EVALUATION OF ANTICATARACT ACTIVITY OF CITRULLUS COLOCYNTHIS (L.) FRUIT EXTRACT ON GOAT LENS.

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ABSTRACT: The present investigation was aimed to evaluate the in-vitro anti-cataract activity of methanolic fruit extracts of Citrullus colocynthis against glucose induced cataract in goat lens. Clear isolated goat lenses are incubated in the manufactured aqueous humor and divided into six experimental groups. The MFECC at a dose of 15 µg/ml, 30 µg/ml, and 60 µg/ml is incubated simultaneously with glucose (55Mm) for a span of 24 hrs. Ascorbic acid (40 µg/ml) is used as the standard drug. At the end of the incubation lens opacity is measured by photographic evaluation. The methanolic extracts of Citrullus colocynthis Fruit extract show significant prevention of cataractogenesis of eye lenses by MFECC at 60 µg/ml.

KEYWORDS: Cataract, Citrullus colocynthis fruit extract, Ascorbic acid, Antioxidants.

INTRODUCTION

Cataracts are the clouding of the lens of your eye, which is normally clear. Most cataracts develop slowly over time, causing symptoms such as blurry vision. Cataracts can be surgically removed through an outpatient procedure that restores vision in nearly everyone. A cataract is a cloudy area in the lens of the eye that leads to a decrease in vision. Cataracts often develop slowly and can affect one or both eyes. Symptoms may include faded colors, blurry or double vision, halos around light, trouble with bright lights, and trouble seeing at night. This may result in trouble driving, reading, or recognizing faces. Poor vision caused by cataracts may also result in an increased risk of falling and depression. Cataracts cause half of all cases of blindness and 33% of visual impairment worldwide. Cataracts are most commonly due to aging but May also occur due to trauma or radiation exposure, be present from birth, or occur following eye surgery for other problems. Risk factors include diabetes, longstanding use of corticosteroid medication, smoking tobacco, prolonged exposure to sunlight, and alcohol. The underlying mechanism involves accumulation of clumps of protein or yellow-brown pigment in the lens that reduces transmission of light to the retina at the back of the eye. Diagnosis is by an eye examination.

Prevention includes wearing sunglasses, a wide brimmed hat, eating leafy vegetables and fruits, and avoiding smoking. Early on the symptoms may be improved with glasses. If this does not help, surgery to remove the cloudy lens and replace it with an artificial lens is the only effective treatment. Cataract surgery is not readily available in many countries, and surgery is needed only if the cataracts are causing problems and generally results in an improved quality of life. About 20 million people worldwide are blind due to cataracts. It is the cause of approximately 5% of blindness in the United States and nearly 60% of blindness in parts of Africa and South America. Blindness from cataracts occurs in about 10 to 40 per 100,000 children in the developing world, and 1 to 4 per 100,000 children in the developed world. Cataracts become more common with age. In the United States, cataracts occur in 68% of those over the age of 80 years. Additionally they are more common in women, and less common in Hispanic and Black people.

ANATOMY OF CATARACT

A cataract develops when the lens in your eye, which is normally clear, becomes foggy.

For your eye to see, light passes through a clear lens. The lens is behind your iris (colored part of your eye). The lens focuses the light so that your brain and eye can work together to process information into a picture.

When a cataract clouds over the lens, your eye can't focus light in the same way. This leads to blurry vision or other vision loss (trouble seeing). Your vision change depends on the cataract's location and size.

