International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 13-03-2019; Accepted: 15-04-2019
www.pharmacyjournal.in
Volume 4; Issue 3; May 2019; Page No. 78-82

Anti cataract activity of Methanolic extract of *Ixora coccinea flower* leaves on dexamethasone induced cataract by using isolated goat lens

N Jyothirmai[1*], R Vamsi[2], SK Abul Kalam[3], V Prem Kumar[4]
[1-4] Department of Pharmacology, Narasaroaopet Institute of Pharmaceutical Sciences Kottapakonda Road, Yallamandha, Narasaraopet, Guntur, Andhra Pradesh, India

Abstract
Objective: To investigate anti cataract activity of Methanolic extract of *Ixora coccinea flower* on Prednisolone induced cataract by using isolated goat lens.
Methods: Anti cataract activity is done by using isolated goat lens. Goat lens were divided into four groups. Group I lens were incubated in artificial aqueous humour (normal control).Group II lens were incubated with dexamethasone 10mg (toxic control).Group III and IV lens were incubated with dexamethasone and methanolic extract of (50µg and 100µg) *Ixora coccinea flower* and subjected to photographic evaluation for opacity, lens were homogenized by using tris phosphate buffer and sodium, potassium, total protein and catalase concentrations were determined
Results: The grades of opacity was 0,3,1 and 1 in group I,II,III and IV respectively. The present study showed higher total proteins ($P < 0.05$ at all concentration) and K+ ions ($P<0.05$ at all concentration) whereas lower concentrations of Na+ ions ($P<0.05$ at all concentration) with MEIC treated groups. The level of Catalase was found to be less in experimentally induced cataract lenses as compared to normal control group. The lenses treated with EEAS showed significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity.
Conclusion: The Present investigation suggests that Methanolic extract of *Ixora coccinea flower* effectively prevent the cataractogenic condition. Thus, the goat lens model and dexamethasone induced cataract model could be used for testing of various anti cataract agents.

Keywords: Cataract, artificial aqueous humour, lens, Prednisolone, *Ixora cocaine*

1. Introduction
Cataract (lens opacification) is a major contributing factor of blindness. It is defined as a clouding of the natural lens, a part of the eye responsible for focusing and producing a clear sharp image. It is called as a "peril of sight" because cataracts have blinded more people throughout the ages than any other affliction of the eye. It is also called as "Senile cataract". Cataract is derived from the Latin word "Cataracta" meaning waterfall. ARN (Age- Related Nuclear Cataract) is the most common form of cataract which is found in ages more than 45 year and opacity forms in the centre of the lens [1]. Cataract Nothing But visual impairment as a result of a disturbance of lens transparency. It is one of the leading cause of blindness worldwide, it accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 28000 new cases are reported daily worldwide. Approximately 25% of the populations over 65 and about 50% over 80 have serious loss of vision because of cataract [2, 3]. Cataractogenesis is influenced by multiple risk factors, such as aging, diabetes mellitus, drugs, trauma, toxins, genetics, smoking and other ocular diseases. Multiple mechanisms such as osmotic graduation, protein aggregates, oxidative stress, post translational protein changes, phase separation are proposed for cataract formation. Combined factors of heritage, UV light exposure, diet, some metabolic disorders, quality of life, cationic pump malfunction and lens metabolism disorder are believed to have a role in cataract formation. The increased incidence of cataracts, in diabetic patients is also well known [4]. Presently, surgery is the only approach for the treatment of cataract, and while favourable outcomes are quite predictable, the limited number of surgeons is underdeveloped countries and the high cost of surgery have made cataract a major health problem. Drugs developed to delay or prevent lens opacification have failed to give convincing positive results in clinical trials. This stimulates the research towards the experimental work on cataract to understand the all possible pathway and mechanism which is responsible for the generation of cataract. While the main treatment for cataract is surgical intervention, it is associated with certain risks and subsequent suboptimal outcomes [5].
Prolonged use of glucocorticoids is a significant risk factor for the development of posterior subcapsular cataract. This places restrictions on the use of glucocorticoids in the treatment of systemic and ocular inflammatory conditions as well as organ transplantation. Glucocorticoids induce subcapsular cataract by cause the metabolic disturbances, protein modifications, oxidative damage and Inactivation of Na, K-ATPase system [6].
The prophylactic and therapeutic effect of many herbal extract have been reported, Such as *Adhatoda vasica, Allium cepa, Cassia fistula, Citrus aurantium, Cochlospermum religiosum, Curcuma longa, Ginkgo biloba, Momordicacharantia, Ocimum sanctum, Vitex negundo* having anti cataract activity [7, 8]. *Ixora coccinea flower* extract having pharmacological action like Antimicrobial, Hepatoprotective, and Antioxidant. Antiulcer,

