Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL RESEARCH ARTICLE

Polyherbal Formulation Containing Antioxidants may Serve as a Prophylactic Measure to Diabetic Cataract: Preclinical Investigations in Rat Model

Nilesh M. Mahajan[1], Bhushan B. Lokhande[3], Raju R. Thenge[3], Purushottam S. Gangane[1], Nitin G. Dumore[2]

Departments of [1]Pharmaceutics and [2]Pharmacology, Dadasaheb Balpande College of Pharmacy, Besa, Nagpur, [3]Departments of Pharmaceutics, IBSS College of Pharmacy, Malkapur, Maharashtra, India

Submitted: 22-03-2018 Revised: 04-11-2018 Published: 21-11-2018

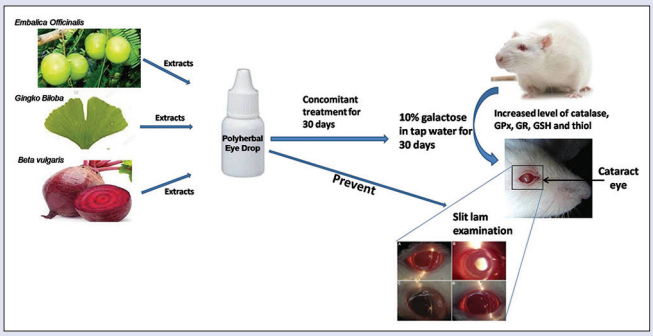
ABSTRACT

Background: Cataract is a major cause of visual impairment in diabetic patients. Due to increasing numbers of type 1 and type 2 diabetics worldwide, the incidence of diabetic cataracts steadily rises. Cataract surgery is the best possible cure for patients suffering from this ailment. However, the elucidation of pathomechanisms to delay or prevent the development of cataract in diabetic patients remains a challenge. **Objective:** The aim of the present study was to develop a polyherbal eyedrops containing potent antioxidant herbal extract and study the effectiveness as prophylactic treatment against galactose-induced diabetic cataract. **Materials and Methods:** Formulations were prepared by using extracts of *Ginkgo biloba* leaves, beet root (*Beta vulgaris*), and amla (*Emblica officinalis*) fruits. The viscosity enhancers were used to increase the retention time. Formulations (F1–F5) were prepared by using carboxy methyl cellulose and poly ethylene glycol-400 as thickening agents and propylene glycol as a solubilizer. Preliminary evaluation showed that formulations have passed clarity, sterility, and eye irritancy tests. Viscosity and pH of formulation were within the normal range. Diabetic cataract was induced in Wistar rats by 10% galactose drink (for 30 days) and anticataract activity was evaluated. Formulation was installed in eye as a prophylactic treatment from day 1 of galactose drinking and continued for 30 days. **Results:** Slit-lamp photography of eyes of rats showed clear lens of rats without any trace of opacity. On the other hand, galactose-treated rats developed dense nuclear opacity in lens as an indication of diabetic cataract. Rats which received prophylactic treatment showed less percent opacity as compared to that of cataract control group and decreased vacuoles. **Conclusion:** We may conclude that polyherbal formulation containing extracts of *Ginkgo biloba* leaves, beet root (*Beta Vulgaris*), and amla (*Emblica officinalis*) fruits may prevent the development of cataract in diabetic patients.

Key words: antioxidant, diabetic cataract, lens opacity, polyherbal formulation, prophylactic measure, prophylaxis

SUMMARY

- Polyherbal eye drops prepared from the extracts of *Ginkgo biloba* leaves, Beta vulgaris and *Emblica officinalis* fruits containing potent antioxidant actives were formulated and developed. All formulations were tested for the parameters like pH, viscosity, sterility and eye irritancy. All the parameters were found within the range of acceptance. Formulations were tested preclinically for the prophylaxis of diabetes induced cataract in Albino wistar rats. Diabetic cataract was developed by allowing the animals to drink galactose solution in tap water (4 gm of galactose every other day for the period of 30 days). Formulation was installed in eye as a prophylactic treatment from day one of galactose drinking and continued for 30 days. Level of different antioxidant enzymes were estimated in the lens of control, and cataract rats treated with either saline solution or formulation [Figure 3]. It was seen that level of catalase, GPx, GR, GSH and thiol level were significantly increased in the lens of rats following galactose administration as compared to control rats ($P < 0.01$). This indicated the role of these enzymes in development of cataract. Prophylactic treatment with formulation significantly restored level of these enzymes in cataract rats to normal level. Effect of significant as compared to vehicle treated cataract rats ($P < 0.01$). Biochemical findings were supported by slit lamp photography of eyes of rats that showed clear lens of rats without any trace of opacity. On the other hand galactose treated rats develops dense nuclear opacity in lens as an indication of diabetic cataract. Rats which received prophylactic treatment showed less percent opacity as compared to cataract control group and decreased vacuoles. This suggests that our polyherbal formulation may prevent development of cataract in diabetic patients.



