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ANTICATARACT POTENTIAL OF COCCINIA GRANDIS LINN. VOIGT: AN IN-VITRO AND IN-VIVO ANALYSIS

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ABSTRACT: Cataracts are one of the primary causes of blindness worldwide. Pharmacological methods for preventing and treating cataracts are scarce and centered on surgery. Thus, this study was designed to evaluate the anticataract potential of Coccinia grandis Linn. Voigt using in-vitro and in-vivo models. The EE and FSJ of C. grandis were investigated for protecting goat lenses from glucose-induced cataractogenesis, as well as naphthalene-induced cataracts in Albino rats. Phytochemical screening of C. grandis contained alkaloids, flavonoids, carbohydrates, phytosterols, proteins, and tannins, therefore indicating the antioxidant and potential medicinal properties of the extract. In the in-vitro model, EE (100 µg/ml) and FSJ (10 ml) resulted in a marked reduction in glucose-induced lens opacity. The treatment restored enzymatic antioxidants like superoxide dismutase and catalase, and the nonenzymatic antioxidants reduced glutathione, which indicated less oxidative damage. In the in-vivo model of naphthalene-induced cataracts, rats treated with EE at 200 mg/kg and FSJ at 10 ml/kg exhibited significant protection against cataract formation. Slit-lamp biomicroscopy revealed a decrease in opacification of the lens, and biochemical assays showed development in the levels of antioxidant enzymes and a decrease in lipid peroxidation. EE was proved to be significantly more effective than FSJ in both models and its activity was comparable to the standard drug-Vitamin E. This study concludes that C. grandis can circumvent oxidative stress, lipid peroxide, and protein aggregation determinants of cataractogenesis. It proves C. grandis is a promising natural agent to prevent cataracts. Future research should target the isolation of the active constituents and the exploration of mechanisms of action to establish new pharmacological approaches against cataracts.

KEYWORDS: *Coccinia grandis*, cataractogenesis, antioxidants, glucose-induced cataract, naphthalene-induced cataract.

1. INTRODUCTION

India has a long history of traditional medicine. The conventional approach eventually reveals that plants have offered humans a diverse range of patent medications to reduce sickness symptoms [1]. Plants conduct such important and broad services for humans that it is difficult to understand their whole relevance [2]. The Indian Materia Medica has around 2000 natural pharmaceuticals, with approximately 400 of mineral and animal origin. These resources help develop medical formulations, nutraceuticals, and cosmeceuticals to meet current needs [3]. C. grandis has been reported to be an anti-diabetic, stomachic, anodyne, aperitif, and antioxidant as well as a folk medicine for the prevention of cataracts, skin diseases, and fever. In Indian folk medicine, C. grandis is used as an anti-malarial and as a magic potion in anemic therapy [4]. The plant selected for study is Coccinia grandis Linn. Voigt to confirm its activity on cataracts. It is an opacity within the eye's clear native crystalline lens that causes eyesight to deteriorate over time. The WHO estimates that in 1990, out of the thirty-eight million blind persons globally, cataracts accounted for 41.8, about sixteen million [5].

Age-related cataracts are the most frequent kind of cataract in adults, commencing around the ages of 45 and 50. In children, causes are generally hereditary and metabolic. In adults, the most prevalent kind is age-related cataracts, which appear between the ages of 45 and 50. In youngsters, genetic and metabolic reasons are common [6]. Vision can become fuzzy or hazy. Colors may appear different; for example, people may experience difficulty with glare from the sun, which becomes a major problem when driving at night as well as from lamps. Most patients with cataracts, if untreated, will eventually develop profound disabling visual losses [7]. New eyeglasses, improved illumination, anti-reflective sunglasses, or lenses that magnify can all improve the symptoms of early cataracts. If these approaches are ineffective, the operation is the only viable option. Supplementation with a prospective cataract preventive agent is foreseen as an additional therapy to assist protect vision, and further clinical trials are necessary to establish the profitability of pharmaceutical interventions in decreasing the possibility of cataract formation [8].

