EXTRACTION AND EVALUATION OF *IN-VITRO* ANTI-CATARACT ACTIVITY OF *Blepharis maderaspatensis* (L).Hyene ex Roth ON GLUCOSE INDUCED GOAT LENS CATARACT MODEL

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ABSTRACT:

The primary goal of this study was to determine whether *Blepharis maderaspatensis*. Hyene ex Roth plant leaves had any anti-cataract properties (L). The current work addresses the toxicity analysis of the plant leave ethanolic extract using the Zebra fish (Danio rerio) model using the glucose induced goat lens cataract model. The ethanolic extract is initially investigated for its phytochemical components using a variety of qualitative assessment techniques. From recently butchered goat eyeballs, lenses were obtained for anti-cataract action. Those lenses developed cataracts as a result of the 55mM glucose in the synthetic aqueous humour. Enalapril is a commonly standard anti-cataract drug [(2)]. Following doses of ethanolic extract of Blepharis maderaspatensis leaves (20µg, 50µg, 100µg, 250µg, and 500µg) are evaluated for the anti-cataract efficacy in goat lens model using glucose 5.5mM concentration with artificial aqueous humour as a standard reference. Against the high glucose concentration, several of those concentrations exhibit good action.

DISEASE INTRODUCTION

CATARACT

The Greek term for "waterfall" is where the word "cataract" originates. It was believed that cataracts were generated by opaque material streaming, much like a waterfall, until the mid-1700s. into the pupil A cataract is brought on by the lens fibres deteriorating or becoming opaque, the development of aberrant lens fibres, and the deposition of materials within the lens.

Cataracts, which are an opacity of the eye's natural crystalline lens, are still the most common reason for blindness in the modern world.50% of blindness worldwide is caused by cataracts, making them the main cause of blindness.

PLANT DESCRIPTION

Botanical classification

Kingdom : Plantae

Phylum : Tracheophytes

Class : Magnoliopsida

Order : Lamiales

Family : Acanthaceae

Genus : *Blepharis*

Species : *Blepharis maderaspatensis* (L).Hyene Ex Roth

Phytoconstituents

These phytochemical study reveals the presence of Alkaloids, carbohydrates, Anthraquinone glycoside, sterols, flavonoids, mucilage, saponins and absence of proteins, amino acids, volatile oil, fixed oil, gum

Toxicity study by zebra fish (Danio rerio) model [3]

An aquarium favourite, the zebrafish (Danio rerio) is a tropical freshwater fish from the minnow family (Cyprinidae) in the group Cypriniformes. Zebrafish are becoming more and more popular as an animal model in recent years, particularly for in vivo drug discovery studies on their embryonic and larval stages, which can be carried out quickly and affordably utilising multi-well plates. Since the early 1970s, this species has gained popularity as a useful experimental model used in research on the development of vertebrates, biological development, and genetic diseases. In addition to being genetically compatible with 70% of the disease genes found in humans, D. rerio is widely known for its capacity for regeneration. This makes it a perfect model for reproducing diseased states in humans and an important tool for scientific research.

The zebrafish embryo is a highly well-liked and trustworthy animal model because of its quick developing processes, transparency, high fecundity, low maintenance in lab settings, adaptability to experimental manipulation, and similar embryonic development to higher vertebrates.

Due to its capacity to produce numerous non-adherent and transparent eggs under the right circumstances, zebrafish have a fairly high availability and reproduction rate. It is currently being utilised for drug development and is a viable and affordable substitute for various mammalian models.