Abbreviations used: ARI: Aldose reductase inhibitors; GPx: Glutathione peroxidase; GR: Glutathione reductase; DTNB: Dithio-bis-nitrobenzoic acid; GSH: Thiol.

Correspondence:

Dr. Nilesh M. Mahajan,
Department of Pharmaceutics, Dadasaheb Balpande College of Pharmacy, Besa, Nagpur – 440037, Maharashtra, India.
E-mail: nmmahajan78@gmail.com
DOI: 10.4103/pm.pm_622_17

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Cataract is a clouding that develops in the crystalline lens of the eye or in its envelope resulting in slight-to-complete opacity and obstruction in the passage of light.[1] This progresses slowly to cause vision loss and potentially blindness, if untreated. An estimated 200 million people worldwide are suffered with a cataract, which is the leading cause of approximately 42% of blindness.[2] Many factors such as age, nutrition, heredity, medications, toxins, healthy habits, and sunlight exposure influence the development

Cite this article as: Mahajan NM, Lokhande BB, Thenge RR, Gangane PS, Dumore NG. Polyherbal formulation containing antioxidants may serve as a prophylactic measure to diabetic cataract: Preclinical investigations in rat model. Phcog Mag 2018;14:572-7.

of cataract.[3] Cataract is also widely diagnosed in individuals with lifestyle diseases such as hypertension, renal failure, and diabetes. Cataract is a major cause of visual impairment in diabetic patients. Due to increasing numbers of type 1 and type 2 diabetics worldwide, the incidence of diabetic cataracts steadily rises.[4] Cataract surgery is the best possible cure for patients suffering with this ailment. However, the elucidation of pathomechanisms to delay or prevent the development of cataract in diabetic patients remains a challenge. In addition, there is no drug treatment currently available in the market as a prophylactic measure for cataract.

Eye is exquisitely delicate and should be protected from foreign substances. The most important challenge to the formulation scientist is to circumvent the protective barriers of the eye without causing permanent tissue damage which otherwise may lead to blindness. Ocular drug delivery is one of the most fascinating and challenging tasks. Therapeutic efficacy of an ocular drug can be greatly improved by prolonging its contact with the corneal surface.[5] The viscosity-enhancing agents are used in the eyedrop preparations or the drug is formulated in water-insoluble ointment formulations to sustain the duration of intimate drug–eye contact. The present study aimed at the development of polyherbal eyedrops preparation containing extracts of *Ginkgo biloba* leaves, beet root (*Beta vulgaris*), and amla (*Emblica officinalis*) fruits. *G. biloba* is used as an antioxidant to treat diabetic-associated cataract. It contains glutathione (GSH) which is the important component of the innate antioxidant system in the lens and its deficiencies are observed in cataractous lenses.[6] It also contains important flavonoid, i.e., quercetin. It also contains important flavonoids, quercetin, which acts as aldose reductase inhibitor (ARI) responsible for the conversion of glucose to sorbitol. This also prevents oxidation induced sodium and calcium influx and loss of lens transparency in diabetic cataract.[7,8] Carotenes such as lutein and zeaxanthin are antioxidants which are abundantly found in beet root.[9] Amla is a rich source of Vitamin C, the well-known antioxidant. It also prevents aggregation and insolubilization of lens proteins caused by hyperglycemia.[10] Aldose reductase plays a role in the development of secondary complication of diabetes including cataract. Amla also inhibits aldose reductase.[11,12]

This study was conducted to evaluate the suitability of polyherbal formulation for the prophylactic treatment of diabetic cataract to avoid the surgery for its removal. Cataract was induced in adult Wistar rats by oral galactose consumption through tap water for 30 days. For prophylactic assessment, formulations were administered as an eyedrop concomitantly with galactose drinking. Opacity index and other biochemical parameters were calculated as an indicator of cataract in rats.

MATERIALS AND METHODS

Extraction of leaves of *Ginkgo biloba*

The *G. biloba* leaves were purchased and authenticated from the JK Medicinal Plants Introduction Centre, Pampore (Jammu and Kashmir, India). 100 g of coarsely powdered dried leaves was mixed with 900 ml of ethanol, refluxed for 30 min, and then kept aside to cool for overnight. The solution was filtered and again the residue was washed with 100 ml of ethanol two times. The filtrates were combined and allowed to evaporate the ethanol to give the dark green greasy extract which was stored at room temperature and protected from light.[13]

Extraction of beet root

Beet roots weighing about 1 kg were washed in water to remove adhering dirt, and the upper layer of the root was removed and chopped into small pieces. Pieces were grinded, and the coarse powder mass of roots was extracted exhaustively with 95% ethanol in a Soxhlet apparatus by continuous heat.[14] The ethanol extract was concentrated to a small volume and then evaporated to dryness.