Antidepressant, Antineoplastic, activity is also reported [8]. With this background the objective of current study was to evaluate the Anti cataract activity of Methanolic extract of *Ixora coccinea flower*

2. Materials and Methods

2.1 Collection and Identification

Ixora coccinea flower were collected from the local medicinal garden of Narasaroaopeta Institute of Pharmaceutical Sciences, Andhra Pradesh, India. Authenticated by the Department of Botany, ANU, Guntur, voucher specimens kept for future reference.

2.2 Drying and Grinding

The collected plant part (Flowers) was separated from undesirable material and The leaves were dried under shade at room temperature for two week. The dried leaves were ground into a coarse powder with a suitable grinder. The powder was stored in air tight container until the analysis was commenced.

2.3 Extraction

Coarsely powdered leaves (500 g) were successively extracted with petroleum ether (60-80ºC) for 7 days to remove fatty matter. The defatted marc was then subjected to Soxhlet extraction with 75% ethanol to obtain methanolic extract. The methanolic extract was evaporated under reduced pressure at low temperature (30ºC) to dryness and brownish yellow colour extract was obtained [9].

2.4 Preliminary Phytochemical Screening

Methanolic extract of *Ixora coccinea flower* was subjected to preliminary Phytochemical for the detection of various constituents [10].

2.5 Ex- Vivo evaluation of anti-cataract activity

In this study, goat lens was used as they were easily available. Fresh goat lens were collected from slaughter house from Guntur.

a) Lens culture

Fresh goat eyeballs were obtained from slaughter house was immediately transported to the laboratory at 0-40 c. The lens were removed by extra capsular extraction and incubated in artificial aqueous humour(Sodium chloride:140mM, Hydrochloric acid:5mM, Magnesium chloride: 2mM,Sodium Bicarbonate:0.5mM,Sodium dihydrogen phosphate:0.5mM,Calcium chloride:0.4mM and glucose:5.5mM) at room temperature and PH 7.8.Cefixime 500mg were added to the culture media to prevent bacterial contamination [11].

b) Induction of Ex-Vivo Cataract

Prednisolone 10mg was used to induce cataract. Prednisolone induced posterior subcapsular cataract by oxidative stress, osmotic change, hydration and conformational change of proteins. A total of 16 lenses were used for the study. These lenses were incubated in artificial aqueous humour with Prednisolone 10mg/kg served as toxic control for 5 days [12].

c) Study group

A total 16 lenses were divided into following groups.(n= 4 in each group).

Group I: Aqueous humour (Normal control).
Group II: Aqueous humour + Prednisolone 10mg (Toxic/model control).
Group III: Aqueous humour + Prednisolone 10mg + EEAS 250µg/ml.
Group IV: Aqueous humour + Prednisolone 10mg + EEAS 500µg/ml.

d) 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.

e) Preparation of Lens Homogenate

After 5 days of incubation, homogenate of lenses was prepared in tris buffer (0.23 M, pH 7.8) containing 0.25 × 10-3 M EDTA and homogenate was adjusted to 10% w/v which was centrifuged at 10,000 G at 4ºC for 1hour and the supernatant was used for the estimation of biochemical parameters [12].

f) Biochemical Parameters

Electrolyte (Na+) and Potassium (K+) estimation was done by flame photometry method and protein estimation was done by Modified Biuret End Point Assay method. Estimation of Catalase in lens homogenate was done by Aeibe *et al*. [13-19].

2.6 Statistical Analysis

Results were expressed as mean ± S.E.M. The statistical significance of the difference between groups for the various treatments were determined by one way analysis of variance (ANOVA) followed by Dunnets test. $P<0.05$ was considered statistically significant.

