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IN-VITRO ANTI-CATARACT ACTIVITY OF *MORUS ALBA* DRIED LEAVES EXTRACT IN CATARACT INDUCED GOAT LENSES

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Abstract

Cataract is a lens disorder which is the opacification of the crystalline lens of the eye affects transparency of the vision. It is a reversible eye disorder and a leading cause of blindness globally. *Morus alba* plant is native of India, Japan and China. It is cultivated in many parts of world. Phytochemical such as coumarins, stilbenoids, phenolic acids, flavonoids, alkaloids and terpenoids can be identified in *Morus alba* plant. In the present paper dried leaf extract of *M. alba* were evaluated for in vitro anti-cataract activity in induced goat lens.

Key-words: *Morus alba,* Cataract, Goat lens

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Introduction

Human eye most sensitive and complex organ which allow us to have a sight of this beautiful world by optimizing vision for varying lights and for different conditions. Eyes contribute about 80% of all the information received by the brain. Cataract is a human eye lens abnormality which results in cloudiness and affects transparency in vision. It is a reversible vision disease. Causes and risk factors: Majority of cases of cataracts are due to age related factors. But they may occur due to environmental factors and due to some trauma or as a secondary cause from some another disease. Cataract occurs when crystallin protein which is responsible for making up of lens undergoes precipitation, aggregation or some modifications. Treatment methods for cataracts are mainly divided into two categories – nonsurgical treatment methods and surgical treatment methods. The non-surgical treatment methods mainly focuses on reducing the worsening of cataract. These methods cannot provide complete cure to cataract. Surgical methods are only option available in present time which can provide complete and permanent cure form cataract. Medicinal plants have been used from time immemorial for better health, removing pain, as a source of flavours, fragrances and food by mankind. In present time also, a large population of world depends on medicinal plants for the primary health care. Medicinal plants provide a good basis for the investigation of new compounds which can be helpful in developing new drug candidates (Rasool Hassan, 2012). *Morus alba* also known as white mulberry belongs to Moraceae family. In India, it is famous by name 'Tut' (Devi *et al*., 2013). Generally, this plant is grown for the cultivation of silkworms, as silk worms only grow on leaves of *Morus alba*. Though in many other countries, this plant is cultivated for fruits and foodstuffs (Butt *et al*., 2008). The breeding of

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mulberries in focused to enhance the production of silkworms in countries like India and China. It can be found in regions from temperate to subtropical of Northern hemispheres regions to Southern hemisphere. It can well adapt various climatic conditions as well as soil types(Ercisli & Orhan, 2007).

Material and Methods

Preparation of extracts

250 gm of shade dried coarsely powdered plant material were loaded in Soxhlet apparatus and was extracted using petroleum ether, chloroform, ethanol and water in soxhlet apparatus untill extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined. (Harborne, 1984; Kokate, 1997)

In-vitro Studies (Cataract induced in goat lenses) (Kumar et al., 2011; Kurmi, 2014))

In vitro experimental model of cataract induced in goat lenses using glucose induced cataract model at a concentration of 55 mM, incubated for 72 h at room temperature. At high concentrations, glucose in the lens was metabolized through sorbitol pathway and accumulation of polyols (sugar alcohols), causing overhydration and oxidative stress. This leads to cataractogenisis.

Drugs and chemicals

Glucose and vitamin E were obtained from SD Fine Chemicals, Mumbai. Nitro blue tetrazolium chloride (NBT), 1- amino-2-naphthol-4-sulfonic acid, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), nicotinamide adenine dinucleotide reduced salt (NADH), nicotinamide adenine dinucleotide phosphate reduced tetra sodium salt (NADPH), oxidized glutathione, reduced glutathione and adenosine-5'-triphophate (ATP) were obtained from HiMedia Laboratories Ltd., Mumbai. Fresh goat lenses were purchased locally from the Corporation slaughter house inIndore. All other chemicals used in the study were obtained commercially and were of analytical grade.

Glucose-induced cataract

Fresh goat eyeballs were obtained from a local slaughterhouse within two hours after killing of the animals and the lenses were isolated. They are preserved and carried to the laboratory at 0-4°C. The isolated lens was incubated in artificial aqueous humor at 37°C and pH 7.8 for 72 h. Glucose at a concentration of 55 mM was used to induce cataract. A total of 42 goat lenses were used and divided into seven experimental groups consisting of 6 in each group. Group I : Artificial aqueous humor alone (Normal control); Group II : Glucose 55 mM alone (Negative control); Group III: Glucose 55 mM + PEEMAL (100 µg/ml); Group IV: Glucose 55 mM + CEMAL (100 µg/ml); Group V-: Glucose 55 mM + EEMAL (100 µg/ml); Group VI: Glucose 55 mM + AEMAL (100 µg/ml) and Group VII : Glucose 55 mM + Vitamin E (100 µg/ml, Positive control) At the end of the experiment, the lenses were removed from the medium and rolled on filter paper to remove medium, adhering non lens tissue, and vitreous humor.