Extraction of amla

Fresh fruits were freed from dust and other organic matter. Fruits were kept in water tank overnight. Seeds were separated from thalamus and thalamus was sun dried. 100 g of the dried thalamus material was further grounded in an electrical grinder, soaked in 500 ml distilled water, and was left for 24 h with slow stirring at room temperature. The mixture was strained out using a fine sieve and the crude extract was concentrated for 3 days.[15] The extract was collected, packed immediately, and stored in a cool and dark place at room temperature and capped air tight. The extract used is shown in Table 1.

Formulation of polyherbal eyedrops

The weighed quantity of all herbal extracts were taken and mixed with propylene glycol/polyethylene glycol 400 in a glass mortar. Tween 80 and benzyl alcohol were added into it and solution was stirred properly with the help of overhead stirrer until a homogeneous mixture was obtained. Separately, the aqueous base was prepared by dissolving methyl cellulose, sodium chloride, and potassium dihydrogen orthophosphate into water. The solution was continuously stirred for 15 min with the help of a magnetic stirrer. The drug solution was gradually added into aqueous phase using syringe with continuous stirring. The prepared ophthalmic solution was passed though membrane filter in an aseptic condition. Finally, the filtered solution was transferred aseptically into sterile container in the laminar air flow and capped airtight. The prototype formulations (F1–F5) are disclosed in Table 2.

Evaluation of ophthalmic solution

Physical properties

The prepared formulations were inspected visually for clarity, color, and presence of any foreign particles. pH and viscosity of formulation were determined by using pH meter and Brookfield viscometer, respectively.

Sterility test

The sterility test was performed by membrane filtration method as per the Indian Pharmacopoeia. Formulation solution was passed through a membrane filter of diameter 47 mm with normal porosity of 0.45 µm. After the filtration, the membrane was removed aseptically

Table 1: Preliminary observation of different extracts

Name of extracts	Nature	Color
Ginkgo biloba	Ethanolic	Dark green
Amla	Aqueous	Creamish or faint yellow
Beet root extracts	Aqueous	Deep wine red

Table 2: Composition of ophthalmic solution (% w/v)

Ingredients	F1	F2	F3	F4	F5
Ginkgo biloba extract	0.1	0.1	0.1	0.1	0.1
Amla fruit extract	0.1	0.1	0.1	0.1	0.1
Beet root extract	0.1	0.1	0.1	0.1	0.1
CMC	1	1	1	-	-
Benzyl alcohol	0.1	0.1	0.1	0.1	0.1
Propylene glycol	7.5	6.25	5	2.5	1
PEG 400	-	-	-	0.5	1
Potassium dihydrogen orthophosphate	2.4	2.4	2.4	2.4	2.4
NaCl	0.9	0.9	0.9	0.9	0.9
Tween 80	1	1	1	1	1
WFI	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml

CMC: Carboxy methyl cellulose; PEG: Polyethylene glycol; WFI: Water for injection

from the metallic holder and divided into two halves. The first half was transferred into 100 ml of culture media meant for fungi and incubated at 20°C–25°C. The other half was transferred into 100 ml of fluid thioglycolate medium and incubated at 30°C–35°C. The incubation was allowed for 7 days and the growth of microorganism was observed in the media. The sterility test results were compared with positive and negative controls.[16]

Eye irritancy test

Formulations were individually instilled into one eye of an albino Wistar rat. Rats were observed for 7 days following application of formulation. During the eye examination, cornea, iris, and conjunctivae were observed for signs of opacity, ulceration, hemorrhage, redness, swelling, and discharge.[17]

Evaluation of anticataract activity

Animals used

Adult female albino Wistar rats were obtained from the animal facility of Dadasaheb Balpande College of Pharmacy, Nagpur. Rats were grouped and housed (three rats/cage) in a climate-controlled room (temperature 22°C and humidity 50%–60%) with a 12 h light/dark cycle. All animals were provided *ad libitum* water for drinking and standard laboratory chow. Institutional and national guidelines for the care and use of animals were followed. All animal experiments were approved by the Institutional Animal Ethical Committee.

Induction of cataract by galactose and treatment protocol

Rats were randomly divided into four groups (n = 6). Normal control group (I) received tap water for drinking. Cataract control group (II) received 10% galactose in water for 30 days. Rats in Group III (preventive treatment group) received 10% galactose in water for 30 days and concomitant treatment with prepared ophthalmic formulation (2 drops in each eye) 4 times per day at interval of 4 h.