Toxicity assay

Using a zebrafish embryo screening assay in a Petri dish with preceding maintenance and spawning procedures and care in accordance with Organization for Economic Co-operation and Development (OECD) guidelines, the toxic effect of the B. maderaspatensis leaf ethanolic fraction was examine

upkeep for zebrafish

Danio rerio spawning and embryo protection Male and female mature zebrafish were chosen and placed in a glass aquarium with a continuous recirculation system at a ratio of 1:2 under a 12:12 h dark: light cycle. Both the water and the air were kept at a constant 27 1 °C. Once fertilised, the embryos were removed from the spawn tanks, placed in clean petridishes with embryo water (made by combining 1 L of Milli-Q water with 0.21 g of Instant Ocean® H salt), and left to develop for 6 hpf (hours post fertilisation). The embryos were meticulously cleaned to get rid of any debris, and any unhealthy or dead embryos were aspirated out with a disposable pipette. Prior to treatment, microscopic inspection of the embryonic development was carried out at 6 hpf.

Procedure for preparing and treating fractions

The fertilised, healthy embryos must be transferred into a dish containing various concentrations of an ethanolic extract of Blepharis maderaspatensis leaves (EEBML), with 20 embryos in each treatment group. Each petridish had a total volume of 30 ml, which was made up of 15 ml of embryo water aspirated from an embryo at 12 hpf (hours post fertilisation) and 15 ml of the ethanolic extract fraction prepared in 2% DMSO. These two components resulted in final concentrations of 1000, 500, 250, 125, 62.5, and 31.25 g/ml. In order to create a control group, embryos were just given 2% DMSO without any sample. All plates must be incubated at 27°C in a room with a controlled temperature.

Microscopic observations

The harmful effects of samples on zebra fish embryos were noticed at 24, 48, 72, and 96 hpf, which corresponds to 12, 36, 48, and 60 hpt (hours post treatment), respectively. During the course of treatment, the survival and sub-lethal endpoint were evaluated. The quantification of hatching success and mortality rate, delayed developmental, oedema frequency, and body malformations are among the sub-lethal endpoint evaluation measurements.

1.Percentage of Mortality (%)

No. of dead embryos/ Total embryos x 100

2.Percentage of Hatchability (%)

No. of hatched embryos/ Total embryos x 100

ASSESSMENT OF IN VITRO ANTICATATARACT ACTIVITY

Preparations Of Artificial Aqueous Humor [4][5]

1. Glucose-5.5mM
2. NaCl-140mM
3. KCl-5mM
4. $MgCl_2$-2mM
5. NaHCO3-0.5mM
6. $NaH(PO_4)_2$-0.5mM
7. $CaCl_2$-0.4mM
8. PH-7.4 for 72 hrs
9. Penicillin-32mg/ streptomycin-250mg

Preparation of buffer solution [6]

(a) 0.1 M Sodium phosphate monobasic; 13.8 g/L (monohydrate, M.W. 138.0)
(b) 0.1 M Sodium phosphate dibasic; 26.8 g/L (heptahydrate, M.W. 268.0)

8.5 ml of sodium phosphate monobasic and 91.5 ml of sodium phosphate dibasic and adjust the final volume to 200 ml with deionized water. Adjust the final PH using sensitive PH meter.

Goat Lens Collection [5]

Goat lenses were used in the study because they were readily available from the Nelpettai slaughter house in Madurai. Fresh goat eyeballs were procured from the slaughterhouse and transported right away to the lab at 0-40^{0}C. The lens was then removed using surgical tools, and the eyeballs were cultured in artificial aqueous humour at room temperature for 72 hours while maintaining a PH of 7.4–7.8. To prevent bacterial contamination, the culture medium was given a dose of streptomycin 250 mg and penicillin 32 mg.

In Vitro Cataract Induction

A 55mM concentration of glucose was employed to cause cataracts. When glucose levels are high, the lens metabolizes it through the sarbitol pathway and accumulates polyols (sugar alcohol), which results in overhydration and oxidative stress. cataract genesis resulted from this. For the study, a total of 'n' numbers of lenses were employed. These lenses underwent a 72-hour incubation period in artificial aqueous humor containing two different concentrations of glucose (5.5 mM served as the normal control and 55 mM as the hazardous control). [5]

Study design and groups

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

1. Group-I - glucose 5.5mM (normal control)
2. Group- II - glucose 55mM (toxic control)
3. Group- III - glucose 55mM + Enalapril 5ng/ml[7]
4. Group -IV - glucose 55mM + Blepharis maderaspatensis extract 100 µg/ml
5. Group -V - glucose 55mM + Blepharis maderaspatensis 250 µg/ml

Morphological and photographic evaluation:

Lens opacity was assessed according to the number of squares that were clearly visible through the lens when the lenses were put on a wired mesh with their posterior surfaces touching the mesh.