2. MATERIAL AND METHODS

2.1 COLLECTION AND AUTHENTICATION OF PLANTS

Fresh and healthy stem parts of C. grandis were obtained in August 2012 from the metropolitan districts of Chennai, and Tamil Nadu, India. Dr. P. Jayaraman, Ph.D., Director of the Plant

Anatomy Research Centre in Chennai, Tamil Nadu, India, identified and taxonomically verified the stem. The certificate's unique register number is PARC/2013/1473.

2.2 PREPARATION OF ETHANOLIC EXTRACT

Plant material was dried at room temperature in a well-ventilated area with uniform distribution of the material. Following comminution, approximately 500 grams of air-dried and powdered C. grandis stem were extracted with ethanol using a cold maceration procedure for 7 days with repeated shakings in tightly sealed jars at room temperature. The extract was concentrated at 50°C after filtering it through a cotton cloth. The leftovers were then vacuum-dried and stored in a desiccator for use in the future. Ethanolic extract yielded 14.6% w/w. Two hours before the experiment, fresh stem juice was prepared [9] [10].

2.3 PRELIMINARY PHYTOCHEMICAL STUDIES

A preliminary phytochemical examination of C. grandis EE and FSJ was performed to detect the presence of flavonoids, alkaloids, phytosterols, carbohydrates, proteins, and tannin components.

2.4 EXPERIMENTAL ANIMALS:

Albino rats weighing between 120 and 150 grams were used in these tests. The purchase animals were weighed, assigned a number and kept in cages at a standard room humidity and temperature of 55 ± 5%. During the research, rats were fed a pelleted diet from the Poultry Experimental Station at Nandanam, Chennai-35, and provided access to potable water at any time. Before beginning the experiment, the animals had been adjusted to laboratory settings. The research was conducted according to the ethical principles authorized by the Ministry of Social Justice and Empowerment, Government of India, and the Institutional Animal Ethics Committee Guide. (Approval No: AJ/ IAEC/ 12/ 01).

2.5 *IN-VITRO* GLUCOSE-INDUCED CATARACT

Within two hours of the goat's slaughter, new eyes were obtained from a nearby slaughterhouse in Tambaram, Chennai. The samples were preserved with synthetic aqueous humor at 0 - 4 C̊ and transported to the testing facility using an ice box. Extracapsular extraction is a surgical technique that separates the lenses from every eyeball without damaging them. Isolated lenses were cultured with synthetic aqueous humor at 37°C and pH set to a rate of 7. for seventy-two hours. The antibiotic penicillin G 32mg and streptomycin 250mg were employed as

preservatives to avoid bacterial contamination. A glucose level of fifty-five mM was used to create cataracts [10] [11] [12].

30 goat lenses were utilized, separated into five groups. Each set has 6 lenses.

Group I: Solvent control - Artificial aqueous humor

Group II: Negative control - Glucose 55mM

Group III: Test I - C. grandis Ethanolic extract (100µg/ml) + Glucose 55mM

Group IV: Test II - C. grandis Fresh stem juice (10ml) + Glucose 55mM

Group V: Standard control - Vitamin E(100µg/ml) + Glucose 55mM

As the last stage in the experiment, all of the incubation lenses were withdrawn from the media and rolled on filter paper to remove any excess medium that had adhered to nonlens tissue and vitreous humor.

1. Photographic Investigation

A patterned square mesh was used to check the measure of lens opacity by placing each lens on the square mesh in such a way that the posterior surface touched the pattern. The opacity of the lenses was observed by several squares visible through the lens.

- **Lens Homogenate preparation**

After incubation, the lenses were homogenized with 10 volumes of a pH 7.0 20mM potassium phosphate buffer solution before being centrifuged at 10,000g for 1 hour. The supernatant was collected using a micropipette and used to calculate biochemical parameters such as enzymatic and non-enzymatic antioxidants [13].

2. Assessment of Biochemical parameters:

- **Estimating total protein content.**

Allow 4.0 mL of alkaline copper solution to stand in 0.1 mL of lens homogenate for 10 minutes. Then, 0.4 ml of phenol solution was quickly added and stirred before incubating at room temperature for 30 minutes to obtain the hue. The V-visible spectrophotometer recorded values

at 610 nm against a distilled water blank. The protein content was calculated using a standard curve using albumin from bovine serum and shown as µg/mg lens tissue [14].