Assessment of development of cataract by slit-lamp examination

The eyes (dilated pupils) were examined every week for opacities using slit-lamp ophthalmoscope for 30 days. Initiation and progression of lens opacity was graded into the following five categories as described previously.[18]

- 0 – Clear lenses with no vacuoles
- 1 – Vacuoles cover approximately one-half of the surface of the anterior pole
- 2 –Some vacuoles have disappeared and the cortex haziness
- 3 – A hazy cortex remains and dense nuclear opacity is present
- 4 – A mature cataract.

Assessment of biochemical parameters

Lens preparation

On day 30 of galactose and formulation treatment, animals randomly selected from each group were sacrificed by decapitation. Eyeballs of each rat were removed and soaked in 0.9% normal saline for biochemical evaluation. The lenses were dissected by the posterior approach and then placed into preweighed Eppendorf tubes and frozen at –40°C until further analysis.

Thiol determination

Thiol was measured using the dithio-bis-nitrobenzoic acid (DTNB) method at 25°C and 412 nm.[19] The clear supernatant liquid following lens homogenization was used for thiol content determination. Thiols react with DTNB to give a yellow compound that has a high absorption of light at 412 nm. This absorption was measured. Through this colorimetric method, the content of thiol in each lens (from GSH and protein) was measured.

Determination of glutathione reductase

The method described by Linetsky *et al*. . was employed for Glutathione reductase (GR) activity. The reaction was initiated by the addition of 20 µl of lens homogenate. Oxidized GSH (GSSG) was reduced to GSH catalyzed by GR with nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. The decrease in the optical density at 340 nm was recorded at 25°C for 2 min. The units of enzymatic activity were calculated using an extinction coefficient of 6.22 mM/cm for NADPH. One unit was equivalent to the oxidation of 1 mmol of NADPH per min.[20]

Catalase activity

Lens catalase activities were determined by Goth's colorimetric method, in which serum was incubated in H_2O_2 substrate, and the enzymatic reaction was stopped by the addition of ammonium molybdate.[21] The intensity of the yellow complex formed by molybdate and H_2O_2 was measured at 405 nm.

Determination of glutathione peroxidase activity

GSH peroxidase (GPx) catalyzes the reduction of hydrogen peroxides, by reduced GSH and functions to protect the cell from oxidative damage. Except for phospholipid-hydroperoxide GPx, a monomer, all of the GPx enzymes are tetramers of four identical subunits. Each subunit contains a selenocysteine in the active site which participates directly in the two-electron reduction of the peroxide substrate. The enzyme uses GSF as the ultimate electron donor to regenerate the reduced form of the selenocysteine. The Glutathione Peroxidase Assay Kit measures GPx activity indirectly by a coupled reaction with GR. GSSG, produced upon reduction of an organic hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in the A340 is directly proportional to the GPx activity in the sample.

RESULTS

Preliminary observations

All the formulations acquired reddish brown color by blending the extracts. All the formulations were found to be clear with respect to any objectionable undissolved foreign material [Table 3].

Evaluation test of different formulations

All the five formulations were observed for clarity and other parameters were measured. Data are presented in Table 3. The foremost test is appearance of formulation. Although all the formulations were reddish brown colored (F1, F2, F3, F4, and F5), all were found to be clear with respect to the presence of any solid particulate matter. pH and viscosity of different formulations were in the range of 6.8 (F1)–7.2 (F4) and 16.2 ± 0.40 (F1)–22.6 ± 0.66 (F5), respectively. Sterility test was performed by observing the growth of microorganisms or colonies following 7 days of incubation in both the media, i.e., culture media meant for fungi and thioglycolate medium. No growth of microorganism suggested that all the formulations passed the sterility test. There were no signs of opacity, ulceration, hemorrhage, redness, swelling, and discharge in eyes of rats following application of these formulations. This indicates that all formulations passed the eye irritancy test.

Table 3: Evaluation of formulation

Formulations	Clarity	Color	pH	Viscosity (cps)	Sterility test	Eye irritancy test
F1	Clear solution	Dark red	6.8	16.2±0.40	Passed	Passed
F2	Clear solution	Dark red	6.9	17.4±0.97	Passed	Passed
F3	Clear solution	Reddish	6.8	19.0±0.30	Passed	Passed
F4	Clear solution	Dark red	7.2	21.9±0.90	Passed	Passed
F5	Clear solution	Dark red	7.1	22.6±0.66	Passed	Passed

