% w/v which was centrifuged a 10,000 g at 4°C for 1 hr and the supernatant was used for the estimation of biochemical parameters .(18)

Estimation of Biochemical parameters:
Protein estimation was done by modified biuret end point assay method. Glutathione estimation was done as reported by Ellman method. Estimation of catalase in lens homogenate was done by Aeibe et al. (19)

Statistical Analysis:
Statistical analysis Results were expressed as Mean± SEM (standard Error of the Mean). The statistical significance of the difference between groups for the various treatments was determined by one-way analysis of variance followed by Dunnett's test. $p<0.05$ was considered statistically significant.

RESULTS

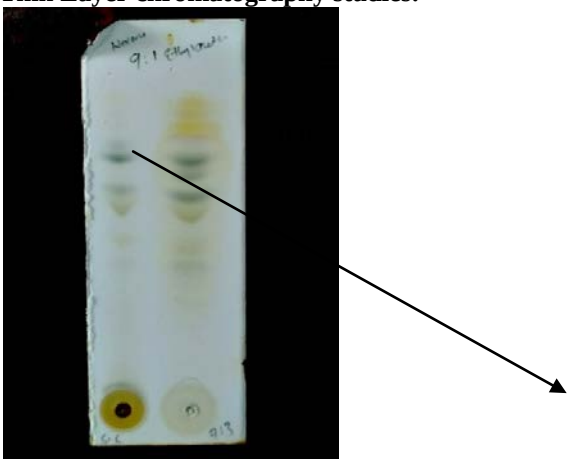
Phytochemical studies of Fraction 95:5ratio (Hexane: Ethyl acetate) revealed the Fraction presence of fattyacids ,phenolics and flavonoids.Table.No:2 Lenses incubated in aqueous humour remained transparent, whereas, the lens incubated with olanzapine (10 mg)developed dense opacities. The opacity increased towards centre with complete opacification at the end of 5 days. Incorporation of Fraction of (H:EA) of HEMBR(250 µg/mL and 500 µg/mL) retarded the development of opacity [Fig.1].

Table.No:2: Phytochemical analysis of various fractions

S.NO	Phytochemical tests	H:EA (95 : 5)	H:EA (70 30)	H:EA (50 : 50)	EA only	H only	M only
1	Alkaloids	-	-	-	-	-	-
2	Glycosides	-	-	-	-	-	-
3	Tannins	-	-	-	-	-	-
4	Phenols	++	-	-	-	-	-
5	Flavonoids	++	++	-	-	-	-
6	Saponins	-	-	-	-	-	-
7	Steroids	++	++				

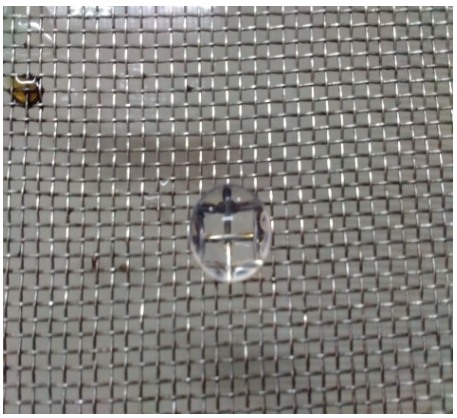
Identification of phytochemical constituents:
Where (+) Indicates presence, and (-) Indicates absence
H- Hexane,EA-Ethyl acetate,M-methanol

Thin Layer chromatography studies:



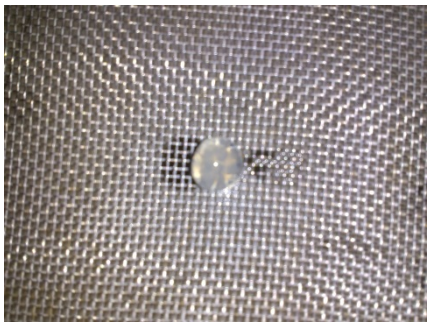
Movement of Hexane: Ethyl acetate(95:5) Fraction