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Examination of lens opacity

Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of hexagons clearly visible through the lens) was observed through the lens as a measure of lens opacity.

Preparation of lens homogenate

After incubation, lenses were homogenized with 10 volumes of 0.1M potassium phosphate buffer, pH 7.0. The homogenate was centrifuged at 10,000 g for 1 h and the supernatant was used for estimation of biochemical parameters.

Biochemical parameters

Estimation of total protein content

To 0.1 ml of lens homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 min for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as μg/mg lens tissue.

Estimation of malondialdehyde (MDA)

Lenses were homogenized in10% (w/v) 0.1 M Tris-HCl buffer (pH 7.5) (24). One ml of the homogenate was combined with 2 ml of TCA-TBA-HCl reagent (15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl) and boiled for 15 min. Precipitate was removed after cooling by centrifugation at 1000g for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The levels of MDA were calculated using extinction coefficient calculation. The values are expressed as nmoles of MDA/ min/ mg lens protein.

Estimation of lipid hydroperoxides (LH)

About 0.1ml of lens homogenate was treated with 0.9 ml of Fox reagent (49 mg of ferrous ammonium sulfate in 50 mL of 250 mM H_2SO_4, 0.397 g of butylated hydroxyl toluene, and 38 mg of xylenol orange in 950 mL of methanol) and incubated for 3 min. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/ mg lens protein.

Inhibition of Cu^{2+} induced lipoprotein diene formation

Lens homogenate was diluted to 0.67% in phosphate buffered saline. Control experiments consisted of identical assay conditions but without the sample. Oxidation was initiated immediately after addition of sample by the addition of 12 μM final concentration of Cu^{2+} added as $CuSO_4.5H_2O$ dissolved in deionized distilled water. Oxidation was determined by measuring the absorbance at 234 nm using a UV–Visible Spectrophotometer. Absorbance was taken after 120 min at 37ºC. The lipoprotein diene formation was measured from the absorbance at a time. The absorbance provides an indication of protection of tissue lipoprotein against oxidation.

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Assay of Ca2+-ATPase activity

To 0.1 ml of the lens homogenate (10% w/v in 0.25 M sucrose) add 0.2 ml of the substrate, ATP. The tubes were incubated for 30 min in a water bath at 37ºC. The enzyme activity is stopped by adding 2 ml of 10% TCA and the same kept in ice for 20 min. All the tubes were then centrifuged at 2500g for 10 min and the supernatant collected. The protein free supernatant was analyzed for inorganic phosphate. For that, 3 ml of the supernatant was treated with 1 ml of ammonium molybdate and 0.4 ml 1-amino-2-naphthol-4-sulfonic acid (ANSA). The colour developed was read at 680 nm after 20 min and the inorganic phosphate value expressed as nm and three parallel experiments were conducted.

Determination of enzymatic antioxidants

Assay of catalase

The reaction mixture contained 2.0 ml of homogenate (lens were homogenized in 10% w/v 50 mM phosphate buffer, pH 7.0) and 1.0 ml of 30 mM hydrogen peroxide (in 50 mM phosphate buffer, pH 7.0). A system devoid of the substrate (hydrogen peroxide) served as the control. Reaction was started by the addition of the substrate and decrease in absorbance monitored at 240 nm for 30 seconds at 25°C. The difference in absorbance per unit time was expressed as the activity and three parallel experiments were conducted. One unit is defined as the amount of enzyme required to decompose 1.0 M of hydrogen peroxide per minute at pH 7.0 and 25°C.

Estimation of glutathione peroxidase (GPx)

The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2 ml of lens homogenate supernatant incubated at 37°C for 10 min. The reaction was arrested by the addition of 10% TCA and the absorbance was taken at 340 nm. Activity was expressed as nmoles/min/mg lens protein.