3. Results

Phytochemical analysis was performed for various phytochemical constituents' present Methanolic extract of *Ixora coccinea flower* and results were shown in table-1

The grades of opacity was 0,3,1 and 1 in group I,II,III and IV respectively and results were shown in table-2. The present study showed higher total proteins ($P < 0.05$ at all concentration) and K+ ions ($P<0.05$ at all concentration) whereas lower concentrations of Na+ ions ($P<0.05$ at all concentration) with EEAS treated groups. The level of Catalase was found to be less in experimentally induced cataract lenses as compared to normal control group. The lenses treated with MEIC showed significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity and results were shown in table-3.

3.1 Photographic Evaluation

Incubation of lenses with Prednisolone 10mg showed moderate opacification starting after 2 days at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 5 days as compared to lenses incubated in normal

aqueous humour where transparency maintained and squares were clearly visible. Incubation of lenses with EEAS at (250 µg/ml, 500 µg/ml) concentrations seems to retard the progression of lens opacification.

Group I (Normal)

Group II (Toxic control)

Group III (EEAS 250µg/ml)

Group III (EEAS 500µg/ml)

4. 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. 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 like aldose reductase inhibitors, restatin, sulindac, aspirin, etc. Cataract is one of the universal processes of ageing and is consequence of cumulative effect of various insults to the lens. The oxidation of lens proteins by free radicals and reactive oxygen species play an important role in the process leading to lens opacification. This oxidative crisis is one of the reasons for generation of cataract.

Ex-vivo model for inducing cataract using Prednisolone 10mg provides an effective model on isolated lenses of goat. Incubation of goat lenses in the media containing dexamethasone (10mg) concentration induce cataract it has shown to cause considerable drop in Na+/K+-ATPase activity, with progression of opacity. The impairment of Na+/K+-ATPase causes accumulation of Na+ and loss of K+ with hydration and swelling of the lens fibers leading to cataract genesis. This alteration in the Na+, K+ ratio change the protein content of the lens, leading to a decrease in total proteins causing lens opacification. The present study showed higher total proteins ($P < 0.05$ at all concentration) and K+ ions ($P<0.05$ at all concentration) whereas lower concentrations of Na+ ions ($P<0.05$ at all concentration) with EEAS treated groups. The imbalance of Na+ and K+ is prevented due to an action of EEAS which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentrations. Catalase is an important part of the innate enzymatic defence system of the lens which is responsible for the detoxification of H2O2. Decrease in the activities of this enzyme in tissue has been linked with the build-up of highly reactive free radicals leading to injurious effect such as loss of integrity and the function of the cell membranes. The catalase keeps the level of free radicals below toxic levels. In this study the level of Catalase was found to be less in experimentally induced cataract lenses as compared to normal control group. The lenses treated with EEAS showed significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity.

Table 1: Phytochemical analysis of Methanolic extract of *Ixora coccinea flower*

Name of the Test	Results
Carbohydrates	++
Steroids	- -
Alkaloids	++
Glycosides	++
Tannins	++
Flavonoids	- -
Saponins	++
Gums	- -

Table 2: Grades for lens

Study Groups	Grade
Group I(Normal control)	0
Group II(Model control)	3
Group III(MEAS 250µg/ml)	1
Group IV(MEAS 500µg/ml))	1

Table 3: Effect of MEIC on Sodium, Potassium, Total protein and Catalase levels in Prednisolone induced Cataract

Groups	Sodium levels µg/ml Mean ± SEM	Potassium levels µg/ml (Mean ± SEM)	TPC level gm/dl (Mean ± SEM)	Catalase levels µm of H_2O_2/min (Mean ± SEM)
Group I	105.5±+2.10	10.8±0.44	3.25±0.01	228.3±0.85
Group II	227.3±3.30	6.17±0.11	1.87±0.03	143±0.91
Group III	165.8±1.93	8.95±0.12	2.52±0.04	267.5±1.04
Group IV	116±1.29	10.18±0.15	2.86±0.04	304±1.86