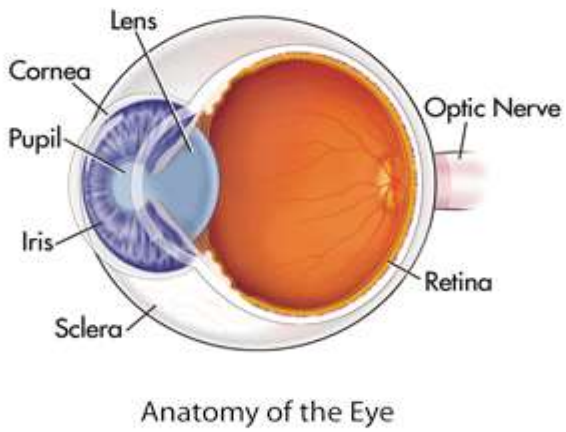

Figure No 1. Anatomy of eye

TYPES OF CATARACT

. A) TYPES OF CATARACTS BY LOCATION-

Nuclear Sclerotic Cataract

A nuclear sclerotic cataract is the most common type of age-related cataract.[2] this type of cataract causes gradual yellow cloudiness and hardening of the central part of the lens called the nucleus. Changes in vision are usually gradual. In some cases, patients may see an actual improvement in near vision before their vision deteriorates to a significant degree.[3]Referred to as "second sight," this stage is usually only temporary.

Cortical Cataract

A cortical cataract generally appears as a cloudy opacity in the part of the lens called the cortex.[4] the cortex consists of the peripheral, or outer part, of the lens. These cataracts often resemble wheel spokes that point inward toward the center of the lens. Light tends to scatter when it hits the spoke-like opacities.

Posterior Sub capsular Cataract

Often referred to as a PSC, a posterior sub capsular cataract is an opacity that develops on the back surface of the lens, directly underneath the lens capsular bag that houses the lens. This type of cataract causes light sensitivity, blurred near vision, and glare and halos around lights. They are more common in diabetic patients and patients who have taken steroids for extended periods of time

TYPES OF CATARACTS BY ORIGIN

Age-related Cataract

Most cataracts develop as we age. Although signs can be seen as early as your 40s to 50s, cataracts usually do not become significant until the late 60s or 70s.

Secondary Cataracts

Cataracts can sometimes develop after undergoing eye surgery, such as surgery for glaucoma or retinal surgery. Patients with diabetes sometimes develop cataracts earlier than normal. Also, patients who are taking steroids for an extended period of time may develop cataracts.

Traumatic Cataract

Cataracts sometimes result from direct injury or trauma to the eye. Cataracts may develop immediately or years after an event that damages the eye. Traumatic cataracts often occur after blunt trauma to the eye or from exposure to certain chemicals.

Congenital Cataract

Some children are born with cataracts. In some cases, the inherited cataract is not significant enough to affect vision. If significant, however, the cataract should be removed in order to avoid vision problems, such as strabismus or amblyopia

Radiation Cataract

Although rare, cataracts sometimes form after exposure to certain types of radiation. This type of cataract may be caused by exposure to ultraviolet light from the sun and other forms of radiation.

CAUSES AND RISK FACTORS OF CATARACTS

The lens of your eye consists mostly of protein and water. The lens is located behind your pupil and iris (the black and colored areas of your eye), which are covered by your cornea (the clear outer layer of your eye).

The proteins in your lens are arranged in a way that keeps the lens clear. But because of normal changes that occur with age, these proteins can begin to clump together, resulting in cloudy areas of vision.

Over time, these protein clumps can grow larger, affecting a greater area of vision.

A number of factors can increase your risk of developing cataracts, including:

- Older age
- Family history of cataracts
- Greater exposure to sunlight
- Living at a higher altitude
- Diabetes
- High blood pressure
- History of eye injury, inflammation, or surgery
- Radiation treatments on your upper body
- Smoking
- Drinking alcohol in excess
- Long-term use of corticosteroid drugs.

While most cataracts are related to aging, some people are born with cataracts or develop them during childhood. These are known as congenital cataracts.

Sometimes congenital cataracts have genetic causes, or they may be due to trauma or infection in the uterus. Conditions that can cause them include:

- Myotonic dystrophy, a type of muscular dystrophy
- Galactosemia, a rare metabolic disorder

- Neurofibromatosis type 2, a disorder in which tumors grow on nerves
- Rubella, sometimes called German measles
- Toxoplasmosis, a parasitic infection
- Cytomegalovirus, a viral infection
- Syphilis
- Herpes simplex virus

Congenital cataracts don't always affect your vision, but if they do, they're usually treated with surgery soon after they're diagnosed.