Assay of superoxide dismutase (SOD)

The assay mixture contained 1.2 ml sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml of 186 μM Phenazonium methosulphate (PMS), 0.3 ml of 300 μM NBT, 0.2 ml of 780 μM NADH, 1.0 ml homogenate (lens were homogenized in 10% w/v 0.25 M sucrose buffer) and distilled water to a final volume of 3.0 ml. Reaction was started by the addition of NADH and incubated at 30°C for 1 min. The reaction was stopped by the addition of 1.0 ml glacial acetic acid and the mixture stirred vigorously. 4.0 ml of n-butanol was added to the mixture and shaken well. The mixture was allowed to stand for 10 min, centrifuged, the butanol layer taken out and the absorbance was measured at 560 nm against a butanol blank. A syste devoid of enzyme served as the control and three parallel experiments were conducted.

Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidised glutathione and 0.1 ml (10 mg/ml) o bovine serum albumin. The reaction was started by the addition of 0.02 ml of lens homogenate with mixing and the decrease in absorbance at 340 nm was measured for 3 min

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against a blank. Glutathione reductase activity was expressed as nmoles NADPH oxidized/min/ mg lens protein at 30ºC.

Determination of non enzymatic antioxidant

Estimation of reduced glutathione (GSH)

Lenses were homogenized in10% (w/v) cold 20 mM EDTA solution on ice. After deproteinization with 5% TCA, an aliquot of the supernatant was allowed to react with 150 µM DTNB. The product was detected and quantified spectrophotometrically at 416 nm. Pure GSH was used as standard for establishing the calibration curve and three parallel experiments were conducted.

Statistical analysis

Results are expressed as mean ± SEM of six lenses in each group. The groups were compared using one-way analysis of variance (ANOVA) with post-hoc Dunnett's test using glucose 55 mM group as control. P values < 0.05 were considered significant.

Results and Discussion

Preparation of extracts

The shade dried coarsely powdered leaves of *Morus alba* Linn was extracted with ethanol. The extracts obtained were evaluated for pH, color and % yield. The results are presented in table 1.

Table 1 Estimation of % yield of various extract of *Morus alba* Leaves

S/No.	Extract	Parameters			
		Nature of Extract	Color	pH	% Yield (w/w)
1.	PEEMAL	Semi Solid	Light greenish	6.9	1.12
2.	CEMAL	Semi solid	Greenish Black	7.11	1.94
3.	EEMAL	Solid Powder	Light Green	7.04	8.43
4.	AEMAL	Solid Powder	Light Green	7.02	10.68

In-vitro Studies (Cataract induced in goat lenses)

The various leaves of *M. alba* extract i.e., PEEMAL, CEMAL, EEMAL & AEMAL were evaluated for in vitro studies using goat lenses. The results of the studies were presented below for examination of lens opacity & lens enzymatic and non enzymatic antioxidants.

Cataractogenesis

Incubation of goat lenses with 55 mM of glucose showed opacification starting at the periphery, at the end of 8 h, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification resulting at the end of 72 h.

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Examination of lens opacity

After 72 h of incubation in glucose 55 mM, lens becomes completely opaque (Fig. 5.3b) as against lenses in normal control (Fig. 5.3a). Incubation of lenses with PEEMAL, CEMAL, EEMAL & AEMAL (Figs. 5.3c, 5.3d, 5.3e & 5.3f) at both the concentrations used, seem to retard the progression of lens opacification, compared with lenses incubated in glucose 55 mM (negative control, Fig. 5.3b). The effect of vitamin E, the positive control groups is showing considerable retardation in the progression of lens opacification (Fig. 5.3g) which is near normalcy when compared to negative control (Fig. 5.3b).

Table 2: Effect of *Morus alba* Leaf Extract on lens protein, MDA, LH, Ca^{2+}ATPase & Cu^{2+} induced lipoprotein

Groups	**Protein** (mmoles/min/mg lens tissue)	**MDA** (μmoles/min/mg protein)	**LH** (nmoles/min/mg protein)	**Ca2+-ATPase** (μmoles/min/mg inorganic phosphate)	**Cu2+-**induced Lipoprotein diene absorbance
Normal Control	96.88±2.10	0.193±0.021	6.32±0.29	4.54±0.11	0.6742±0.03
Glucose Control	53.43±2.29[a]	0.689±0.058[a]	12.77±1.21[a]	1.21±0.13[a]	0.1478±0.01[a]
Glucose 55 mM + PEEMAL	70.29±1.74[b]	0.428±0.028[b]	9.22±1.57[b]	2.12±0.11[c]	0.3877±0.05[b]
Glucose 55 mM + CEMAL	71.20±1.11[b]	0.389±0.027[b]	8.98±1.11[b]	2.98±0.1[b]	0.4239±0.02[b]
Glucose 55 mM + EEMAL	74.21±2.11[b]	0.310±0.001[b]	8.58±0.12[c]	3.40±0.22[b]	0.5819±0.02[b]
Glucose 55 mM + AEMAL	78.21±2.89[b]	0.301±0.10[b]	8.16±1.21[c]	3.98±0.28[b]	0.5780±0.11[b]
Glucose 55 mM + Vit. E	83.39±3.11[b]	0.244±0.017[b]	7.78±1.10[c]	4.16±0.19[b]	0.6324±0.04[b]