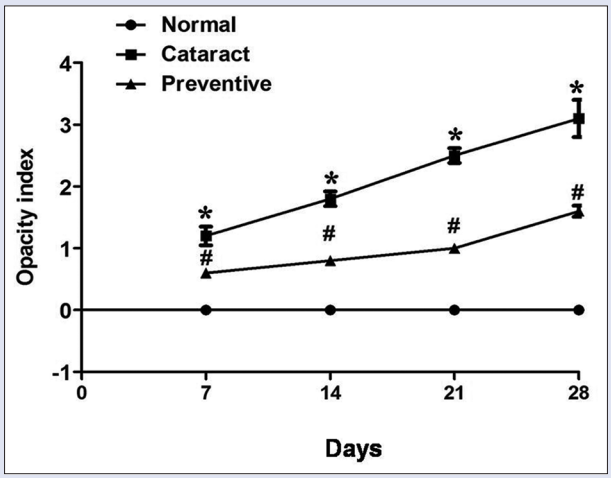


Figure 1: Effect of polyherbal eyedrops on development of diabetic cataract in rats. Opacity index was statistically analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test. *P < 0.001 versus normal rats. #P < 0.01 versus cataract group

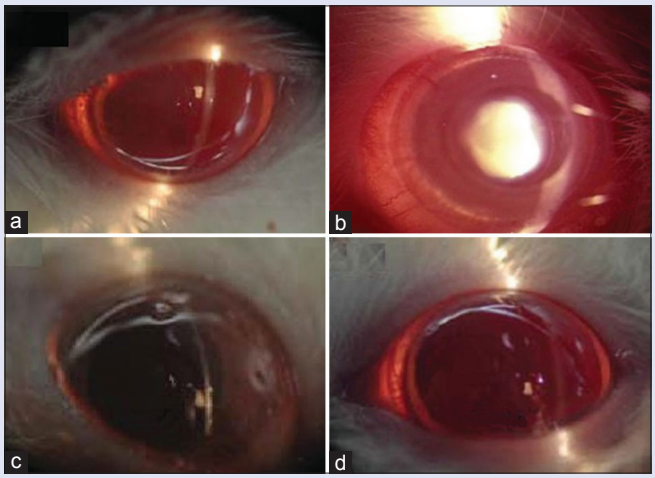


Figure 2: Photographs showing stages of cataract in lens of normal rats (a), rats with cataract (b and c), and cataract rats that received prophylactic polyherbal treatment (d)

Preventive effect of formulations on cataract development in rats

Cataract was evaluated in terms of opacity index and represented in Figure 1. Ophthalmoscopic examination of the eyes showed no opacity in the lenses of normal control rats throughout the duration of experimental period (A). Lens was observed by slit-lamp photography. Galactose-treated cataract control rats showed dense nuclear opacity [Figure 2].

We found a gradual increase in the opacity index from 1.2 (day 1), 1.8 (day 14), 2.5 (day 21), and 3.1 (day 28). Vacuoles and capsular deposits (B and C) were observed in these rats. Prophylactic treatment with formulation prevented the development of cataract. There was decrease in vacuoles. Opacity was significantly decreased following prophylactic treatment on days 7–28 (all $P < 0.001$).

Effect of polyherbal ophthalmic solution on different biochemical parameters

Level of different antioxidant enzymes was estimated in the lens of control and cataract rats treated with either saline solution or formulation [Figure 3]. It was observed that levels of thiol, GPx, GR and catalase were significantly increased in the rats following galactose administration as compared to control rats ($P < 0.01$). This indicated the role of these enzymes in the development of cataract. Prophylactic treatment with formulation significantly restored level of these enzymes in cataract rats to normal level. Prophylactic treatment with formulation significantly ($P < 0.01$) restored level of these enzymes to normal level in cataract induced rats as compared to untreated group of animals.

DISCUSSION

Diabetic cataract is an important secondary complication which may cause blindness if remain untreated. Treatment with oral hypoglycemic and insulin does control the blood glucose level, but it fails to prevent the occurrence of cataract. Although surgery is the first-line approach to remove cataract, this may develop severe complication in diabetic patients.[22] Despite this tricky situation, currently, there is no drug therapy available in the market as a prophylactic or curative measure for cataract. This suggests the urgent need of medicine that may prove effective for the prevention of development of diabetic cataract. Galactose-fed rats are well known used rat model for cataract.[23] Galactose produces large amounts of its reduced form, galactitol, than glucose. Galactose-induced cataract in rats shares similar disease initiation factors with the human cataractous condition.[24] The three mechanisms possibly involved in galactose cataract formation are the polyol pathway, oxidation, and nonenzymatic glycation.[25] Accumulation of sugar alcohol inside the lens leads to osmotic stress. Nonenzymatic glycation is also involved in cataractous changes; under hyperglycemic conditions, excess glucose reacts nonenzymatically with proteins. Accumulation of advanced glycation end products in diabetic eyes contributes to accelerate cataractogenesis in hyperglycemic experimental animals and diabetic humans.[26] Sorbitol-lowering agents were shown to delay cataract development in galactose-fed rats.[27] Lycopene (a potent antioxidant but not antihyperglycemic agent obtained from tomatoes) also afforded protective effect in the galactose model of cataract but not in hyperglycemic rats.[28] This implies that in the galactose model, drugs with antioxidant activity might show a protective effect but not the drugs that would counter the central features of diabetes. Since development of cataract is a slow process, chronic drug therapy is requisite to prevent as well as to cure the condition. Synthetic agents may produce harmful effects on long-term usage. Medicinal plants and related natural products