0 - No presence

+ - Slight presence

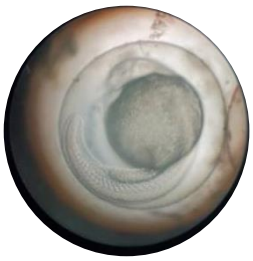
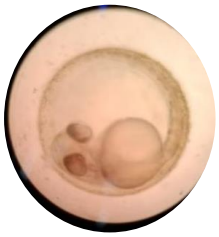
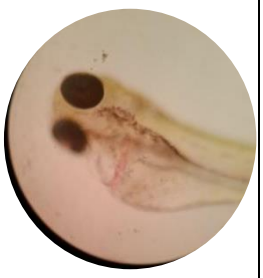
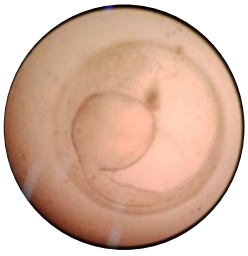
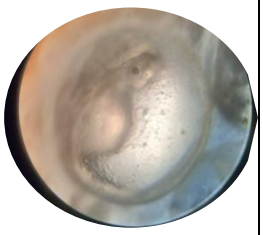
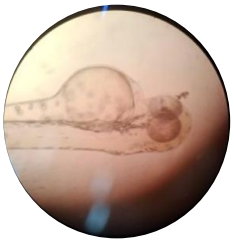
++ - the presence of diffuse opacity

+++ - the presence of a substantial amount of thick opacity

maderaspatensis leaf extraction results in morphological abnormalities in zebrafish embryos

The ethanolic extraction generated from *B. maderaspatensis* leaves was given in six different doses to the treated groups and a control group, respectively, and the harmful effects of each were monitored. At 72 hpf, the teratogenicity parameters and of treated and untreated embryos/larvae containing various concentrations of EEBML (ethanolic extract of Blepharis maderaspatensis leaves) were noted.

In contrast to the untreated group, observations revealed that none of the larvae treated with the lowest plant concentration (B) had any noticeable morphological flaws. The embryos were shown to be toxic to the ethanolic extract at 1000 g/mL, with all of the embryos dying after 48 hpf, and in 500 g/mL of EEBML, some of the embryos in these groups showed abnormalities with crooked backbones and oedema. Embryos in the untreated and lowest dosage (32.5 g/mL) groups lacked the aforementioned criteria.