Values are expressed as mean ± SEM; n=6 in each group; [a]P<0.01 when compared to normal control; [b]P<0.01, [c]P<0.05 when compared using one-way ANOVA with post-hoc Dunnett's test using glucose 55 mM group as control.

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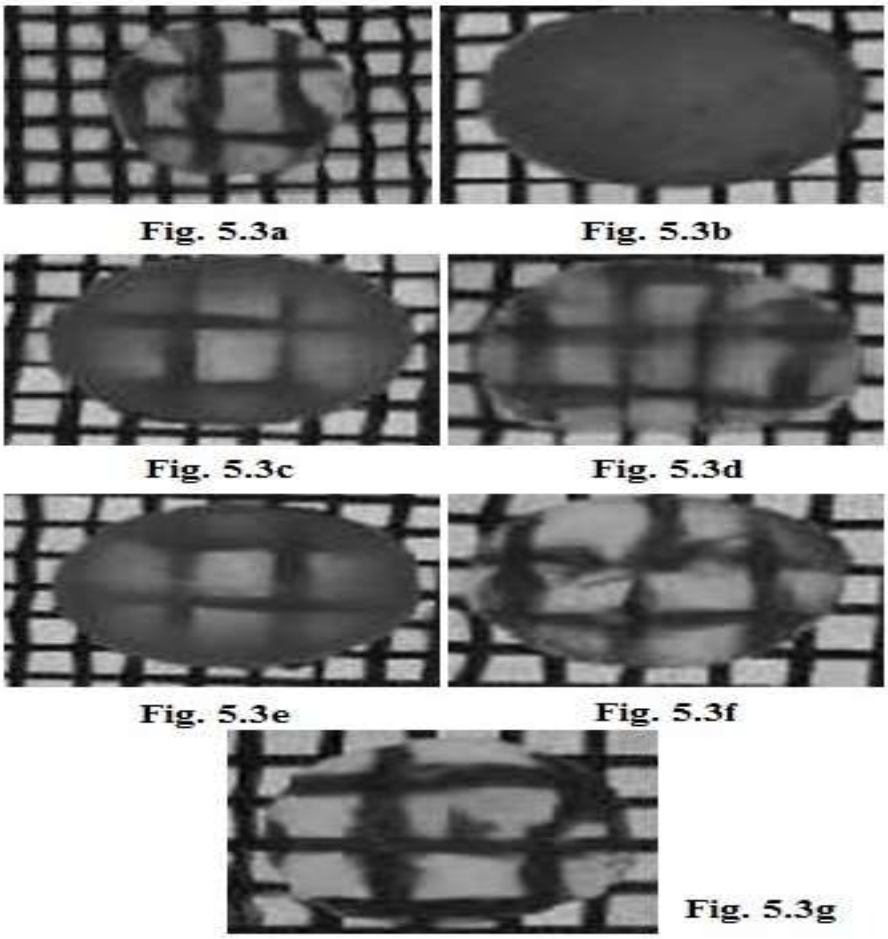
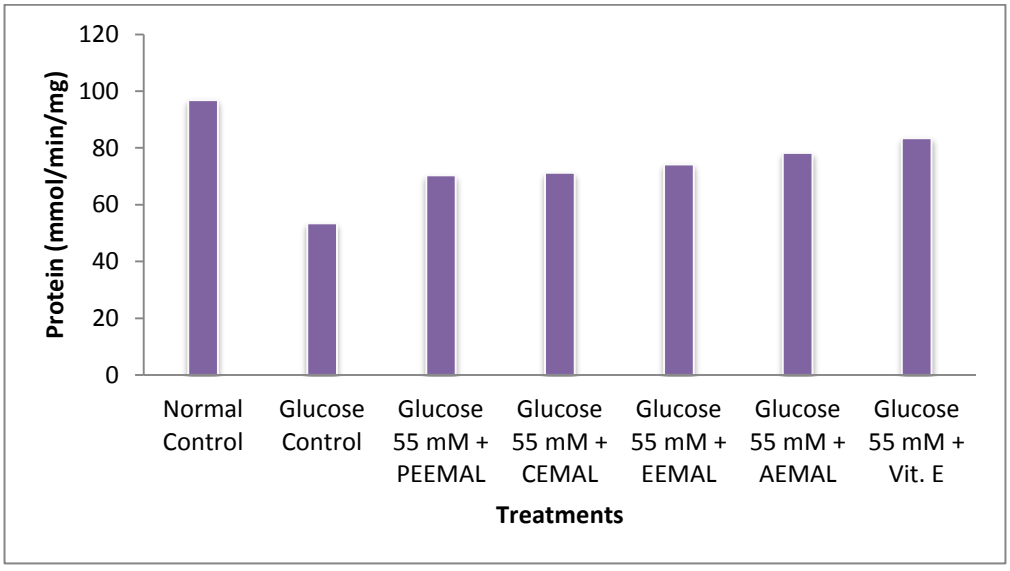


