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Ex-Vivo & *In-Vitro* Evaluation of Anti-Cataract Activity of *Coriandrum sativum* & *Withania somnifera*

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Abstract : A cataract is haziness or clouding in crystalline lens of living being. It disturbs lens transparency by changes in lens properties like precipitation of crystalline protein, increase in reactive oxygen species, increase in sorbitol accumulation, etc. There is no pharmacology treatment for cataract. Surgery is only option to get rid from cataract. Coriander is used since ancient time in eyesight weakness and in conjunctivitis. Ashwagandha is used as anti diabetic and as anti oxidant in market. As we know Polyol pathway is main cause of cataract, anti diabetic drug might have better action in cataract. To evaluate this (1) *Ex-vivo* Photographic evaluation of goat eye lens and (2) Biochemical parameters testing of same lens was carried out. The hydroalcoholic extract of both the drugs were prepared of 250µg/ml , 500µg/ml and 1000µg/ml concentration. After treatment with test drugs the total protein content increases, decrease in MDA level, Increased in catalase activity was observed in significant amount. This puts light on cheaper and efficient use of herbal preparation in cataract.
Keywords : Coriander, Ashwagandha, Hydroalcoholic, MDA level, Catalase activity, Protein content.

Introduction

The eyes are termed as a window to the soul for all the living being. The optic image which helps us to connect with world is formed with the help of Cornea and crystalline lens jointly on the retina.(1) Cataract is defined as opacity in the disease free transparent lens which becomes hazy leading to interferes with clear site.(2)It alters the visibility by stopping passage of light.(3) It disturbs lens transparency by changes in its properties. It is characterized by Cloudy or blurry vision, Faded Colours, Poor night vision, Double vision or multiple visions in single eye, Hazy appearance surrounding light, frequent prescription changes in eye glasses.(4) It may occurs due to parameters like older age, habits like smoking and alcohol consumption, change in diet, Chemical exposure, medical conditions like diabetes and hypertension, etc.(5) Mainly two mechanism is involved in the formation of cataract i.e. Sorbitol-Aldose reductase Pathway and Non enzymatic Glycation.(6)

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The enzyme aldose reductase catalyzes the reduction of glucose to sorbitol through polyol pathway which decreases the activity of sorbitol dehydrogenase causing accumulation of sorbitol which leads to apoptosis of lenticular epithelial and generation of free radical. Both increases the cataract formation.[6]Glycation end product like superoxide radicals, carboxymethyl lysine & urea is main concern in cataract.[6] It all get accumulated cause protein degradation of lens and accumulation of same in lens which blur the lens.[7]Till date the only option to treat and cure cataract is surgery. Few experiment was carried out on the drugs which have capacity to decrease the sorbitol level. The agents preventing glucose conversion to sorbitol have also given positive results in retardation of diabetic cataract. [8] Non opioid drug like aspirin was also tested. It shows positive result but required therapeutic dose of 1500 mg which is not safe due to serious general side effects, which makes an aspirin as a questionable candidate as an anticataract drug. [8] Since ages, coriander is used as treatment to protect eye and decrease the need of glass. Improving the human eyesight and fighting against conjunctivitis is the many use of coriander since long. [9] Seeing such pharmacological action of coriander, idea of testing in cataract came out. Also some research shows that Coriander has antioxidant, aldose reductase inhibition and antiglycating activity which is main countering action of cataract. [10] Ashwagandha since long has numerous positive pharmacological properties. Currently used as antidiabetic, Anti-ageing and as Antioxidant in market. Many studies have shown that Ashwagandha is potent aldose reducatse inhibitor. Citing this further research is useful for validating its use as anticataract agent. [11]

Materials and Methods

Collection of plants: Whole plant of *Coriandrum sativum* (Coriander) &*Withaniasomnifera* (Ashwagandha) was collected from Medicinal Garden of School of pharmacy, RK University, Rajkot.

Authentication of plants: Authentication of plant was done at Department of Botany School of Science, RK University, Rajkot.