SIGNS AND SYMPTOMS OF CATARACTS

The most common initial symptom of a cataract is a small area of blurred vision. Over time, this area of blurred vision is likely to grow as your lens becomes more clouded. Eventually, you may get the sense that your overall vision is dull or blurry.

Along with vision that gets progressively blurrier, you may also experience color changes in your lens that cause everything you look at to take on a yellowish or brownish cast.

Eventually you may have trouble distinguishing colors, particularly shades of blue and purple, as well as performing tasks that require distinguishing colors, including reading.

Other than cloudy vision and color changes, symptoms of cataracts may include:

- Poor night vision
- Sensitivity to lamps, headlights, or sunlight
- Halos around lights
- Double vision
- Frequent changes to your glasses or contacts prescription.

It's a good idea to schedule an eye exam if you notice any changes in your vision over time. If you suddenly develop a change in your vision, see an eye doctor right away.

PATHOPHYSIOLOGY

Changes in the lens proteins (Crystalline) affect how the lens refracts light and reduce its clarity, therefore decreasing visual Activity. Chemical modifications of these lens proteins lead to the change in lens color. New cortical fibres are produced concentrically and lead to thickening and hardening of the lens in nuclear sclerosis, which often appears yellow and can Increase the focusing power of the natural lens. Increasing Myopia can also be evidence of a progressing nuclear sclerotic Cataract. Cortical cataracts are most often seen as whitish spokes peripherally in the lens, separated by fluid. Vacuoles and water Clefts can also be seen in these lenses. Posterior sub capsular Cataracts are due to the migration and enlargement of lens epithelial cells. Diabetes mellitus is a major factor in the formation of this type of cataract. Oxidative stress was connected with slow developing cataracts.

GENERAL MECHANISM OF CATARACT FORMATION

The extreme opacification affects the transparent nature of Lens. Lens transparency is necessary to enter incoming light Passes through lens and falls on retina to form image. Opacification of lens causes light scattering, as light passes through lens and then to the retina where diminished focus of Light impairs vision. The visual activity is lost in cataract due To the absorption of light by less transparent lens. The

Generalized mechanism of cataract includes lens fibre cells Disruption, cellular protein aggregation and lens cell cytoplasm dysfunctioning.

PLANT INTRODUCTION:

Citrullus colocynthis, with many common names including colocynth, bitter apple, bitter cucumber, desert gourd, egusi, vine of Sodom, or wild gourd, is a desert viny plant native to the Mediterranean Basin and Asia, especially Turkey, and Nubia. C. Colocynthis is a desert viney plant that grows in sandy, arid soils. It resembles the watermelon, which is in the same genus. It is native to the Mediterranean Basin and Asia, and is distributed among the west coast of northern Africa, eastward through the Sahara, Egypt until India, and reaches also the north coast of the Mediterranean and the Caspian Seas. C. Colocynthis, a perennial plant, can propagate both by generative and vegetative means. However, seed germination is poor due to the extreme xeric conditions, so vegetative propagation is more common and successful in nature. In the Indian arid zone, growth takes place between January and October. Colocynth has been widely used in traditional medicine for centuries. In Arabia the colocynth had numerous uses in traditional medicine, such as a laxative, diuretic, or for insect bites. The powder of colocynth was sometimes used externally with aloes, unguents, or bandages. Troches made of colocynth were called "troches of alhandal" used as an emetic. In traditional Arab veterinary medicine, colocynth sap was used to treat skin eruptions in camels.

Taxonomy

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Angiospermae
Category: Fabids
Order: Cucurbitales
Family: Cucurbitaceae
Genus: Citrullus
Species: Citrullus colocynthis
Part used: seed, leaves, fruit, bark, roots, flowers.