GROUPING	DAY 1	DAY 2	DAY 3	DAY 4
CONTROL				
31.25 µg/ml				

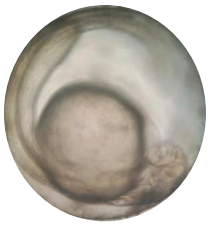
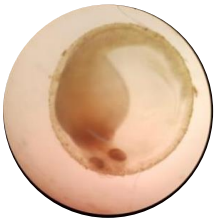
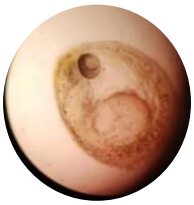
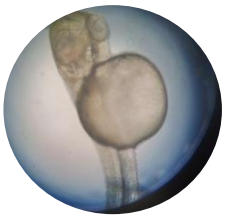
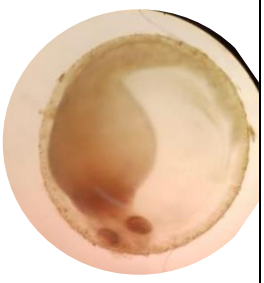
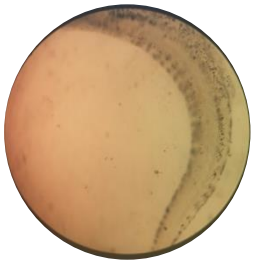
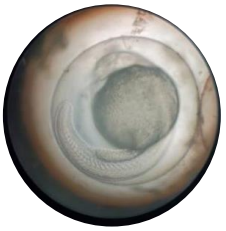
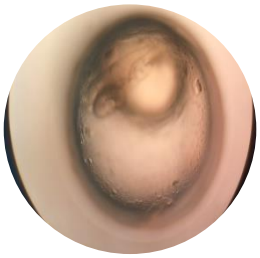
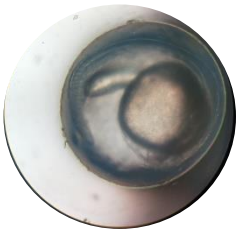
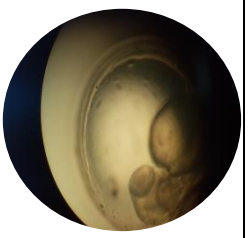
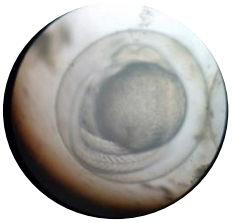
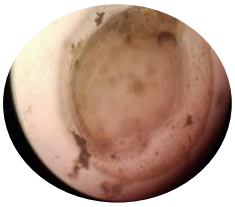
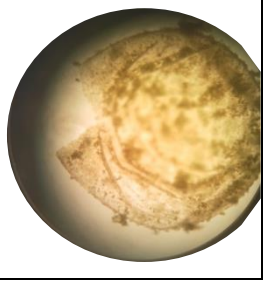
62.5µg/ml				
125µg/ml				
250 µg/ml				
500 µg/ml				
1000 µg/ml				

Table 1

Rate of mortality:

Coagulation and the absence of heartbeat in zebra Fish embryos are indicative of mortality towards the end of the study at 96 hpf. In contrast, the embryos treated with the highest dose of 1000 µg/ mL had an almost 95% mortality rate at 24 hpf.. Treatment with the lowest dose of the plant extraction caused minimal mortality rate 5% (48 hpf and 72 hpf), A 100 % mortality rate was observed & Coagulation can be seen in the embryos treated in 500 &1000 µg/mL of EEBML, by contrast, the embryos immersed in the solution with an EEBML concentration of 250 µg/mL suffered from oedema and the absence of heartbeat

Hatchability of zebraFish embryos :

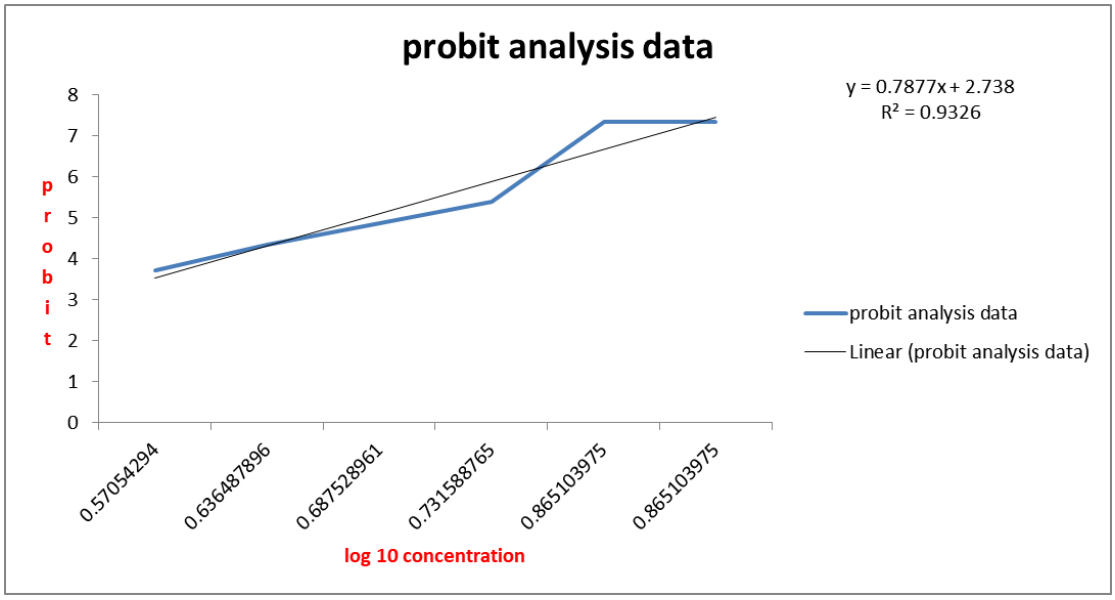
Hatching indicates the successful development of the embryo into larvae, which occurs between 48 and 72 hpf. However, as the concentration increased, the hatchability rate of EEBML treated embryos became significantly lower than the control groups. Table 1 depicts the percent hatchability for the normal and the EEBML treated embryos at 72 hpf. The percent of hatchability of the embryos was observed to be normal and complete in the control group and in the group treated with 32.5µg/mL of the plant extraction. The successful embryonic development of zebraFish embryos is indicated by hatching after 48 hpf . Hatchability could not be measured in the embryos immersed in solution with the EEBML at concentrations of 250 µg/mL , 500 µg/mL and 1000 µg/mL due to the 45 , 75 ,95 percentage of mortality rate respectively with coagulation before 48 and 24 hpf