Preparation of plant extract: Leaves of Ashwagandha & seeds of coriander are allowed to dry & then dried parts are converted to powder form. The powder is kept for hot maceration in hydroalcohol solvent (50% water + 50% ethanol) for 48 hours followed by Filtration & calculation of % yield. [12] [13]

Preparation of different concentrations: Obtained plant extract of both the herbs are weighed accurately and different concentration like 250µg/ml , 500µg/ml and 1000µg/ml are made with the help of water of both the extract.

Preparation of lens Culture: An artificial aqueous humor (NaCl-140mM, KCL-5mM, $MgCl_2$-2mM, $NAHCO_3$-0.5 mM, Na_2HPO_4-0.5mM, $CaCl_2$-0.4mM and glucose 5.5-mM) was prepared and it is allowed to store at room temperature maintaining pH 7.8. To prevent microbial contamination, strict aseptic technique was performed and antibiotic drug streptomycin was added to culture.[14]

Collection and isolation of goat eye balls: Fresh eye balls of young and healthy goats were collected from slaughter house. The eye balls were taken to the laboratory with the help of icebox with artificial aqueous humor filled in it. The temperature was maintained below 4°C.[14]

Removal of Goat eye lens from goat eye balls: The lens was removed by incision of eye balls through surgical method by dissection of cornea and pupil.

Incubation of lens in Culture: The isolated goat eye lens was incubated in artificial aqueous humor (which was prepared before) for 72 hours at room temperature and pH was maintained 7.8 throughout incubation period.

Induction of In Vitro cataract in isolated lens by glucose: Glucose (55mM) was used to induce the cataract In-vitro on goat eye lens. At higher concentration, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration as well as oxidative stress. Thus generation of cataract take place.[15]

Study design:

Groups (n=3)	Treatment
Normal control	Artificial aqueous humor (Glucose content 5.5mM)
Disease control	Artificial aqueous humor (Glucose content 55mM)
250µg/ml ASHW	Artificial aqueous humor (Glucose content 55mM + ASHW(250µg/ml) for 3 days)
500µg/ml ASHW	Artificial aqueous humor (Glucose content 55mM + ASHW (500 µg/ml) for 3 days)
1000µg/ml ASHW	Artificial aqueous humor (Glucose content 55mM + ASHW (1000 µg/ml) for 3 days)
250µg/ml CORI	Artificial aqueous humor (Glucose content 55mM + CORI (250µg/ml) for 3 days)
500µg/ml CORI	Artificial aqueous humor (Glucose content 55mM + CORI (500 µg/ml) for 3 days)
1000µg/ml CORI	Artificial aqueous humor (Glucose content 55mM + CORI (1000 µg/ml) for 3 days)

ASHW: Hydroalcoholic extract of ashwagandha

CORI: Hydroalcoholic extract of coriander

Methods:

1. Morphological and photographic Evaluation: After the incubation period of 72 hours, Lenses were placed on a graph paper with the posterior surface touching the paper. The patterns of square line clearly visible through the lens were observed to measure lens opacity and also size of lens were measured. [16]

- The degree of opacity was graded as follows:

0 : Absence/ clear visibility
1 : Slight degree of opacity
2 : Presence of diffuse opacity
3 : Presence of diffuse to moderate diffuse opacity
4 : Presence of extensive thick opacity

Homogenate preparation: After the incubation period of 72 hours, lenses were homogenized in Tris buffer (23 M) having pH 7.8 and containing 0.25 x 10^{-3} EDTA with the help of sonicator and stirrer. The homogenate was adjusted to 10 % of w/v. The homogenate prepared was allowed to centrifuge at 10,000 rpm/min for 1 hour. Supernatant was separated, rest of things discarded. With the help of supernatant, estimation of parameter takes place. [16]

2. Estimation of MDA level (Malondialdehyde):[17]This method estimates Malondialdehyde (MDA), which is a product of lipid per oxidation process. It generally increased in stress conditions.

Reagent: 1. Thiobarbituric acid (0.067% in Tris hydrochloride, PH 7): 0.067 gm of thiobarbituric acid is dissolved in 100 ml of Tris hydrochloridebuffer to get pH 7**(A)**.