Phytochemical screenings

The seed extracts of Citrullus colocynthis were analysed for the Presence of alkaloids, glycosides, tri terpenoids, steroids, Saponin, flavonoids, tannins and carbohydrates according to Standard methods.

Test for alkaloids

2ml of dilute hydrochloric acid was added to the 5 ml of Extract then treated with dragondroff's reagent, appearance of an orange brown precipitate showed the presence of alkaloids.

Test for glycosides

The extract was hydrolyzed with dilute hydrochloric acid for few hours on a water bath. 1ml of pyridine and a few drops of Sodium nitroprusside solution were added. Then 2-3 drops of Dilute NaOH was mixed. Pink colour produced which turn into Red indicated presence of glycosides.

Test for triterpenoids

About 5 ml of extract was mixed in 2 ml of chloroform; 2 ml of acetic anhydride and a few drops of conc. H2so4 were added. Reddish violet colors indicated the presence of triterpenoids.

Test for steroids

10ml of chloroform was mixed with 2ml of extracts and conc. H2so4 was added to form lower layer. A reddish yellow Colour at the interface was an indicative of the presence of Steroidal ring.

Test for Saponins

15 ml of distilled water was added to the extract and shaken vigorously until formation of a stable persistent froth which Indicates presence of saponins

Test for Flavonoids

Few drops of dilute naoh was mixed with 2 ml of extract. A yellow solution that turns colorless showed the presence of Flavonoids.

Test for Tannins

In a test tube containing little quantity of extract few drops of 1 % lead acetate were added. Yellow precipitate appeared it showed the presence of tannins.

Test for Carbohydrates

The small portion of extract was mixed with 2ml of Molisch's Reagent and the mixture was shaken properly. After that 2ml of Concentrated H2SO4 was poured carefully along the side of the test tube. Violet ring at the interphase was not formed which indicates absence of carbohydrate.

These are the phytochemical tests used for the evaluation of phytochemical parameters in Citrullus colocynthis.

MATERIAL AND METHODS

PLANT MATERIAL

Citrullus colocynthis (Cucurbitaceae) fruits were collected from Pune district, Maharashtra (India). The plant has been identified morphologically and authenticated by R.P. Ganorkar head of department of botany of chandmal tarachand college shirur, Pune, Maharashtra (India). The fruits were cleaned and dried in the shade, then powdered to 0.422 mm mesh size and stored in an air-tight container at 25^{o}C.

EXTRACTION PROCESS

Citrullus colocynthis fruits (100 g) in powdered form were extracted with methanol using a Soxhlet assembly for 48 h, filtered and last traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The yield was 2.78 g of dry extract.

Requirements

Test drug: Citrullus colocynthis methanolic fruit extract.

Chemicals:
Sodium chloride (NaCl), Potassium chloride (KCl), Magnesium chloride (MgCl2), Sodium bicarbonate (NaHCO3), Calcium Chloride (CaCl2), Glucose, Penicillin, Streptomycin , Ascorbic acid.

Instruments: Incubator, Wired mesh, Petri dish.

Dose selection: Citrullus colocynthis fruit extract –15, 30 and 60µg/ml.

Standard Ascorbic Acid: 40µg/ml.

Collection of eyeballs:

Goat eye balls were used in the present study. They were obtained from the slaughterhouse instantly after slaughter and transported to laboratory at 0-4o C. (2)

PROCEDURE:

LENS CULTURE:

A Fresh goat eyeballs were obtained from the slaughter house and instantly transported to the laboratory at 0-4°C. The lens were detached by extra capsular extraction and incubated in unreal aqueous humor (NaCl 140 mM, KCl 5mM, MgCl2 2 mM,NaHCO3 0.5 mM, NaHPO4 0.5 mM, CaCl2 0.4 mM and glucose 5.5 mM) at room temperature and maintain pH 7.8 by addition of NaHCO3). Penicillin G 32% and streptomycin 250 mg% added to the culture media to inhibit bacterial contamination. At high concentration, glucose the lens was metabolized through sorbitol pathway and accumulation of polyols causing over hydration and oxidative stress. This leads to cataractogenesis.