Photographic evaluation

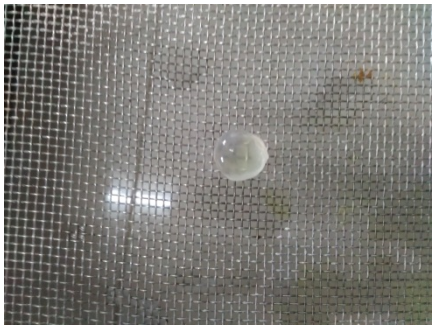


Group I: Normal control

Group II: Model control (olanzapine10mg)



Group III: Test control (Fraction 250ug/mL)



Group IV: Test control (Fraction 500ug/mL)

Fig:2: Olanzapine induced cataract:

Table: 3: Grades of Lens

Study Groups	Grade
Group I(Normal control)	0
Group II(Model control)	3
Group III(Test I)	1
Group IV(Test II)	1

Table: 3: Effect of Fraction (Hexane: Ethyl acetate) of MEBR on weight of the lens

Groups	Wt. of lens before drug treatment(gm)	Wt. of lens after drug treatment(gm)
Group I	0.41	0.41
Group II	0.45	0.73
Group III	0.47	0.65
Group IV	0.37	0.55

Protein content

The protein level of olazapine treated lens (Group II) showed significant decrease as compared to normal control group (Group I) HEMBR at concentration of 250 µg/mL and 500 µg/mL showed significant increase in lens protein as compared to toxic control (Group II) [Fig.3].

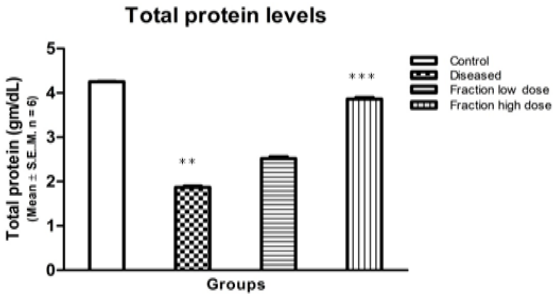


Figure 3: Effect of Fraction (Hexane: Ethyl acetate) of MEBR on Total protein content on isolated goat lens; all values are mean ± SD, $n = 6$; Statistical comparison was performed using analysis of variance (ANOVA) followed by Dunnett's test test (***$p < 0.05$)

Catalase levels

In this study, incubation by olanzapine resulted in a time dependent inactivation of the enzymes.[23] Lens catalase activities were also significantly lower in the lens of Group II as compared to Fraction treated groups.[Fig.4]

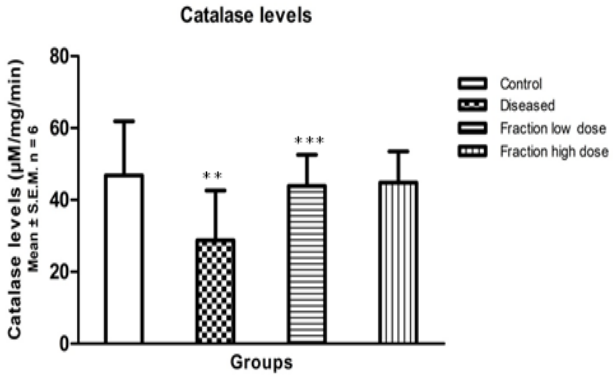


Figure: 4: Effect of Fraction (Hexane: Ethyl acetate) of MEBR on Catalase levels on isolatedgoat lens; all values are mean ± SD, $n = 6$; Statistical comparison was performed using analysis of variance (ANOVA) followed by Dunnett's test test (***$p < 0.05$)

Glutathione level

The glutathione level of olanzapine treated lens (Group II) showed significant decrease compared to navie control group (Group I) HEMBR at the concentration of 250µg/mL and 500 µg/mL showed significant increase in lens glutathione as compared to toxic control (Group II). Only treated group (Group IV) showed almost the same levels of glutathione as that of control [Fig. 5].

