International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 779-783
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Evaluation of Anti Cataractogenic Activity of Cerium Oxide Nanoparticles in Corticosteroid Induced Cataract

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Received: 14 Mar 2019 / Accepted: 16 Apr 2019 / Published online: 1 Jul 2019
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Abstract

Aim: To investigate the anti cataractogenic activity of Cerium oxide nanoparticles on Prednisone 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 humor (normal control). Group II lens were incubated with Prednisone 100mg (toxic control). Group III and IV lens were incubated with Prednisone and Cerium oxide nanoparticles (10mg and 20mg) and subjected to photographic evaluation for opacity, lens was 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 in group I, II and III 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 Cerium oxide nanoparticles 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 Cerium oxide nanoparticles showed significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity. **Conclusion:** The Present investigation suggests that Cerium oxide nanoparticles effectively prevent the cataractogenic condition. Thus, the goat lens model and prednisone induced cataract model could be used for testing of various anti cataract agents.

Keywords
Cataract, artificial aqueous humour, lens, prednisone, nanoparticles.

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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 is 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 restriction on the use of glucocorticoids in the treatment of systemic and ocular inflammatory conditions as well as organ transplantation. Glucocorticoids induce sub-capscular cataract by cause the metabolic disturbances, protein modifications, Oxidative damage and Inactivation of Na, K-ATPase system. (6) Cerium compounds have recorded uses in drugs and pharmaceuticals as early as the mid nineteenth century. In 1854, cerium nitrate was first reported to relieve vomiting. Cerium(III) oxalate was administered for many following decades for anti-vomiting effects in cases of sea sickness, gastrointestinal and neurological disorders, and especially in pregnant women. Cerium(III) oxalate was used as an antiemetic until the mid-1950s when it was replaced by the antihistamine meclizine which is still in use today (7)

Trivalent cerium exhibits similar size and bonding properties to Ca2+, an extremely biologically important cation. Ce3+ can replace Ca2+ in biomolecules due to their similar ionic radius, thus Ce3+ compounds strongly exhibit the ability to act as anticoagulant, or anti-clotting, agents. Several of the lanthanides, including cerium, are well-known for their anticoagulant properties and have been employed as antithrombic drugs. Cerium compounds are also known in particular for their uses in topical burn treatments due to their bacteriostatic and bactericidal effects. By the end of the 19th century, Ce compounds were used in both human and veterinary medicine; most commonly used were Ce(III) acetate and Ce(III) stearate treatments. Later, studies confirmed the antiseptic effects of cerium(III) chloride, Ce(III) nitrate, and Ce(IV) sulfate and demonstrated particular susceptibility of both gram-negative and gram-positive bacteria (which tend to coat burn wounds) to their effects. Cerium (III) nitrate in particular is a widely used treatment for burn wounds, exhibiting nearly a 50% reduction in death rate for patients with life-threatening burns when compared to patients who were administered silver nitrate treatments (8)

Promising results have been obtained using cerium (Ce) oxide nanoparticles (CNPs) as antioxidants in biological systems. CNPs have unique regenerative properties owing to their low reduction potential and the coexistence of both Ce (3+)/Ce (4+) on their surfaces, based on this property we select the cerium oxide nanoparticles for anti cataractogenic activity because free radicals is also one of the causative factor for cataract.

Present study I used cerium oxide nanoparticles for evaluation of anticataract activity in corticosteroids induced cataract.

MATERIALS AND METHODS:

Chemicals and drugs

Cerium Nitrate, Prednisone and Cefixime.

Cerium oxide nanoparticles(25nm) are purchased from sigma Aldrich company.

Acute toxicity:

Acute toxicity study was performed as per with OECD 423. 2000mg/kg single oral dose proved to be nontoxic**.**

Dose selection

1/10th acute toxic dose is generally selected as therapeutic dose for experimentation. As present study is focused on evaluating nanoparticles, 20mg/kg is selected as therapeutic dose.

Ex- Vivo evaluation of anti-cataract activity:

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

Lens culture:

Fresh goat eyeballs were obtained from slaughter house was immediately transported to the laboratory at 0-40 c. The lens was 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. (9)

Induction of Ex-Vivo Cataract:

Prednisone 100mg was used to induce cataract. Prednisone induced posterior sub capsular cataract by oxidative stress, osmotic change, hydration and conformational change of proteins. A total of 12 lenses were used for the study. These lenses were incubated in artificial aqueous humour with Prednisone 10mg/kg served as toxic control for 5 days. (10)

Study group:

A total 12 lenses were divided into following groups. (n= 4 in each group).

Group I: Aqueous humour (Normal control).

Group II: Aqueous humour + Prednisone 100mg (Toxic/model control).

Group III: Aqueous humour + Prednisone 100mg + Cerium oxide nanoparticles 20mg.

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 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. (11)

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 Aeibeet al. (12-17)

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 Dunnetstest. $P<0.05$ was considered statistically significant.

RESULTS:

The grades of opacity were 0, 3 and 1 in a group I, II, III 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 Cerium oxide nanoparticles 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 Cerium oxide nanoparticles showed significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity and results were shown in table-1.

Table: 1 Effect of Cerium oxide nanoparticles on Sodium, Potassium, Total protein and catalase levels in Prednisone induced Cataract

Groups	Grade	Sodium levels µg/ml Mean ± SEM	Potassium levels µg/ml (Mean ± SEM)	TPC level gm/dl (Mean ± SEM)	Catalase levels µm of H2O2/min (Mean ± SEM)
Group I	0	101.5±+2.10	9.8±0.44	3.15±0.01	218.3±0.85
Group II	3	227.3±3.30	6.17±0.11	1.87±0.03	143±0.91
Group IV	1	110±1.29	9.18±0.15	2.75±0.04	208±1.86

Data presented as mean ±S.E.M.(n=3). Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnet test. *P<0.05. Significant When compared to model control

Photographic Evaluation

Incubation of lenses with prednisone 100mg showed moderate opacification starting after 3 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 Cerium oxide nanoparticles at 20 mg concentrations seems to retard the progression of lens opacification.

Group I(Normal)

Group II (Toxic control)

Group III (cerium oxide nanoparticles)

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 Prednisone 100mg provides an effective model on isolated lenses of goat. Incubation of goat lenses in the media containing Prednisone 100mg 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 cataractogenesis. 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 Cerium oxide nanoparticles treated groups. The imbalance of Na+ and K+ is prevented due to an action of Cerium oxide nanoparticles 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 Cerium oxide nanoparticles showed significant rise in enzyme level

suggesting maintenance of antioxidant enzyme integrity.

CONCLUSION:

The Present investigation suggests that Cerium oxide nanoparticles 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 Cerium oxide nanoparticles was confirmed by increase catalase levels in lens. In conclusion all the above finding lends credence to leaves of Cerium oxide nanoparticles in the treatment of cataract.

ACKNOWLEDGMENT:

The authors are thankful to Shri Vishnu College of Pharmacy, Bhimavaram, for providing the facilities to conducting the study.

CONFLICT OF INTEREST:

Authors are no Conflict of interest.

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