• Calculating lipid hydroperoxide levels

Incubate 0.1 mL of lens homogenate in 0.9 mL of Fox reagent for three minutes at room temperature. A colorimeter was used to measure the color generated at 560 nm. The findings are shown as moles per mg lens protein [15].

3. Enzymatic antioxidant analysis

• Catalase assay

The reaction mixture comprises 2.0 mL of homogenate and 1 mL of 30 mM hydrogen peroxide. The control system did not contain the substrate. The reaction was started by adding the substrate, and the decrease in absorbance was measured at 240 nm for thirty seconds at 25°C. The activity was estimated by dividing the absorbance variance per unit time, and three such tests were performed. A unit is the quantity of enzyme required to break down one milligram of hydrogen peroxide per minute at a pH of 7.0 and 25 degrees Celsius [16].

• Superoxide dismutase assay.

The test mixture consisted of 1.2 ml sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml PMS, 0.3 ml NBT, 0.2 ml NADH, 1.0 ml homogenate, and 3.0 ml distilled water. The reaction was begun by adding NADH and then incubated for 1 minute at 30°C. The reaction was halted by adding 1.0 ml of glacial acetic acid, and the liquid was violently agitated. After adding 4.0 mL of n-butanol, the liquid was shaken thoroughly. After standing for 10 minutes, the mixture was processed, the butanol layer was removed, and the absorbance at 560 nm was compared to a butanol blank. A system without enzymes acted as the control, and three parallel tests were undertaken [17].

4. Non-enzymatic antioxidant determination:

• Estimating reduced glutathione levels.

On ice, lenses were homogenized with a 10% (w/v) cold 20 mM EDTA solution. After deproteinizing with 5% TCA, a supernatant aliquot was permitted for reaction with 150 µM DTNB. The product was identified and measured using a spectrophotometer at 416 nm. The

calibration curve was established using pure GSH as the standard, and three parallel tests were carried out [18].

2.6 STATISTICAL EVALUATION:

The usual evaluation was carried out using one-way ANOVA followed by the Student's t-test. Statistical significance was defined as $P < 0.05$.

2.7 NAPHTHALENE INDUCED CATARACTOGENESIS

Number of animals: The study comprised 30 Albino rats of weight 100- 120grams

Number of groups: Five groups consisting of 6 animals each.

Duration of study: 28 days

Standard drug: Vitamin E

Animal grouping:

Group I: Normal control has been Served as Liquid paraffin 10ml/kg bodyweight p.o.

Group II: Cataract control Served as Naphthalene 1g/kg body weight p. o

Group III: Test I Served as Ethanolic extract of *C. grandis* 200mg/kg body weight + Naphthalene p.o

Group IV: Test II Served as Fresh stem juice of *C. grandis* 10ml/kg body weight + Naphthalene p. o

Group V: Standard control Served as Vitamin E 50mg/kg body weight + Naphthalene p. o

The lens was examined regularly throughout the trial, twice a week for the first two weeks and then weekly after that. The pupil was examined using a torchlight after being dilated with one drop of 1% Tropicamide to look for morphological alterations in the lens.

(I) Slit lamp Examination:

Later the experiment is terminated, on the 28th day all the animals were taken for eye examination for lens opacification using a slit lamp biomicroscope and photographs were taken. A drop of 1% Tropicamide was used as mydriatic.

The depth of cataract developed was graded according to below mentioned dissimilar stages,

Stage One: Clear normal lens, Stage Two: peripheral vesicles, Stage Three: Peripheral vesicles and cortical opacities, Stage Four: diffuse central opacities, Stage Five: mature cataract

The inclusion of cataracts was regarded as when the red cornea reflex was completely undetectable from any portion of the lens and looked plain white to the bare eye [19].

(II) The percentage incidence of cataracts and the opacity index were computed using the formula below.