S.No	Concentration of EEBML (µg/ml)	Total embryos	No. of embryos hatched	No. of embryos dead	% Hatchability	% Mortality
1	Control	20	20	0	100	0
2	31.25	20	19	1	95	5
3	62.5	20	18	2	90	10
4	125	20	14	6	70	30
5	250	20	11	9	55	45
6	500	20	5	15	25	75
7	1000	20	1	19	5	95

Lethal concentration dose (LC50) of the EEBML in Zebra fish embryo (*Danio rerio*).

concentration of EEBML in µg/ml	log10 concentration	Percentage of dead	probit value
15.625	1.193820026	5	3.36
31.25	1.494850022	10	3.72
62.5	1.795880017	25	4.33
125	2.096910013	40	4.75
250	2.397940009	55	5.13
500	2.698970004	90	6.28
1000	3	95	6.64

Median lethal concentration (LC50) value of the EEBML based on probit analysis.



The above chart shows the logarithmic estimation of the LC50 value using the concentration and mortality rate of the embryos. The LC50 value for EEBML is indicated by the statistical estimation of the amount of toxicant (g) per body weight (kg) required to induce the death of 50% of the population of the animal tested. According to the OECD guidelines, any toxicants are considered harmful, toxic, and extremely toxic if the LC50 ranges between 31.25 and 1000 g/L. Based on their scale, the LC50 of ethanol extraction is estimated to be 143.3838 g/L, making it significantly detrimental.

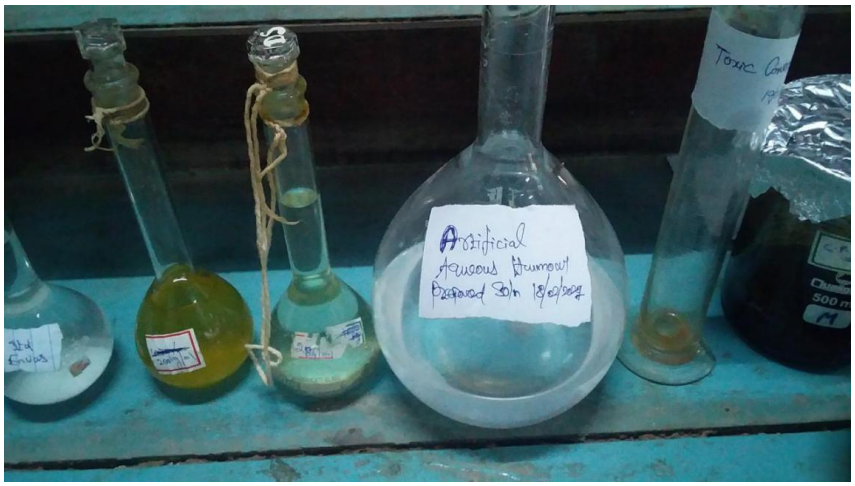
Teratogenic defects of varying concentrations of *B.maderaspatensis* leaves extract at 72 hpf in *D. rerio* larvae