2. Trichloroacetic acid (20%): 20 ml of trichloroacetic acid was dissolved in 100 ml of distilled water **(B)**.

Procedure: Take 1.5 ml of reagent B and mix with 1.5 ml lens homogenate in test tube. Allow to centrifuge for 15 minutes. Take supernant and add 3 ml of reagent A. Prepare blank solution by same method adding 1.5 ml distilled water instead of lens homogenate. Take absorbance of test against blank at 532 nm & at 600 nm at interval of 3 minutes.

Calculation: (Absorbance at 532 nm) - (Absorbance at 600nm).

Extinction coefficient of this MDA-TBA, abduct at 532nm is $155nM^{1}cm^{-7}$.So, Concentration of MDA (mM) = (A532 – A600)/155.

3.Total protein Estimation [18]**:** Total protein level decreases in the cataract due to degradation.

Reagents:

1) 48 mL of 2% sodium carbonate in 0.1 N NaOH**(A)**,
2) 1mL of 1 gm sodium potassium tartrate in 100ml water**(B),**
3) 1 mL 0.5 gm CuSO4 in 100ml water **(C),**
4) 1-Part Folin-Phenol [2 N]: 1- Part of water **(D)**,
5) Albumin standard - 1 mg/mL.
Solution 1: A + B + C
Solution 2: D

Procedure: 0.2 ml of albumin solution is considered as protein standard and 0.2ml of lens homogenate as test solution. Both taken in different test tube and makeup volume upto1 ml with distilled water. 4.5 ml of solution 1 was added to all of above test tube and kept for incubation for 10 minutes. After incubation, 0.5 ml of solution 2 was added in all test tubes and incubated for 30 minutes. Distilled water has been served as blank for both. Absorbance was measured at 660 nm in UV spectrophotometer.

Calculation: After absorbance standard graph was plotted, then, the amount of protein present in the given sample was estimated from the standard graph and is expressed in mg/dl

4.Estimation of Catalase Activity:[19] Catalase is the enzyme which protect from the cellular damages. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm.

Reagents: 1.Phosphate buffer pH 7: (A) 6.81 g Potassium dihydrogen phosphate added in 1000ml distilled water and (B) 8.90 gmdisodium phosphatetoo added in 1000 ml distilled water. Mix solutions (a) and (b) in the proportion 1:1.5 (v/v) i.e. 50ml: 75ml.

2. Hydrogen peroxide (30 µmol/L): Take 0.34 ml of 30% hydrogen peroxide and add in 100ml phosphate buffer.

Procedure: Make reaction mixture containing 1 ml phosphate buffer, 1 ml of hydrogen peroxide and 2 ml homogenate solution. Take water as blank. The rate of decomposition of H_2O_2 was followed by decrease in absorbance at 240 nm in UV.

Statistical Data Analysis: To check the significance of data, following statistical tests were performed:ANOVA : to see the variability within all the groups.Tuckey's test: for the same purpose mentioned in above test.P – value, Degree of freedom, Standard deviation, etc.Data were considered statistically significant at $P < 0.05$ and highly significant at $P < 0.001$. Statistical analysis was performed using INSTAT statistical software.

Results

The percentage yield of hydroalcoholic extract of 25gm of dried powder of *Coriandrum satvium* was found to be 1.62% W/W using maceration process.

The percentage yield of hydroalcoholic extract of 25gm of dried powder of *Withania Somnifera* was found to be 9.2% W/W using maceration process.

Photographic and morphological evaluation of lens

(1) Goat lens were observed with naked eye to evaluate degree of opacity. Opacity indicates the level of cataract formed.