Data presented as mean ±S.E.M. (n=4).Data were analysed by one way analysis of variance (ANOVA) followed by Dunnet test.
*P<0.05.Significant When compared to model control

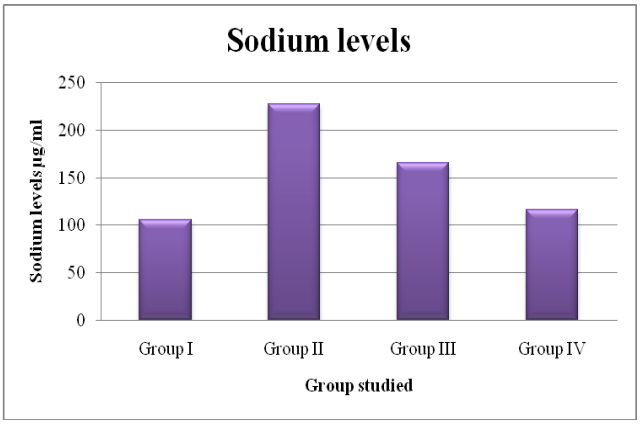

Fig 1: Effect of Methanolic extract of *Ixora coccinea flower* on Sodium Levels

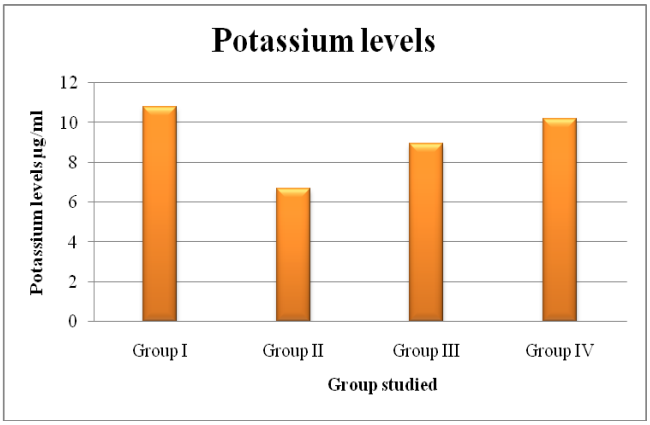

Fig 2: Effect of Methanolic extract of *Ixora coccinea flower* on Potassium Levels

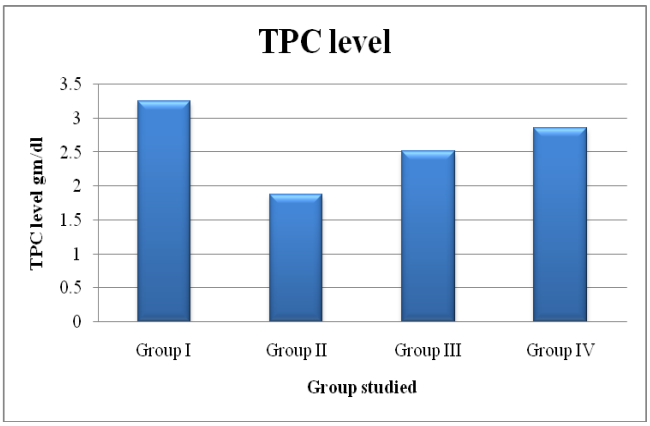

Fig 3: Effect of Methanolic extract of *Ixora coccinea flower* on Total protein Levels

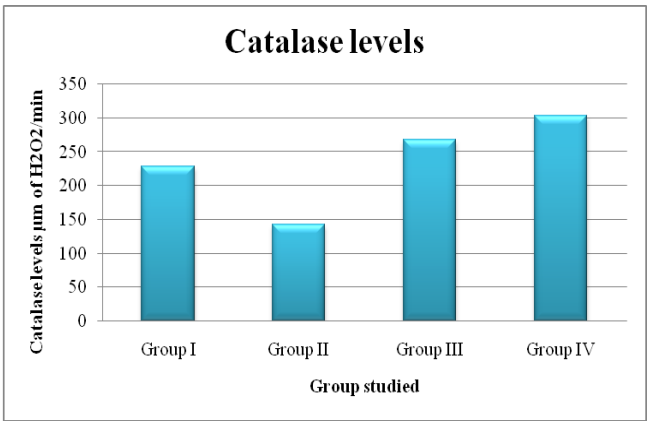

Fig 4: Effect of Methanolic extract of *Ixora coccinea flower* on Catalase Levels