INDUCTION OF IN-VITRO CATARACT:

Glucose at a concentration of 55 mM was used to induce cataracts. At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration and oxidative stress. This generates cataractogenesis. These lens were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM) served as normal control and 55 mM serve as toxic control) for 72hours.

STUDY DESIGN AND GROUPS:

The Anti-cataract activity was carried out with the extracts of Citrullus colocynthis fruit extract. The extract was taken in different doses.

Goat lenses were divided into six groups of six lenses each and incubated as follows

Group I: Aqueous humor + Glucose 5.5 mM (Normal control).
Group II: Aqueous humor + Glucose 55 mM (Negative control)
Group III: Aqueous humor + Glucose 55mM + 40µg/ml Ascorbic acid. (Standard)
Group IV: Aqueous humor + Glucose 55mM + 15µg/ml MFECC (Test I)
Group V: Aqueous humor + Glucose 55mM + 30µg/ml MFECC (Test II)
Group VI: Aqueous humor + Glucose 55 mM + 60 µg/ml MFECC (Test III)

PHOTOGRAPHIC EVALUATION:

Lenses were placed on a wired mesh with the posterior surface touching the mesh, the pattern of mesh number of squares clearly visible through the lens was observed to measure lens 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 72 hours of incubation, homogenate of lenses was prepared in tri 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.

BIOCHEMICAL PARAMETER:
ESTIMATION OF TOTAL PROTEIN CONTENT:

To 0.1 ml of lens homogenate, 4.0ml of alkaline copper solution was added and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for color 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.

STATISTICAL ANALYSIS:

All data were expressed as mean ± SD. All data were analyzed with SPSS/10 student software. Hypothesis testing methods include done way analysis of variance (ANOVA) followed by LSD. The values are expressed as mean ± S.D. and results were considered significantly different if $P<0.05$. Statistical variations are compared as-Normal Goat lens vs. Goat lens + Glucose 55mM, Goat lens + Glucose55mM vs. Goat lens + Glucose55mM +: Methanolic fruit extract of citrullus colocynthis.

ESTIMATION OF TOTAL PROTEIN CONTENT

Total protein content was estimated by the Lowry method. The Method is having the following procedure. 1% of the lens Tissue was homogenized in 0.25M ice cold Sucrose solution. 0.5 ml of the crude homogenate is thoroughly mixed with 1ml of Trichlor acetic acid. Then centrifuged at 1000 rpm for about 15 minutes. Discard the supernatant liquid. The pellet was dissolved in 1ml of 1N naoh. Then 4ml of alkaline copper Reagent was added followed by 0.4 ml of Folin- phenol Reagent (Folin reagent):(Distilled water){1:1}. Read the Colorimeter at 600nm.

Chemicals Required

- 10% TCA: 10 grams of Trichlor acetic acid is dissolved in 100ml of distilled water.
- 1N naoh: 4 grams of naoh is dissolved in 100ml of Distilled water.
- Alkaline copper reagent (ACR):
- SOLUTION A: 0.4 grams of naoh was dissolved in 100ml of distilled water. Then 2 grams of Na2Co3 was Added.
- SOLUTION B: 0.5 grams of cuso4 was dissolved in 100 ml of distilled water. Then 1 gram of sodium Potassium tartrate was added.
- Solution A: 50 ml
- Solution B: 1ml
- Both the solutions were mixed properly.
- Folin Phenol reagent: These is prepared by making 1:1 dilution i.e. 1ml of Folin phenol reagent: 1ml of Distilled water.

RESULT AND DISSCUSSION

PHYTOCHEMICAL PARAMETERS:

Table no: 1 results of phytochemical parameter of methanolic extract.