Score	Level of opacity
0	Absence
1	Slight opacity
2	Diffusive opacity
3	Moderate Diffuse opacity
4	Extensive thick opacity

Score for Degree of Opacity

Day 0 Day 1 Day 2 Day 3

Fig 1 Photographic Evaluation of opacity of lens of Disease control group

Day 0 Day 1 Day 2 Day 3

(A) ASHW-250

Day 0 Day 1 Day 2 Day 3

(B) ASHW-500

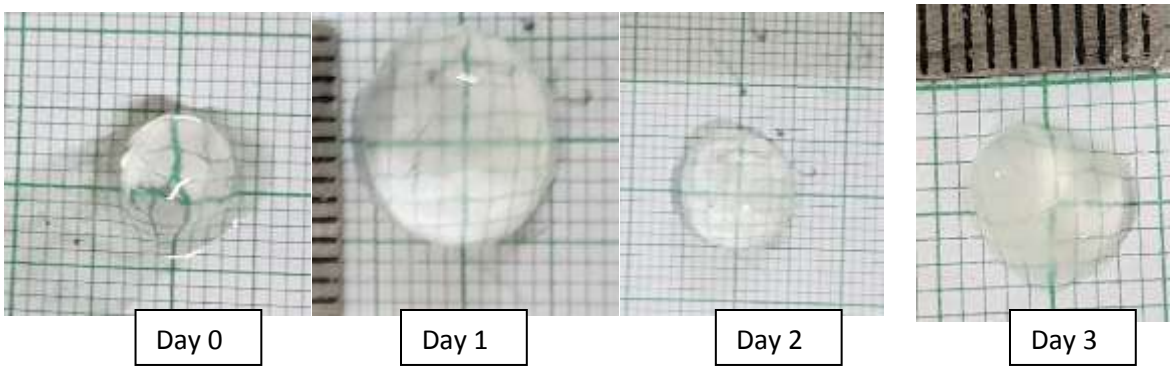
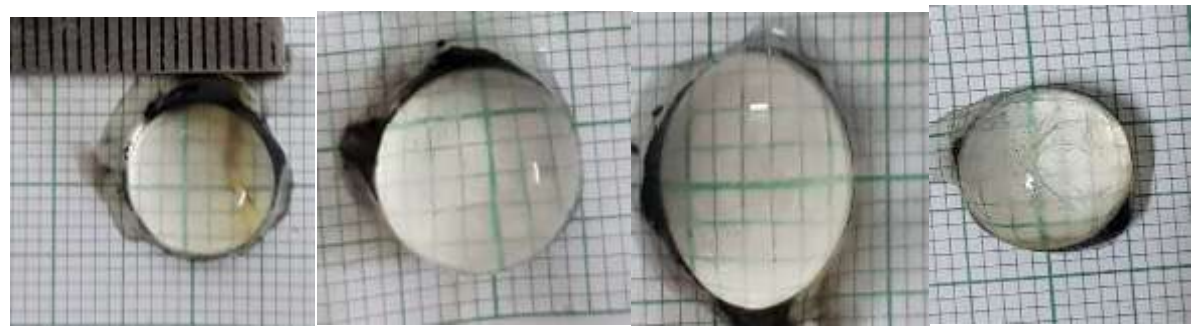
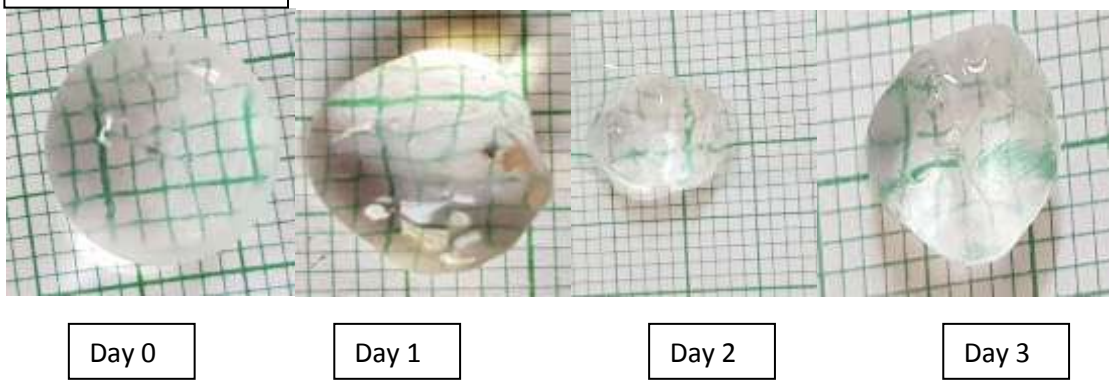