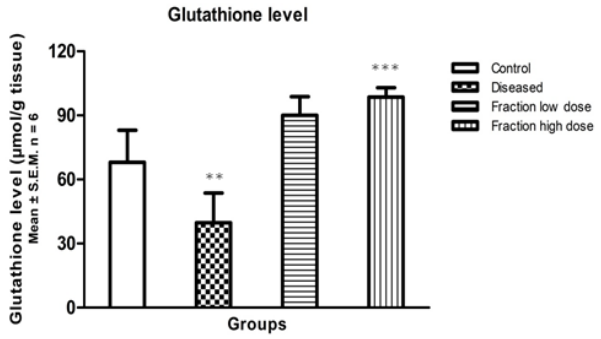


Figure: 5: Effect of Fraction (Hexane: Ethylacetate) of MEBR on Reduced Glutathione levels on isolatedgoat lens; all values are mean ± SD, $n = 6$; Statistical comparison was performed using analysis of variance (ANOVA) followed by Dunnett's test test (***$p < 0.05$)

DISCUSSION:

Cataract is a major cause of blindness worldwide. It is an age-related phenomenon, over and above oxidative stress also plays its role. Surgical treatment has remained the only remedy till now. Hence, if a drug is sought which can either reverse or prevent lenticular opacity it will be a great advance in the treatment of this disorder. A number of drugs have been shown to interfere with the process of cataract formation such as aldose reductase inhibitors, restatin, sulindac, and aspirin. Cataract is one of the universal processes of aging and is a consequence of cumulative effect of various insults to the lens. The oxidation of lens proteins by free radicals and reactive oxygen species plays an important role in the process leading to lens opacification. This oxidative crisis is one of the reasons for generation of cataract.

olanzapine is an atypical antipsychotic which mediates its effects by serotonin-dopamine antagonism. It has been hypothesized that serotonin antagonism results in reduced glucose responsiveness of the pancreatic beta-cells . This explains olanzepine's diabetogenic effect, as was observed in our patient. In diabetic patients, impaired glucose tolerance causes sorbitol accumulation in the lens via the polyol pathway, resulting in oxidative stress and cataract formation. Besides that, non-enzymatic glycation of lens proteins may also contribute to formations of cataract .(20) Diabetic cataracts are characterised by diffuse subcapsular or cortical 'snowflake' opacities at the initial stage, later followed by generalized cortical cataract, as in our patient .Cortical cataract has been found to be associated with diabetes mellitus regardless of glucose control

CAT, SOD, and GSH are important components of the innate enzymatic defenses of the lens. CAT has been shown to be responsible for the detoxification of significant amounts of H_2O_2 SOD catalyzes the removal of superoxide radicals (O–), which would otherwise damage the membrane and biological structures.(21) The enzyme GSH, first demonstrated in the lens by Pirie,(22) has been reported to maintain the integrity of the phospholipid bilayer of membranes by inhibiting lipid peroxidation. CAT and GSH catalyze the transformation of H_2O_2 within the cell to harmless by products, thereby curtailing the quantity of cellular destruction inflicted by products of lipid peroxidation. A reduction in the activities of these enzymes in tissues has been associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes.

Reduction in the levels of reduced glutathione is observed during cataract of any etiology. GSH plays a leading role in preserving lens clarity. It also acts as antioxidant and stabilize proteins in reduced form.(23,24)Phytoconstituents from herbal drugs may ultimately inhibit expenditure of GSH through oxidation leaving the–SH groups intact. On the other hand, they may directly stimulate reduced glutathione synthesis which may be due to a modulating effect on reduced glutathione related enzymes in the lens

The restoration of reduced glutathione levels by HEMBR could be potential reason for its anticataract activity.

During oxidative stress, denaturation and aggregation of proteins is observed which is leads to total protein opalescence in lens. Intestingly HEMBR improved showed decrease in opalescence which is associated with an increasing protein levels

CONCLUSION:

The present study suggests that the HEMBR exhibits potent anti cataract activity in olanzapine induced cataract. Further studies are needed for structural elucidation of HEMBR and determining exact molecular mechanism(s) responsible for anti-cataract activity.

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