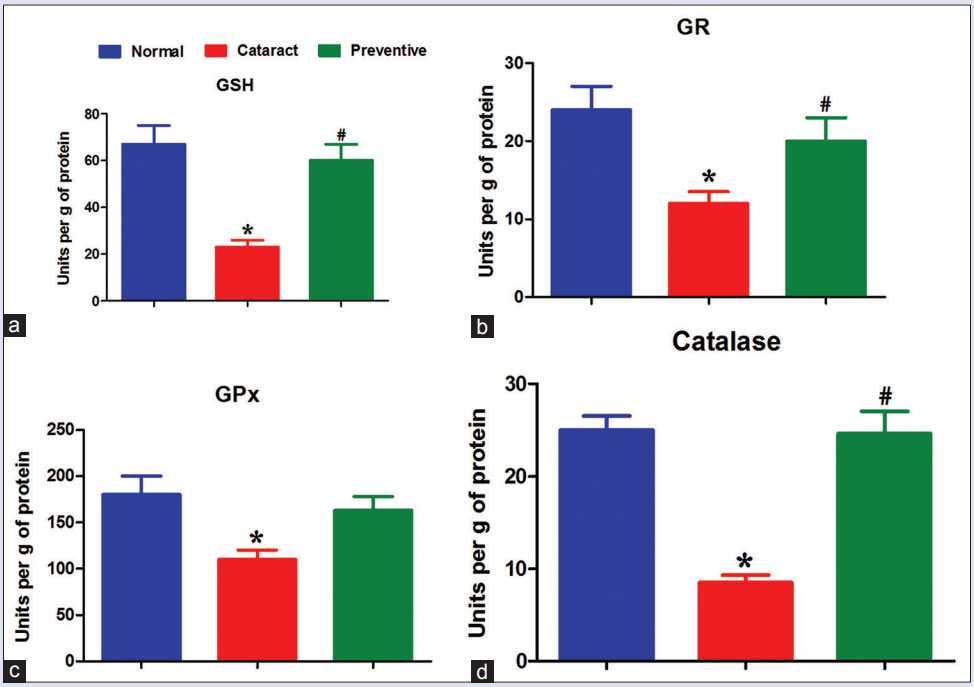


Figure 3: Effect of diabetic cataract and prophylactic treatment of herbal eye drop on biochemical parameters such as level of thiols (GSH) (a) level of Glutathione reductase (GR) (b) level of Glutathione peroxidase (GPx) (c) level of Catalase (d) in rats. Data is expressed as mean ± standard error of mean. Data were analyzed by one way ANOVA follwed by Dunnett's *post hoc* test. *$P < 0.01$ versus naïve rats. #$P < 0.01$ versus cataract group

control the process of cataract generation at various levels, especially as antioxidants and ARIs.[29]

Furthermore, cataract is also a condition that may develop due to defect in antioxidant system in eyes. Reports suggested that Vitamin C and Vitamin E can be effective in preventing the cataract formation by their antioxidant property.[30] To overcome such condition, anticataract formulations contain antioxidant of either synthetic or plant origin.[31] In earlier studies, it was reported that *Ocimum sanctum* and *Camellia sinensis* possess antioxidant properties and offer protection against cataracts.[32-35] *G. biloba*, amla, and beet root are well-known plants which are rich in antioxidant contents. *G. biloba* contains GSH, the important component of the innate antioxidant system in the lens. Deficiency of GSH is also reported in the cataractous lenses.[11] *G. biloba* extract prevents human lens epithelial cells from high glucose-induced apoptosis by inhibiting oxidative stress and suggested to possess a potential protective effect against diabetic cataract formation.[36] It also contains quercetin which inhibits aldose reductase resulting in blockage of conversion of glucose to sorbitol in polyol pathway in diabetic cataract. Carotenes such as lutein and zeaxanthin are present in beet root and act as antioxidants. Amla is a rich source of Vitamin C that preserves GSH level. By virtue of its tannoid supplements delay cataract progression and inhibition of aldose reductase activity as well as sorbitol formation in the lens, which is suggested as a possible mechanism.[9]

In the present study, rats with diabetic cataract showed decreased level of thiols (GSH). In contrast, cataract rats which were treated concomitantly with anticataract herbal formulation expressed GSH level like those of normal rats. GSH protects the eye against chemical and oxidative stress. In cornea, GSH maintains normal hydration level to protect cellular membrane integrity.[37] Our results with support of previous literature[38] indicate that decreased thiol levels may lead to reduced defense system that is responsible for the development of cataract. This may be attributed to the presence of *G. biloba* extract which contains GSH, an important component of the innate antioxidant system in the lens.[11] Amla may add benefit since it contains riboflavin which is a precursor to FAD, a coenzyme for GR that recycles GSH and prevents diabetes-induced GSH loss. Vitamin C in amla is also known to preserve GSH level.