Fig. 5.3: Examination of lens opacity of treatments



Graph 1: Effect of *Morus alba* Leaf Extract on lens protein

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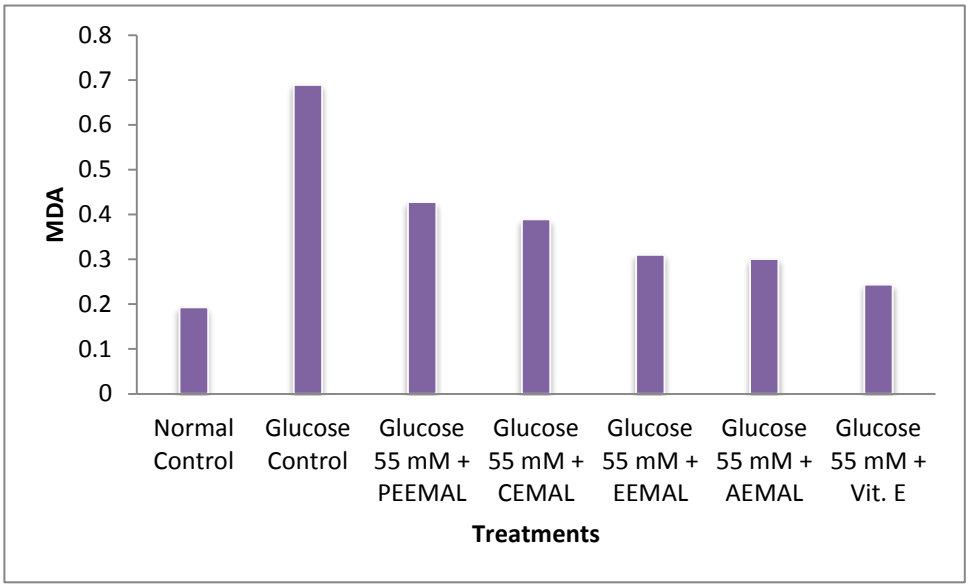
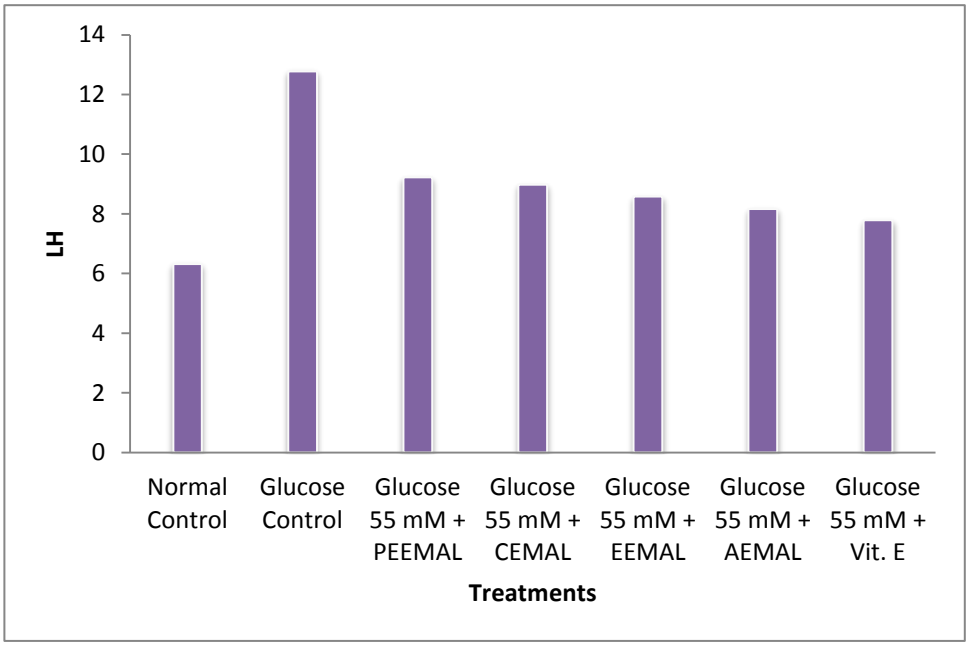