The incidence of cataracts was determined using:

$$\% \text{ Incidence} = \frac{\text{Number of animal in each stage}}{\text{Total number of animals}} X\ 100$$

The Opacity Index was derived by:

$$\text{Opacity Index} = \frac{\text{Number of eyes in each stage } X \text{ Stage of the eye}}{\text{Total number of eye}}$$

(III) Preparation of Lens Homogenate:

Animals were sacrificed with thiopental sodium phosphate at a dosage of 40mg/kg body weight i.p. Eye lenses were gently eliminated using sterile surgical equipment and cleaned with ice-cold saline. Each pair of lenses was homogenized using a glass homogenizer and a Teflon pestle in 1.2ml of ice-cold phosphate buffer (20 mM, pH 7.4). The homogenate was subsequently spun down at 10,000g for 1 hour, and the supernatant obtained using a micropipette was utilized for the assessment of biochemical parameters, enzymatic and non-enzymatic antioxidants [20].

3. RESULTS.

3.1 PRELIMINARY PHYTOCHEMICAL ANALYSES.

Table 1: Preliminary Phytochemical Analysis of EE (Ethanolic Extract) and FSJ (Fresh juice) of *Coccinia grandis* Linn. Voigt

S.NO	PHYTOCHEMICAL TEST	EE	FSJ
01	**FLAVONOIDS** a. Shinoda test b. Conc. Sulphuric acid test	+	+
02	**PHYTOSTEROLS**	+	+
03	**GLYCOSIDES**	-	-
04	**CARBOHYDRATES**	+	+
05	**PROTEINS** a. Xanthoprotein test b. Millon’s test	+	+
06	**ALKALOIDS**	+	+

07	**TANNIN** a. Ferric chloride test b. Gelatin test	+	+
08	**SAPONINS** a. Foam test	-	-

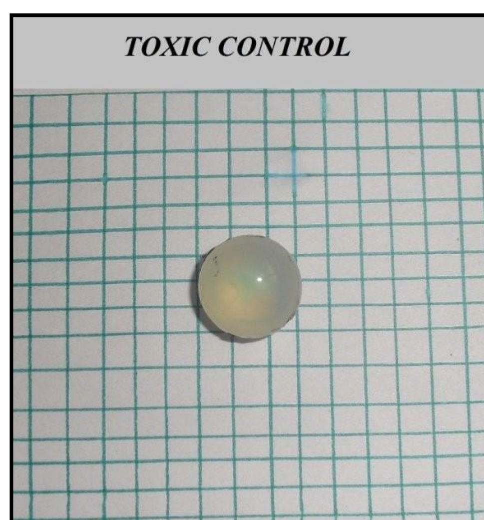
(+) indicates the presence of phytoconstituent

(-) Indicates absence of phytoconstituent

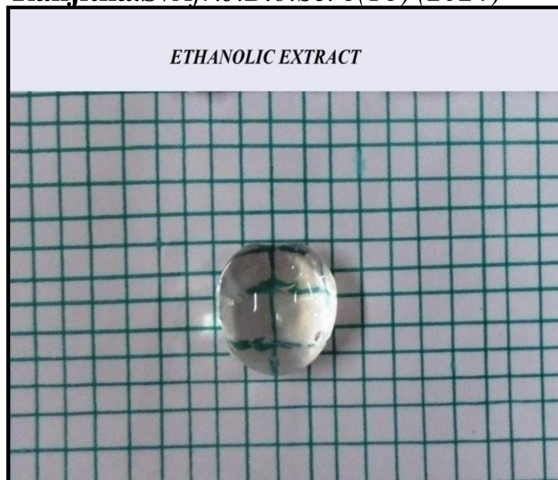
Preliminary phytochemical analysis of EE and FSJ of *C. grandis* has identified the presence of alkaloids, carbohydrates, phytosterols, flavonoids, proteins, and tannin compounds. The preliminary phytochemical analysis reveals that the possible action of *C. grandis* may be due to flavanol glucoside content. Thus, the observed anticataract and antipruritic activity may be attributed to the presence of flavanol glucoside. The phytochemical results are concluded in (Table 1).