GR is the enzyme responsible for the maintenance of cellular GSH homeostasis. A significant reduction in the activity of GR was observed in lens of diabetic cataract rats. However, pretreatment with polyherbal formulation increased the activity of GR in cataractous rats. The decreased activity of GR in diabetic rats as seen in our experiment supports our hypothesis of GSH depletion in the same rats.

Catalase and GPx are antioxidant enzymes that protect cells and tissues against oxidative injury. During cataract development, modifications in decrease in the activity of catalase and GPx lead to an increase in oxygen free radical's production. We also observed reduction in the catalase activity in lens of diabetic cataract rats. This is restored in rats pretreated with polyherbal formulation. Extract of *G. biloba* was found to reduce catalase activity in lens with cataract. This indicates that antioxidant component present in the herbs may prevent the loss of activity of these enzymes.[39]

CONCLUSION

The polyherbal formulation containing *G. biloba*, beet root, and amla extract can prevent the onset, progression, and maturation of galactose-induced diabetic cataract in rats. The anticataract activity of the polyherbal formulation may be attributed to the combined antioxidant effect. Thus, we may suggest the suitability of indigenous polyherbal formulations to prevent the occurrence of diabetic-associated cataract.

Acknowledgements

The authors are thankful to the Management and Principal, Dr. Rajendra Gode College of Pharmacy, Malkapur, and Dadasaheb Balpande College of Pharmacy, Nagpur, for providing necessary facilities to carry out the research work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Rao GN, Sadasivudu B, Cotlier E. Studies on glutathione S-transferase, glutathione peroxidase and glutathione reductase in human normal and cataractous lenses. Ophthalmic Res 1983;15:173-9.
2. Pollreisz A, Schmidt-Erfurth U. Diabetic cataract-pathogenesis, epidemiology and treatment. J Ophthalmol 2010;2010:608751.
3. Head KA. Natural therapies for ocular disorders, part two: Cataracts and glaucoma. Altern Med Rev 2001;6:141-66.
4. Harding JJ, Egerton M, van Heyningen R, Harding RS. Diabetes, glaucoma, sex, and cataract: Analysis of combined data from two case control studies. Br J Ophthalmol 1993;77:2-6.
5. Mitra AK, Anand BS. Drug delivery to The. In: Fischbarg J, editor. The Biology of the Eye. New York: Academic Press; 2006. p. 307-51.
6. Ertekin MV, Koçer I, Karslioğlu I, Taysi S, Gepdiremen A, Sezen O, *et al.* Effects of oral *Ginkgo biloba* supplementation on cataract formation and oxidative stress occurring in lenses of rats exposed to total cranium radiotherapy. Jpn J Ophthalmol 2004;48:499-502.
7. Chan PC, Xia Q, Fu PP. *Ginkgo biloba* leave extract: Biological, medicinal, and toxicological effects. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2007;25:211-44.
8. Sanderson J, McLauchlan WR, Williamson G. Quercetin inhibits hydrogen peroxide-induced oxidation of the rat lens. Free Radic Biol Med 1999;26:639-45.
9. Abdel-Aal el-SM, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. Nutrients 2013;5:1169-85.
10. Suryanarayana P, Saraswat M, Petrash JM, Reddy GB. *Emblica officinalis* and its enriched tannoids delay streptozotocin-induced diabetic cataract in rats. Mol Vis 2007;13:1291-7.
11. Daisy P, Averal HI, Modilal RD. Curative properties of *Phyllanthus* extract in alloxan-induced diabetic rats. J Trop Med Plant 2004;5:21-7.
12. Elobeid MA and Ahmed EA. Antidiabetic efficacy of aqueous fruit extract of Amla (*Emblica officinalis*) in streptozotocin induced diabetes mellitus in male rats. Trop J Pharm Res 2015;14:801-6.
13. Zahradnikov A, Schmidt S, Sekretar S, Janac L. Determination of the antioxidant activity of *Ginkgo biloba* leaves extract. J Food Nutr Res 2007;46:15-9.
14. Chakole R, Zade S, Charde M. Antioxidant and anti-inflammatory activity of ethanolic extract of *Beta vulgaris* roots. Int J Biomed Adv Res 2011;2:124-30.