Sr. no.	Phytochemical tests	Results
1	Alkaloids	++
2	Glycosides	++
3	Triterpenoids	++
4	Steroids	++
5	Saponins	++
6	Flavonoids	++
7	Tannins	++
8	Carbohydrates	--

++ = Present
-- = Absent

IN- VITRO ANTI-CATARACT ACTIVITY:

After 8 hr. of incubation lenses with glucose 55mM shows opacification at periphery, on the posterior surface of the lens. At the end of 72 hrs. Complete opacification is progressively increased to words the center.

PHOTOGRAPHIC EVALUATION:

After 72 hours of incubation, transparency was maintained in the Group I (normal control group) [656fig.2 (A)] but there was complete loss of transparency in the Group II (negative control group) [fig. 2(B)] indicating complete cataractogenesis. Group III (positive control group) [fig. 2(C)] containing lens treated with standard ascorbic acid were squares of the graph paper were visible through the lenses. Goat lenses of groups containing escalated doses of the methanolic fruit extract of Citrullus colocynthis (Group IV, V) were less hazy and the squares of the graph paper were visible through the lenses indicating suppression of cataract formation [fig.2 (D) and (E)]. Group VI (containing 60µg/ml) was more effective in suppressing cataract formation [fig. 2(F)] than Group IV and Group V [fig. 2(D) and (E)].

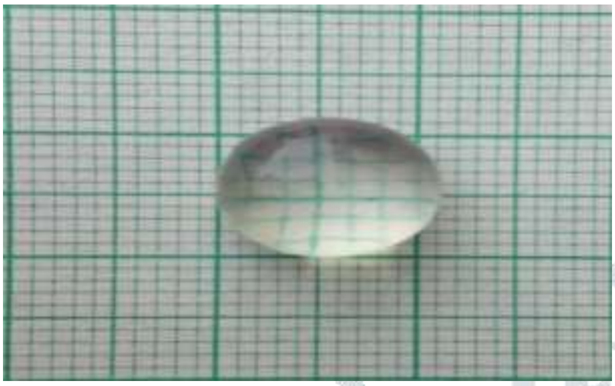

Fig 2. (A): Normal control (group I) Group II) Showed transparent lens.

Fig 2 (B): Negative control Complete cataractogenesis

Fig 2 (C): Positive control (Group III) Standard-ascorbic acid.

Fig. 2 (D): Group IV

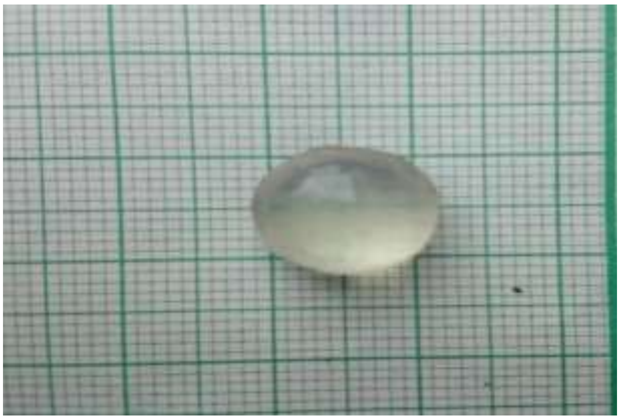

Fig 2 (E): Group V

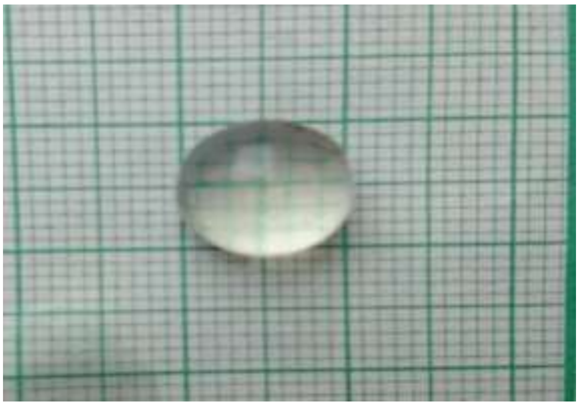

Fig. 5 (F): Group VI

Fig 2: Effect of MFECC on goat lens in glucose induced cataract.