Extracts Concentration (µg/mL)	Teratogenicity Parameters					
	Hyperactive	Delayed hatch	Crooked backbone	Less Pigmentation	Awkward position	Edema
control	-	-	-	-	-	-
31.25	-	+	-	-	-	-
62.5	+	+	-	-	-	-
125	+	+	+	+	+	+
250	-	+	+	-	+	+
500	+	+	+	+	+	+
1000	ND	ND	ND	ND	ND	ND

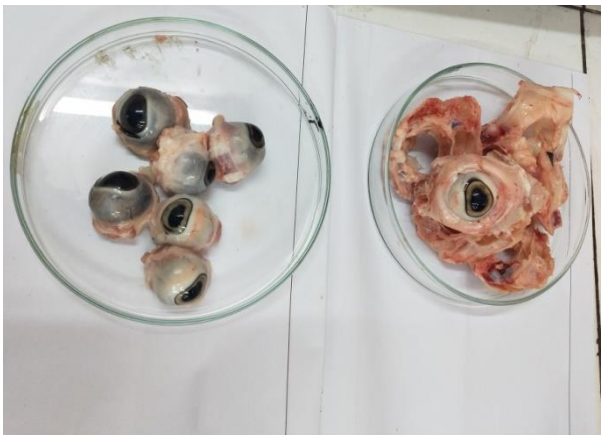
Table 2

(+) - positive, (-) - negative, (ND) - not determined

PREPARATION OF ARTIFICIAL AQUEOUS HUMOUR



COLLECTION OF GOAT EYE



Eyeballs are successively removed from the freshly slaughtered goat

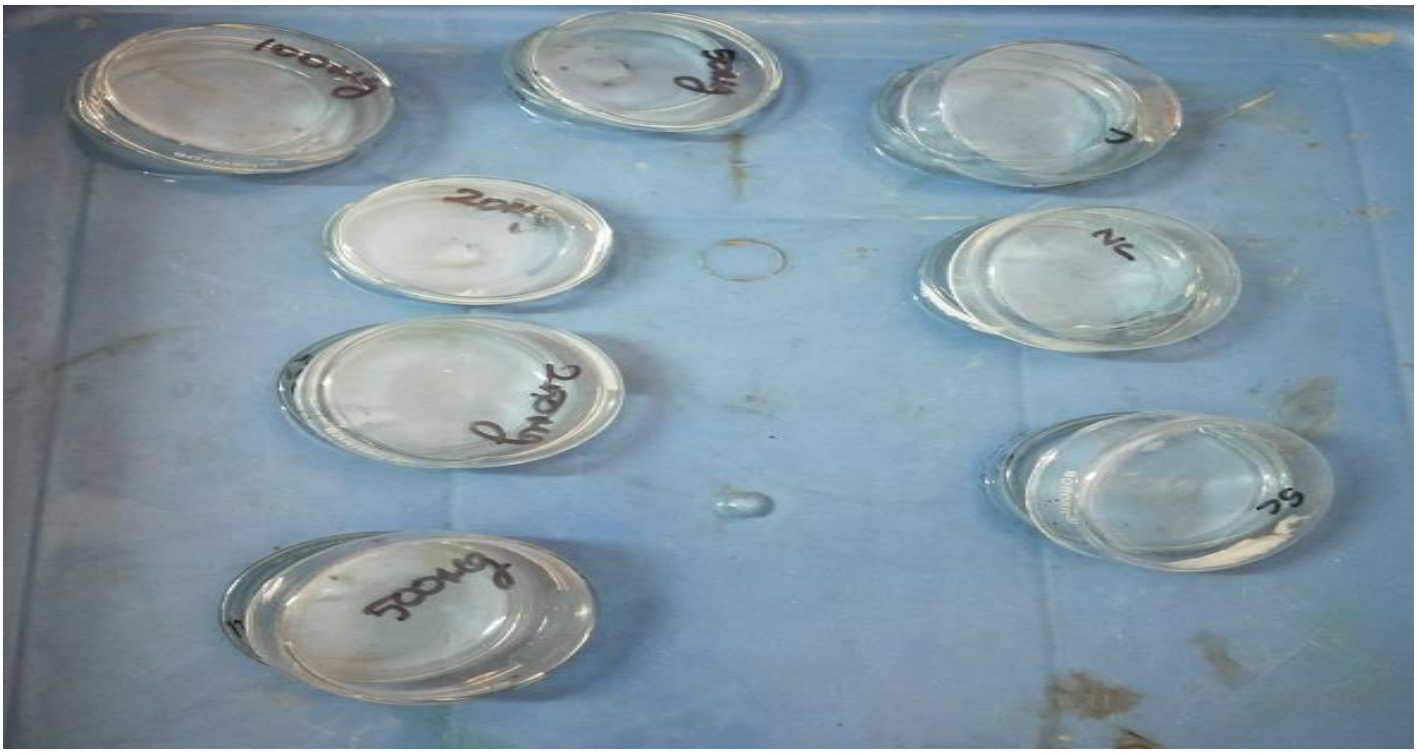
A

B

A. Eye ball with surrounding skeleton of skull

B. lens were removed from the eye balls by dissection of eye