Fig.2 (A)(B)(C) Photographic Evaluation of opacity of lens of Ashwagandha group





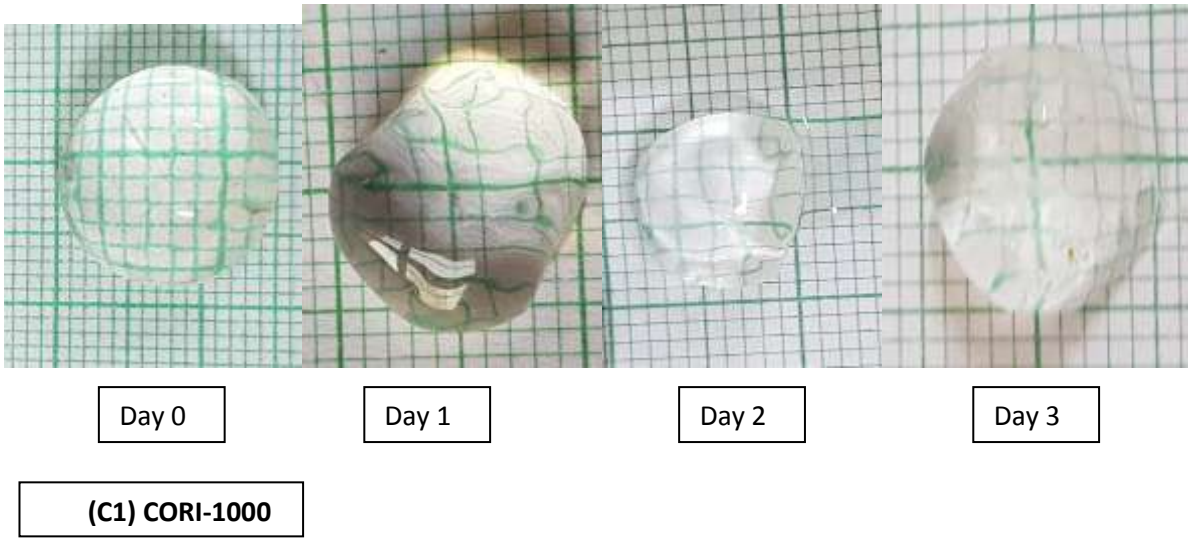


Fig.3 (A1)(B1)(C1) – Photographic Evaluation of opacity of lens of coriander group

Table 1 Evaluation of Degree of opacity in various concentration of extract of ashwagandha & coriander on glucose induced cataract

Sr.no.	Groups	Score of degree of opacity
1	Normal Control	0
2	Disease Control	3.67±0.5774
3	ASHW- 250	3.67±0.5774
4	ASHW-500	3.33±O.5774
5	ASHW-1000	2.67±0.5774
6	CORI- 250	2.33±0.5774
7	CORI-500	2.00± 00
8	CORI-1000	1.67± 0.5774