Table no. 2 Effect of methanolic fruit extract of Citrullus colocynthis (MFECC) on degree of opacity on lens by glucose induced cataract.

Sr. no	Compound	Degree of Opacity
1	Normal	0
2	Negative control (Glucose 55 mM)	3
3	Positive control (Ascorbic acid 40µg/ml)	1
4	Test 1(MFECC 15µg/ml)	2
5	Test 2 (MFECC 30µg/ml)	1
6	Test 3 (MFECC 60µg/ml)	0

Normal control - Zero degree opacity occurred, clear lens is obtained.

Negative control – presence of extensive thick opacity, because of high conc. of glucose induced cataractogenesis.

Positive control (Ascorbic acid 40µg/ml) – lenses show slight degree of opacity, clear lens was not found.

Test 1 (MFECC 15µg/ml) – lenses show slight degree of opacity, clear lens was not found.

Test 2 (MFECC 30µg/ml) – lenses show slight degree of opacity, clear lens was not found.

Test 3 (MFECC 60µg/ml) – Zero degree opacity is occurred, clear lens is obtained. Test drug inhibits cataractogenesis.

Table No.3: Effect of MFECC on total protein levels in Goat lens homogenate after 72 hours of incubation in glucose 55 mM induced cataract:

Sr.no	Treatment	Total protein mg/gm
1	Normal lens Control Glucose 5.5 mM	208.51 ± 2.235
2	Negative control Glucose 55 Mm	168.40± 2.093**
3	Standard Glucose 55 mM + Ascorbic acid 40µg/ml	207.37± 2.639**
4	Test 1-Glucose 55 mM + MFECC 15µg/ml	185.24± 2.149
5	Test 2-Glucose 55 mM + MFECC 30µg/ml	209.31± 1.678**
6	Test 3-Glucose 55 mM + MFECC 60 µg/ml	212.76± 1.667**

N=6, values are expressed as Mean ± SEM. Comparison were made as follows, # $p < 0.05$, < 0.01 when compared with normal control. * $p < 0.05$, ** $p < 0.01$ when compared with negative control. (Values are compared on 72hr by one way ANOVA Dennett t test) N.S. – non significant.

Glucose 55 mm treated lenses (Group-II) showed significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate (P<0.01) compared with normal lenses (Group-I). Ascorbic acid treated lenses (Group-III) and Lenses treated with MFECC (Group-IV, V, VI) showed higher concentrations of proteins (total proteins) (P<0.01) compared with Glucose 55 mm treated lenses (Group-II).

DISCUSSION

Alteration of Na+/K+ ratio due to reduction in Na+/ATPase activity in the lens causes Radical scavenging activity. In Normal control, after 72 hr. of incubation of lens in aqueous humor and 55 mM of glucose. The lens was clear in because of low conc. of glucose that does not show any effect on lens and numbers of squares are clearly visible through lens. The lens showed zero degree of opacity. In Standard group, after 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose+ 40µg/ml Ascorbic acid std. drug, numbers of squares were not clearly visible through lens as compared to Test-3, the lens showed slight degree of opacity. In Test- 1 & Test -2, after 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose + 15 µg/ml and 30µg/ml MFECC test drug, number of squares were not clearly visible through lens as compared to MFECC 60 µg/ml test-3 drug, the lens show slight degree of opacity to cataractogenesis. This alteration in the Na^+, K^+ ratio changes the protein content of In Test- 3, after 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose + 60µg/ml MFECC drug, number of squares were clearly visible through the lens .The lens showed absence of opacity, because the test drug inhibits cataractogenesis and oxidative stress.

CONCLUSION

In the present study the MFECC showed significant reduction of cataract at the dose of 100 µg/ml in goat lenses. The result support the traditional use of this plant in cataract conditions and suggests the presence of biologically active compounds which may be worth for further investigation and elucidation.

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