INCUBATION OF ISOLATED GOAT EYE LENS IN THE RESPECTIVE MEDIUM

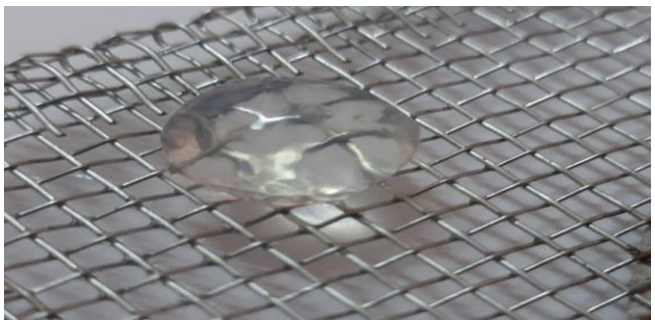


SELECTED DOSE

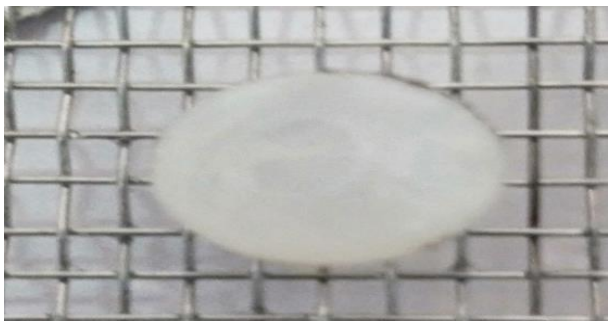
1.Control	Aqueous humour only (glucose 5.5mM)
2.Negative control (toxic control)	Aqueous humour with high concentration of glucose (55mM)
3.Standard control	Negative control with 5mg of enalapril
4.20 µg dose	Negative control with 20 µg dose of EEBML
5.50 µg dose	Negative control with 20 µg dose of EEBML
6.100 µg dose	Negative control with 20 µg dose of EEBML
7.250 µg dose	Negative control with 20 µg dose of EEBML
8.500 µg dose	Negative control with 20 µg dose of EEBML