Graph 2: Effect of *Morus alba* Leaf Extract on MDA



Graph 3: Effect of *Morus alba* Leaf Extract on LH

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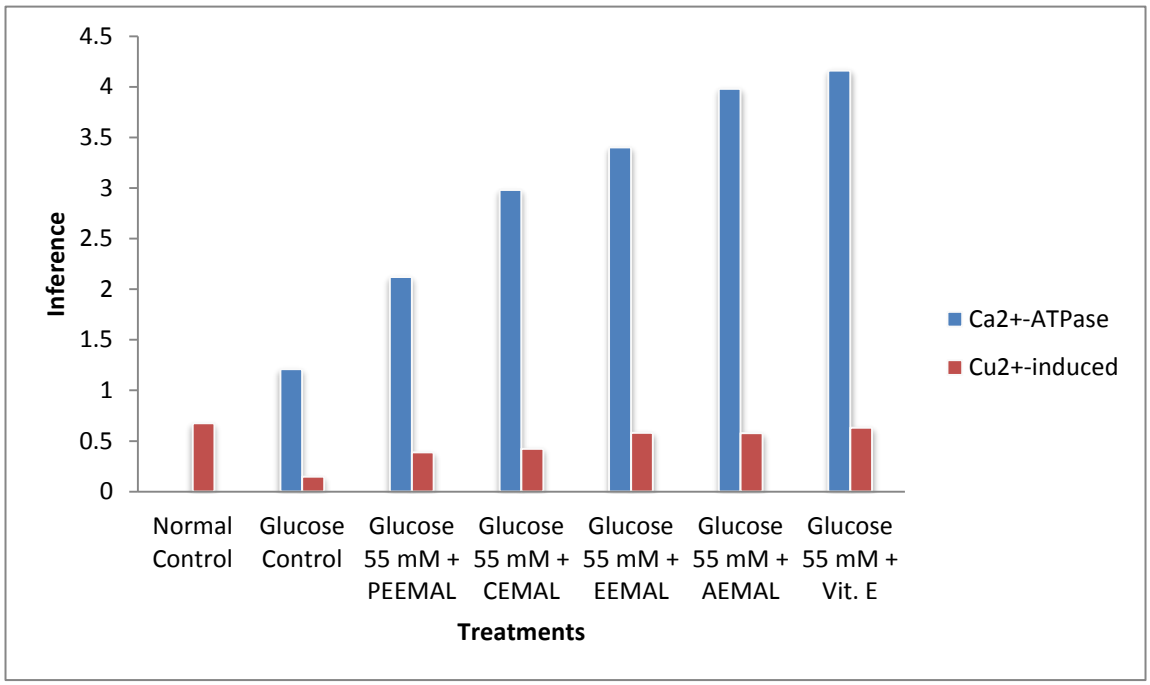

Graph 4: Effect of *Morus alba* Leaf Extract on Ca^{2+}ATPase & Cu^{2+} induced lipoprotein

Biochemical parameters

There was a significant (P<0.01) decrease in the level of total protein, Ca^{2+}-ATPase, Cu^{2+}-induced lipoprotein diene formation and an increase in the level of malondialdehyde and lipid hydroperoxides in lenses incubated with 55mM glucose when compared to normal control. Lenses incubated with 55 mM glucose and simultaneously with PEE, CE, EE and AE of MAL or standard drug, vitamin E caused a reversal of the above effects. Extract used at concentrations (100 µg/ml) showed a dose dependent increase in the effect. PEE, CE, EE and AE of MAL and Vitamin E (100 µg/ml,) showed increase in the total protein, Ca^{2+}-ATPase, Cu^{2+}-induced lipoprotein diene formation and a decrease in the level of malondialdehyde and lipid hydroperoxides.

Enzymatic and non enzymatic antioxidants

Incubation with 55 mM glucose for 72 h produced a significant (P<0.01) decrease in the levels of enzymatic antioxidants like catalase, superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the lens homogenate when compared to normal control. Incubation with the PEE, CE, EE and AE of MAL at doses of 100 µg/ml and Vitamin E (100 µg/ml) simultaneously with 55 mM glucose significantly restored the levels of both enzymatic and non enzymatic antioxidant enzymes which is almost similar to the control group (Table 3).