5. Conclusion

The Present investigation suggests that Methanolic extract of *Ixora coccinea flower* effectively prevent the cataractogenic condition which was indicated by increase in the total protein content, potassium level and decrease in the sodium. However, antioxidant property of Methanolic extract of *Ixora coccinea flower* was confirmed by increase. Catalase levels in lens. In conclusion all the above finding lends credence to flowers of *Ixora coccinea flower* in the treatment of cataract.

6. Acknowledgment

The authors are thankful to the Principal, Chairman and management of Narasaraopet Institute of Pharmaceutical Sciences, for providing the facilities for conducting the study.

Conflict of interest

Authors are no Conflict of interest.

7. References

1. Hammond CJ, Duncan DD, Snieder H, de Lange M, West SK, Spector TD. The heritability of age-related cortical cataract: the twin eye study. Investigative Ophthalmology & Visual Science. 2001; 2(3):601-605.
2. Hyman L. Epidemiology of eye disease in the elderly. Eye. 1987; 1(2):330-341.
3. Klein R, Lee K. Cardiovascular disease selected cardiovascular disease risk factors and age related cataracts. American Journal of Ophthalmology. 1997; 123(3):338-346.
4. Van H, Harding J. A case-control study of cataract in Oxfordshire: some risk factors. British Journal of Ophthalmology. 1988; 72(11):804-808.
1. Gupta SK, Sujata J, Velpandian T, Prakash J. An update on pharmacological prospective for prevention and development of cataract. Indian Journal of Pharmacology. 1997; 29(1):3-10.
5. Urban RC, Cotiler E. Corticosteriod induced cataracts. SurvOpthalmol. 1986; 31:102-110
6. Prasad S, Hemalatha S. Cataract: A major secondary complication of diabetes, its epidemiology and an overview on major medicinal plants screened for anticataractactivity. Asian Pacific Journal of Tropical Disease. 2011; 1(4):323-329.
7. Pharmacological activities *of Alstonia scholarislinn.* (Apocynaceae) - Pharmacognosy Reviews 2007; 1(1).
8. Sazada S, Verma A, Rather AA, Jabeenand F Meghvansi MK. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Advances in biological research. 2009; 3:188-195.
9. Joshi SC, Padalia RC, Bisht DS, Mathela CS. Terpenoid diversity in the leaf essential oils of Himalayan Lauraceae species. Chemistry and Biodiversity, 2009; 6(9):1364-73.
10. Pal A, Rai G, Bhadoriya S. In vitro prevention of cataract by Oyster *muschroom pleurotus florida* extract on isolated goat eye lens. Indian Journal of Pharmacology. 2011; 43(6):667-670.
11. Black RL, Oglesby RB, von Sallmann L, Bunim JJ. Posterior subcapscular cataracts induced by corticosteroids in patients with rheumatoid arthritis. The Journal of the American Medical Association. 1960; 174:150-155.
12. Mahapatra K, Mishra P. Comparison of sodium and potassium content in ORS powders by Flame photometric method. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011; 2(3):263.
13. Gitelman H, Kukolj S, Welt L. The influence of the parathyroid glands on the hypercalcemia of experimental magnesium depletion in the rat. Journal of Clinical Investigation. 1968; 47(1):118.
14. Imaruna R. For determination of Na+/k+ measurement. Analytical Biochemistry. 1958; 2:581.

15. Estimation of total protein by modified Biuret end point assay method., http://btprotocolsmaulik. blogspot.in/ 2007/04/to-performestimation- of protein-by.html
16. Hiroshi O. Assay for lipid peroxidation in Animal tissue by Thiobarbituric Acid Reaction. Analytical Biochemistry. 1979; 95(1):351-358.
17. Misra HP, Fridovich I. The oxidation of phenylhydrazine: superoxide and mechanism. Biochemistry, 1976; 15(3):681-7.
18. Weydert C, Cullen J. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nature Protocols. 2009; 5:51-66.