15. Kavitha Nair N, Patel K, Gandhi T. Effect of aqueous extract of *Embelica officinalis* on selenite induced cataract in rats. Iran J Pharm Res 2010;9:147-52.
16. Ministry of Health and Family Welfare, Government of India. Indian Pharmacopoeia. New Delhi: Ministry of Health and Family Welfare, Government of India; 1996;2. p. A117-47.
17. Wortzman MS. Eye Products in Cosmetic Safety: A primer for Cosmetic Scientists. In: Whittam JH, editor. Eye Products in Cosmetic Safety: A Primer for Cosmetic Scientists. New York: Marcel Dekker; 1987.
18. Suryanarayana P, Saraswat M, Mrudula T, Krishna TP, Krishnaswamy K, Reddy GB, *et al.* Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci 2005;46:2092-9.
19. Zhang S, Chai FY, Yan H, Guo Y, Harding JJ. Effects of N-acetylcysteine and glutathione ethyl ester drops on streptozotocin-induced diabetic cataract in rats. Mol Vis 2008;14:862-70.
20. Linetsky M, Hill JM, Chemoganskiy VG, Hu F, Ortwerth BJ. Studies on the mechanism of the UVA light-dependent loss of glutathione reductase activity in human lenses. Invest Ophthalmol Vis Sci 2003;44:3920-6.
21. Góth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 1991;196:143-51.
22. Stanga PE, Boyd SR, Hamilton AM. Ocular manifestations of diabetes mellitus. Curr Opin Ophthalmol 1999;10:483-9.
23. Pessotto P, Liberati R, Petrella O, Romanelli L, Calvani M, Peluso G, *et al.* In experimental diabetes the decrease in the eye of lens carnitine levels is an early important and selective event. Exp Eye Res 1997;64:195-201.
24. Kinoshita JH. Cataracts in galactosemia. The Jonas S. Friedenwald Memorial Lecture. Invest Ophthalmol 1965;4:786-99.
25. Spector A. Oxidative stress-induced cataract: Mechanism of action. FASEB J 1995;9:1173-82.
26. Kyselova Z, Stefek M, Bauer V. Pharmacological prevention of diabetic cataract. J Diabetes Complications 2004;18:129-40.
27. Harding JJ. Pharmacological treatment strategies in age-related cataracts. Drugs Aging 1992;2:287-300.
28. Pollack A, Oren P, Stark AH, Eisner Z, Nyska A, Madar Z, *et al.* Cataract development in sand and galactosemic rats fed a natural tomato extract. J Agric Food Chem 1999;47:5122-6.
29. Singh B, Kumar D, Singh R. Phytotherapeutics for management and prevention of cataract generation. Phytopharmacology 2012;3:93-110.
30. Watkins R. Foundations of a solution to cataract blindness. Clin Exp Optom 2002;85:59-60.
31. Awasthi S, Srivatava SK, Piper JT, Singhal SS, Chaubey M, Awasthi YC, *et al.* Curcumin protects against 4-hydroxy-2-trans-nonenal-induced cataract formation in rat lenses. Am J Clin Nutr 1996;64:761-6.
32. Gupta SK, Halder N, Srivastava S, Trivedi D, Joshi S, Varma SD, *et al.* Green tea (*Camellia sinensis*) protects against selenite-induced oxidative stress in experimental cataractogenesis. Ophthalmic Res 2002;34:258-63.
33. Gupta SK, Trivedi D, Srivastava S, Joshi S, Halder N, Verma SD, *et al.* Lycopene attenuates oxidative stress induced experimental cataract development: An *in vitro* and *in vivo* study. Nutrition 2003;19:794-9.
34. Gupta SK, Srivastava S, Trivedi D, Joshi S, Halder N. *Ocimum sanctum* modulates selenite-induced cataractogenic changes and prevents rat lens opacification. Curr Eye Res 2005;30:583-91.
35. Agrawal SS, Gupta SK, Kalaiselvan V, Srivastava S, Saxena R. Potential of traditional food supplement, soya bean, as a novel anti cataract agent. Int J Integr Biol 2008;3:1-10.
36. Wu ZM, Yin XX, Ji L, Gao YY, Pan YM, Lu Q, *et al. Ginkgo biloba* extract prevents against apoptosis induced by high glucose in human lens epithelial cells. Acta Pharmacol Sin 2008;29:1042-50.
37. Ganea E, Harding JJ. Glutathione-related enzymes and the eye. Curr Eye Res 2006;31:1-1.
38. Lou MF, Dickerson JE Jr., Garadi R, York BM Jr. Glutathione depletion in the lens of galactosemic and diabetic rats. Exp Eye Res 1988;46:517-30.
39. Salem H, Nageuib A, Gomaa AM, Al Balkini MS, Mohee A. The potential effect of *Ginkgo biloba* extract on development of cataract in selenite induced cataract rat pups. IJCBS Res 2015;2:30-41.