EVALUATION OF ANTI- CATARACT ACTIVITY OF ISOLATED GOAT EYE LENS BY PHOTOGRAPHIC EVALUATION

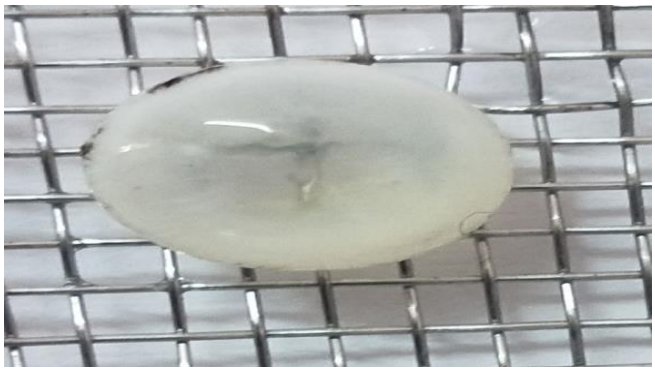
(1) Control



(2) Negative Control



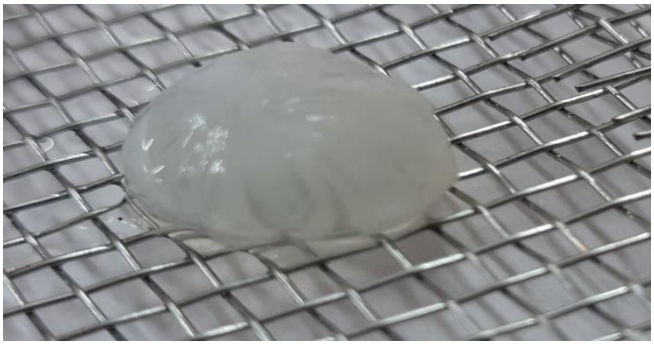
(3).Standard control



(4).20µgdose

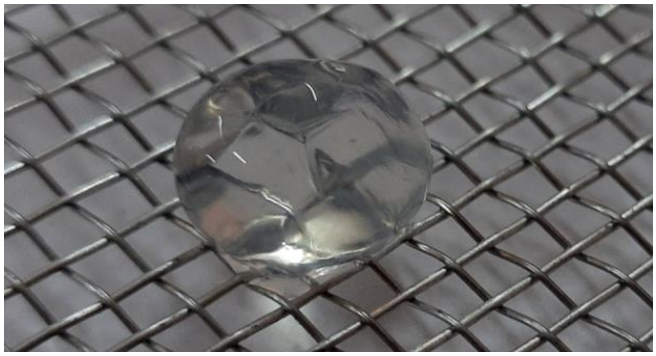
5.50 µg dose

6.100 µg dose



7.250 µg dose

8.500 µg dose



Lens opacity was graded by numbers of squares clearly visible through lens

Table

S.No	Grading of lens	Groups
1)	**0**	normal control (glucose 5.5mM)
		100 µg dose of EEBML in glucose 55mM
		250 µg dose of EEBML in glucose 55mM
		500 µg dose of EEBML in glucose 55mM
2)	+	Standard control in glucose 55mM
3)	++	50 µg dose of EEBML in glucose 55mM
4)	+++	Negative control & 20 µg dose of EEBML in glucose 55mM

Discussion:

The results of the evaluation of anti-cataract activity using the goat eye lens method are as follows: aqueous humour control; excellent anti-cataract results for 100, 250, and 500µg of EEBML; and moderate anti-cataract results for 50µg. 20µg failed to demonstrate the inhibitory activity. The lens's well-formed cataract is visible in the negative control. The development of cataract was actively inhibited by *Blepharis maderaspatensis* in doses of 100, 250, and 500µg in this study.

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1. Kumar, Manish. “In Vitro Anticataract Activity of Zingiber officinale on Goat Lenses.” *International Journal of Pharmaceutical & Biological Archive* 2 (2011): n. pag.

2. In-Vitro Anticataract Activity Of Hibiscus Rosa-Sinensis Linn On Goat Lens SAHID AZIZ[1]*, MAHANJIT KONWAR[2], SWARNAMONI DAS[3] Int J Pharm Pharm Sci, Vol 7, Issue 5, 334-336

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