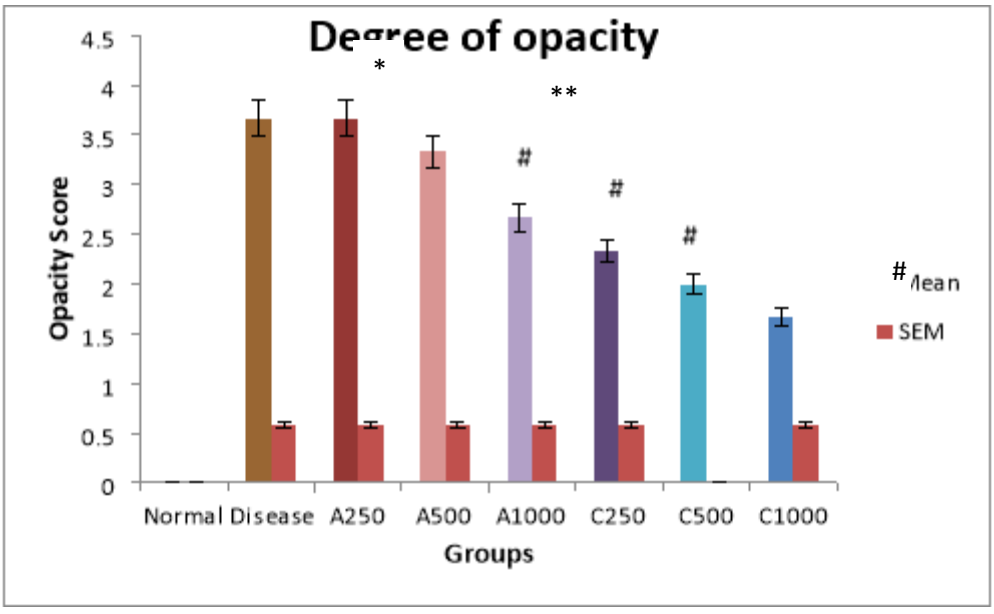


Fig 4 *Ex-vivo* study of effect of Coriander & Ashwagandha on opacity of cataract induced lens

* Indicates significant difference between disease and normal control (P<0.001)
# Indicates significant difference between disease and test group (P<0.001)
** Indicates significant difference between disease and test group (P<0.05)

It was studied that hydroalcoholic extract of coriander works better incontrolling opacity of cataract in lens of goat in comparison of same solvent extract of Ashwagandha. Among 3 different concentrations of both the plants (i.e. 250 µg/ml, 500µg/ml, 1000µg/ml) 1000µg/ml concentration of coriander shows least opacity.Except 250 µg/ml concentration of Ashwagandha, rest all 5 plants extract gives positive effect in comparison of disease control

Estimation of MDA level (Malondialdehyde)

Table 2 Effect of hydroalcoholic extract of ashwagandha and coriander on MAD level on cataract induced goat eye lens

Sr.no.	Groups	MDA level (µmol/mg)
1	Normal	0.1869±0.026
2	Disease	2.109±0.016
3	ASHW-250	1.649±0.018
4	ASHW-500	0.7226±0.004
5	ASHW-1000	0.5041±0.002
6	CORI-250	0.4915±0.003
7	CORI-500	0.4774±0.0005
8	CORI-1000	0.21956±0.0007

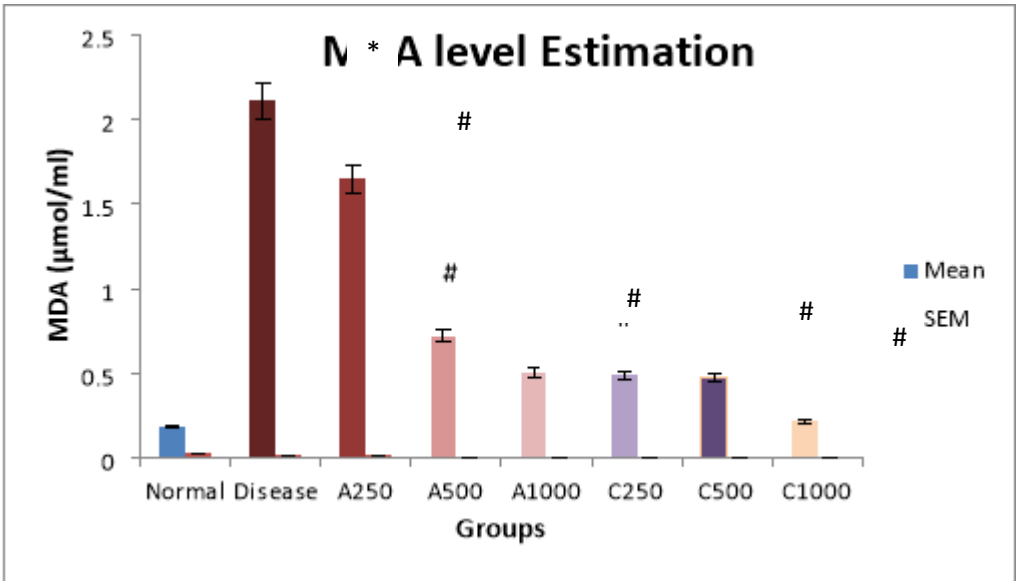


Fig:5 *In-vitro*study of hydroalcoholic extract of aswagandha and coriander on MDA level of diabetic induced cataract of goat lens