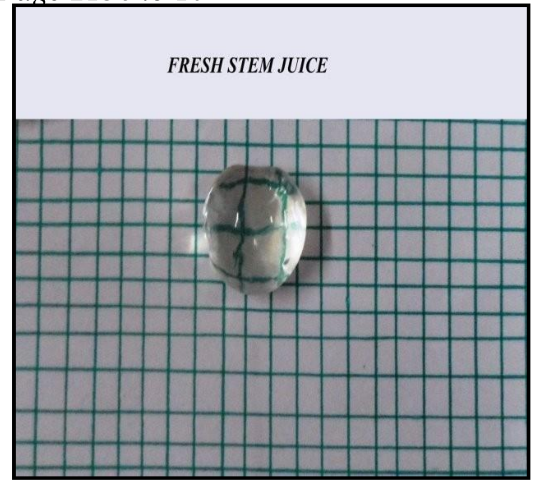
3.2 *IN-VITRO* GLUCOSE-INDUCED CATARACT

(I) Photographic Examination

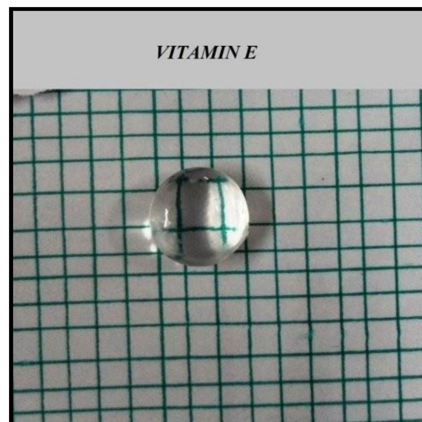


(a) **(b)**





(c) **(d)**



(e)

Fig 1: Normal and experimental goat lenses were treated with glucose.

After 72 hours of incubation in glucose 55 mM, the lens became entirely opaque, resulting in a loss of transparency and the absence of squares (Fig. 1 b), whereas lenses incubated in normal control glucose at 5.5 mM revealed clear transparency and the presence of squares (Fig. 1 a). Incubating lens with 100 µg/ml of EE (Fig. 1 c) and 10ml of FSJ of C. grandis (Fig. 1 d) appears to retard the progression of lens opacification, as the lens appears slightly hazy and more squares are visible, indicating the suppression of cataract formation when compared to lenses incubated in glucose 55 mM (negative control, Fig. 1b).At a dosage of 100µg/ml, vitamin E significantly slows the course of lens opacification (Fig. 1 e), which is close to normality when compared to the negative control (Fig. 1 b).

Table 2: Effect of EE and FSJ of *C. grandis* on *in-vitro* goat lens homogenate SOD, catalase, protein, GSH, and LH in control and experimental groups.

GROUP	Superoxide Dismutase (mmoles/min/mg protein)	Catalase (mmoles/min/mg protein)	Total protein (mg/g tissue)	Reduced Glutathione (mmoles/min/mg protein)	Lipid peroxidation (mmoles/min/ mg protein)
Group I (Normal control)	4.500 ± 0.06325 a***	8.333 ± 0.3777 a***	133.1 ± 0.7259 a***	2.275 ± 0.2835 a***	0.1783 ± 0.08773 a**
Group II (Negative control 55 Mm)	2.217 ± 0.3545	3.467 ± 0.4274	80.18 ± 0.8830	0.9045 ± 0.07545	0.9370 ± 0.07828
Group III (EE-100µg/ml)	3.300 ± 0.2191 b***	6.417 ± 0.3656 b***	89.13 ± 0.7706 b***	1.649 ± 0.1438 b**	0.2850 ± 0.1134 b*
Group IV (FSJ-10ml/kg)	3.700 ± 0.2000 c***	6.383 ± 0.4875 c***	95.45 ± 0.6060 c***	1.873 ± 0.2085 c***	0.2483 ± 0.06940 c*
Group V	4.400 ± 0.2757	7.817 ± 0.2137	104.1 ± 0.7259	2.235 ± 0.2796	0.1583 ±

Standard control (Vitamin E 100µg/ml)	d***	d***	d***	d***	0.05193 d**

The values are provided as mean ± SEM from six animals in each group. Group II compared. Group I (a), Group II vs. Group III (c), Group II vs. Group IV (d), and Group II vs. Group V are the comparison groups. Significance: *** p<0.001, ** p<0.01, *p<0.05, ns-Not significant.