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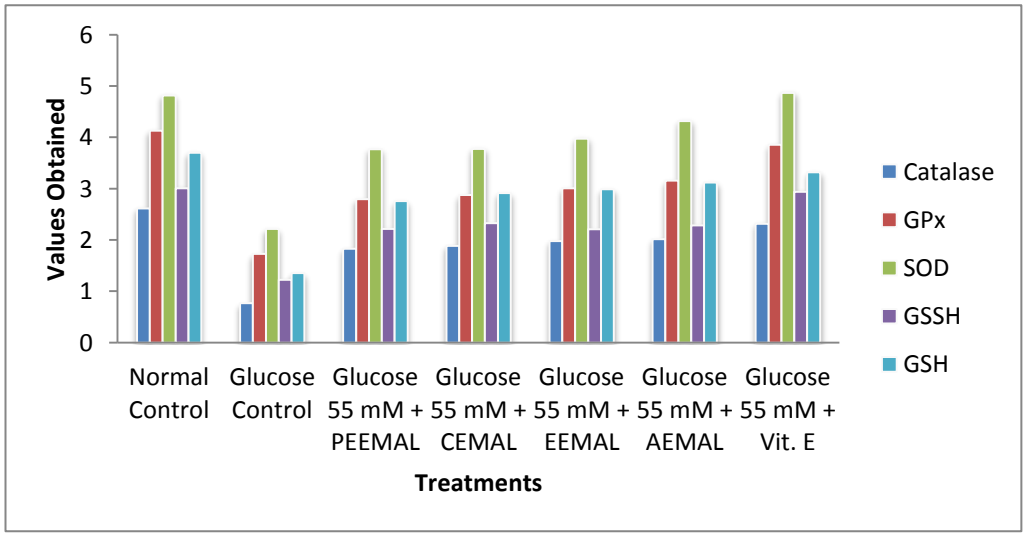
Table 3: Effect of *Morus alba* Leaf Extract on lens enzymatic antioxidants in control and experimental groups

Groups	**Catalase** (mmoles/min/ mg protein)	**GPx** (µmoles/min/ mg protein)	**SOD** (µmoles/min/ mg protein)	**GSSH** (µmoles/min/ mg protein)	**GSH** (µmoles/min/ mg protein)
Normal Control	2.62±0.07	4.13±0.11	4.82±0.17	3.01±0.22	3.70±0.33
Glucose Control	0.77±0.05a	1.73±0.02 a	2.22±0.20 a	1.23±0.02a	1.36±0.12a
Glucose 55 mM + PEEMAL	1.83±0.11b	2.80±0.03 b	3.77±0.21 b	2.22±0.04b	2.76±0.18b
Glucose 55 mM + CEMAL	1.89±0.12b	2.88±0.11 b	3.78±0.23 b	2.33±0.11b	2.92±0.19b
Glucose 55 mM + EEMAL	1.98±0.22b	3.01±0.03 b	3.98±0.02 b	2.21±0.08b	2.99±0.11b
Glucose 55 mM + AEMAL	2.02±0.04b	3.16±0.20 b	4.32±0.22b	2.29±0.11b	3.12±0.18 b
Glucose 55 mM + Vit. E	2.32±0.11b	3.86±0.23 b	4.87±0.24b	2.94±0.19b	3.32±0.11 b

Values are expressed as mean ± SEM; n=6 in each group; aP < 0.01 when compared to normal control; bP < 0.01, cP < 0.05 when compared using one way ANOVA with post-hoc Dunnett's test using glucose 55 mM group as control.



Graph 5: Effect of *Morus alba* Leaf Extract on lens enzymatic antioxidants in control and experimental groups

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Conclusion

For cataract, presently there is no definitive pharmacological therapy available and the only solution for the patient with advanced cataract is surgery. The limitations of cataract surgery have stimulated experimental cataract research mainly focusing on the prevention of cataract formation. Research using plants and plant products are on the rise owing to their natural origin and comparative safety.

Prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism and long-term irreversible changes in stable macromolecules. Non enzymatic glycation, oxidative stress and polyol pathway are the possible mechanisms by which high glucose concentrations induce and accelerate lens opacification leading to cataract formation. High concentrations of glucose contribute to oxidative stress by generating more reactive oxygen species at the mitochondrial level owing to increased intracellular glucose metabolism. Increased reactive oxygen species initiates polyol pathway by stimulating aldose reductase resulting in high levels of sorbitol. Sorbitol does not easily cross cell membranes and accumulates in cells causing damage by disturbing osmotic homeostasis. This intralenticular accumulation of polyols is a major factor in acute models of sugar cataract.