* Indicates significant difference between disease and normal control (P<0.001)
# Indicates significant difference between disease and test group (P<0.001)

It was studied that Ashwagandha and coriander have sufficient ability to improve cataract condition of goat eye lens.After treatment with Ashwagandha and coriander at dose 250µg/ml, 500µg/ml, 1000µg/ml respectively,all show decrease in MDA level in test groups in comparison of disease control group.Dose dependent action of both the extract can be seen (Table).

Estimation of Total Protein Level

Table 3 Effect of hydroalcoholic extract of ashwagandha and coriander on Protein level on cataract induced goat eye lens

Sr. No.	Groups	Total Protein Level (gm/dL)
1	Normal	1.28± 0.001
2	Disease	0.4133± 0.002
3	ASHW-250	0.412±0.004
4	ASHW-500	0.513±0.0017
5	ASHW-1000	0.529±0.003
6	CORI-250	0.9983±0.005
7	CORI-500	1.01±0.001
8	CORI-1000	2.533±0.04

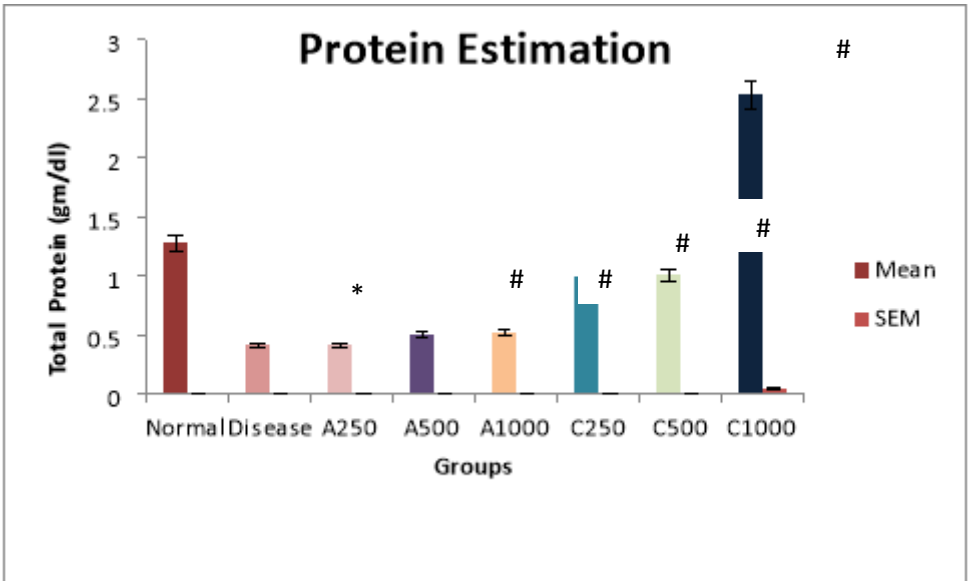


Fig 6 *In-vitro* study of hydroalcoholic extract of aswagandha and coriander on protein level of diabetic induced cataract of goat lens

* Indicates significant difference between disease and normal control (P<0.001)
# Indicates significant difference between disease and test group (P<0.001)

It was studied that Ashwagandha and coriander have ability to treat the cataract which is induced by glucose in goat eye lens by increasing total protein level in dose dependent manner.As cataract occurs it denatured protein and decreases total protein content as seen in disease control group.After treatment with Ashwagandha and coriander at dose 250µg/ml, 500µg/ml, 1000µg/ml respectively, except ASHW-250 all dose increase the total protein level in test group as compared to disease control.CORI-1000 shows better result in protein content in comparison of normal control.

Estimation of catalase activity.

Table 4 Effect of hydroalcoholic extract of ashwagandha and coriander on CAT level on cataract induced goat eye lens

Sr.No.	Groups	Catalase activity(µmol/min/g)
1	Normal	1.9266±0.015
2	Disease	0.5933±0.007
3	A250	0.7434±0.005
4	A500	0.96533±0.003
5	A1000	1.226±0.008
6	C250	1.1926±.0004
7	C500	1.269±0.004
8	C1000	1.443±0.001

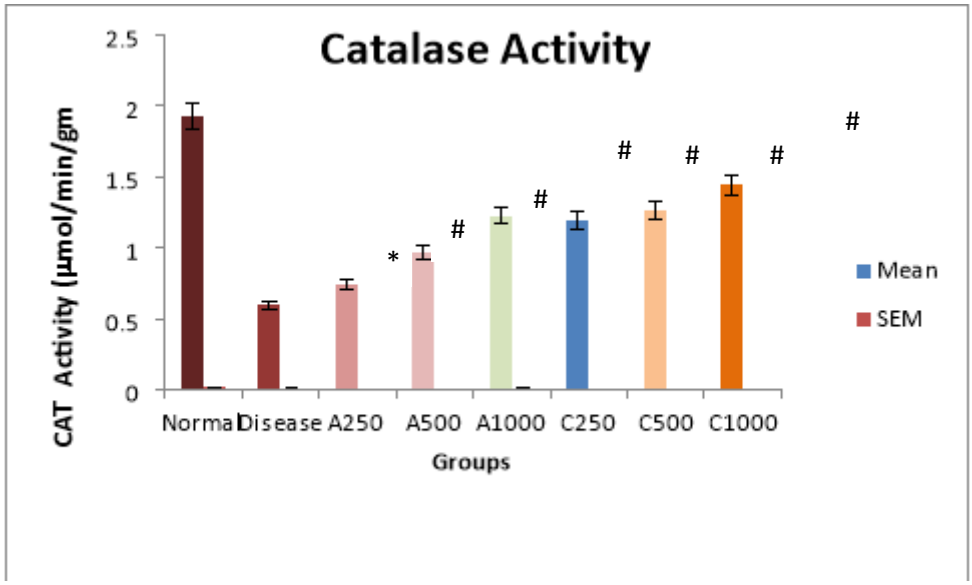


Fig 7: *In-vitro* study of hydroalcoholic extract of aswagandha and coriander in diabetic cataract on goat lens in CAT activity

* Indicates significant difference between disease and normal control (P<0.001)
# Indicates significant difference between disease and test group (P<0.001)

It was studied that Ashwagandha and coriander have ability to treat the cataract which is induced by glucose in goat eye lens by increasing catalase activity in dose dependent manner

As cataract forms, catalase activity of lens decreases as seen in disease control group

After treatment with Ashwagandha and coriander at dose 250µg/ml, 500µg/ml, 1000µg/ml respectively, all doses increase the catalase activity in test group as compared to disease control.

CORI-1000 shows maximum catalase activity among all test groups.

Conclusion

The present study confirmed the anti cataract activity of the test drugs, coriander and ashwagandha respectively. In comparative study of both the test drug, it was concluded that coriander is more potent against

cataract than ashwagandha. Data of our study also confirmed that both the drugs give concentration depedent effect. As concentration of both the drug increase, their efficacy as anticataract drug increases. Maximum anticataract activity at 1000 µg/ml is noted in both the drugs. Polyol pathway inhibition is confirmed by this experiment. The possible mechanism behind this might be aldose reductase inhibition and anti oxidant effect. Data suggest that both the drugs can be used in diabetes induced cataract and also may have potent effect on retinopathy occurred due to complication of diabetes mellitus. This experiment also give rise to hope that hydroalcoholic solvent is useful for extraction process because some photochemical may be soluble in water; some may be soluble in alcohol.Further Pre clinical and clinical studies are needed to establish use of ashwagandha and coriander as anti cataract drug.

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