In the present study investigation of lens opacity was carried out to differentiate the control and experimental lenses. Incubation with glucose in high concentration results in various biochemical changes leading to formation of cataract. However the groups incubated with extract have reversed the lens opacity which is almost similar to the standard drug, vitamin E.

High glucose concentration causes accumulation of Na^+ and loss of K^+ altering the Na^+-K^+ ratio which in turn alters the protein content of the lens, leading to a decrease in the content of water soluble proteins. Extracts of *M. alba* and vitamin E restored the levels of proteins, which may be due to direct action on the Na^+-K^+ ratio or indirectly by their free radical scavenging activity.

Lipid peroxidation is an autocatalytic process, which is a common cause of cell death. The by-products of lipid peroxidation are toxic compounds malondialdehyde (MDA) and lipid hydroperoxides (LH) whose involvement in cataractogenesis has been suggested, mainly due to its cross linking ability. In this study, glucose-induced cataractous goat lenses showed an increase in MDA and LH levels. Incubation of extract simultaneously with glucose reversed these effects. This effect was almost similar to the vitamin E treated group.

The Ca^{2+}-ATPase is a transport protein in the cells that serves to maintain the Ca^{2+} levels in the cell. This protein is sensitive to oxidative damage. In glucose induced cataract there was a significant ($P<0.01$) decrease in the levels of Ca^{2+} ATPase. The extracts and vitamin E increased the levels considerably.

The Cu^{2+}-induced lipoprotein diene formation was used to determine the antioxidant potential. The results provide an indication of protection of lipoprotein by the *M. alba* extracts against oxidation. The effect was similar to the standard drug vitamin E.

Several varieties of toxic species of oxygen are formed in the lens including the superoxide anion, hydrogen peroxide, hydroxyl radical and lipid hydroperoxides. This increase has been proposed as a central mechanism of oxidative injury. Thus the determination of the lens *in vitro*

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antioxidant enzymes like SOD, CAT, GPx, GSSH and non enzymatic antioxidant enzyme, GSH were carried out.

Catalase catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and hydroxyl radical. The first enzyme involved in the antioxidant defence is superoxide dismutase. The oxygen radicals are strongly inhibited by superoxide dismutase. Glutathione peroxidase has a major role in degrading the levels of H_2O_2 in cells and plays a vital role in protecting membrane lipids, and thus the cell membranes from oxidative disintegration The enzyme GSSH catalyses the conversion of oxidized glutathione (GSSH) to reduced glutathione (GSSG), thereby maintaining a constant level of GSH in normal lens . The nonenzymatic antioxidant GSH protects cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. The incubation with 55 mM of glucose severely reduced the levels of both enzymatic and non enzymatic antioxidants in the goat eye lens. Simultaneous incubation with the extracts of *M. alba* and vitamin E considerably increased the levels of all the antioxidants studied.

References

1. Butt, M. S., Nazir, A., Sultan, M. T., & Schroën, K. (2008). Morus alba L. nature's functional tonic. *Trends in Food Science & Technology*, *19*(10), 505–512.
2. Devi, B., Sharma, N., Kumar, D., & Jeet, K. (2013). Morus alba Linn: A phytopharmacological review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(2), 14–18.
3. Ercisli, S., & Orhan, E. (2007). Chemical composition of white (Morus alba), red (Morus rubra) and black (Morus nigra) mulberry fruits. *Food Chemistry*, *103*(4), 1380–1384.
4. Harborne J.B. (1984). Phytochemical Methods", Chapman and Hall, London, I Edition, 138.
5. Kokate C.K. (1997). Practical Pharmacognosy, Vallabh Prakashan, Delhi, 4 Edition, 107-111.
6. Kurmi, R., Ganeshpurkar, A., Bansal, D., Agnihotri, A., & Dubey, N. (2014). Ethanol extract of Moringa oliefera prevents in vitro glucose induced cataract on isolated goat eye lens. *Indian journal of ophthalmology*, *62*(2), 154-157.
7. Kumar, M., Singh, T., Ali, J., & Tyagi, L. K. (2011). In vitro anticataract activity of Zingiber officinale on goat lenses. *Int J Pharm Biol Arch*, *2*(5), 1430-1433.
8. Rasool Hassan, B. A. (2012). Medicinal Plants (Importance and Uses). *Pharmaceutica Analytica Acta*, *03*(10).