1. Biochemical Parameter:

Group II had significantly lower levels of lens protein and LH ($P < 0.005$) in comparison to Group I (Table 6). C. grandis treatment (groups III and IV) significantly increased lens protein and lipid peroxides ($P < 0.05$) compared to group II. Vitamin E (100 µg/ml, $P < 0.05$) also increased total protein and lipid peroxides.

2. Enzymatic antioxidants

- **Lens Catalase level**: Incubating the lens with 55 mM glucose resulted in enzyme inactivation. Group II showed substantially decreased lens catalase activity (Table 2) when compared with EE and FSJ-treated groups at dosages of 100 µg/ml and 10 ml, respectively. Vitamin E therapy (100 µg/ml) brought the levels within range of the control values.
- **SOD Level:** After 72 hours of incubation with 55 mM glucose, the level of enzymatic antioxidant superoxide dismutase in goat eye lens homogenate decreased significantly ($P<0.01$) compared to the control group. Incubation with EE and FSJ at dosages of 100 µg/ml and 10 ml, respectively, and Vitamin E (100 µg/ml) concurrently with 55 Mm glucose considerably ($P<0.01$) recovered the levels of SOD, which were practically identical to the control group. (See Table 2)

3. Non-enzymatic antioxidants

Reduced glutathione level: Group II had considerably lower glutathione levels ($P < 0.005$) compared to the normal control group I (Table 6). C. grandis EE and FSJ at 100 µg/ml and 10 ml doses significantly increased ($P < 0.05$) lens glutathione levels compared

to group II. Vitamin E (100 µg/ml, $P<0.05$) combined with 55 mM glucose substantially ($P<0.01$) restored lowered glutathione levels002E

3.3 *IN-VIVO NAPHTHALENE-INDUCED CATARACTOGENESIS*

(I) Slit lamp examination

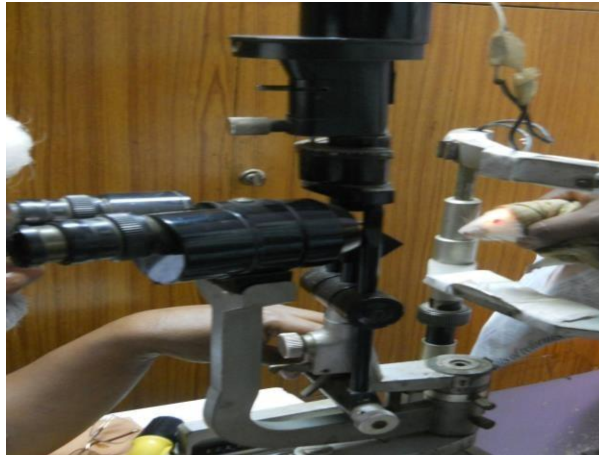
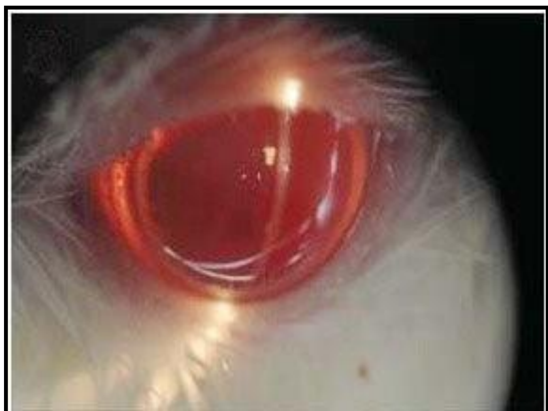
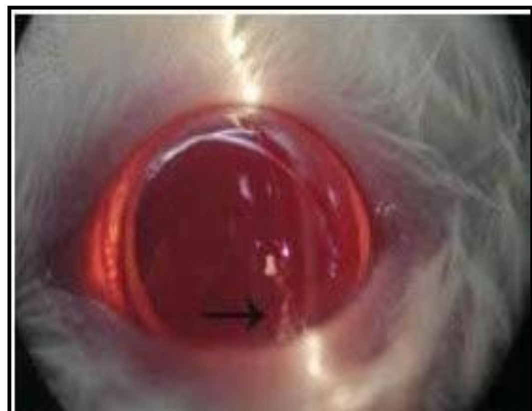
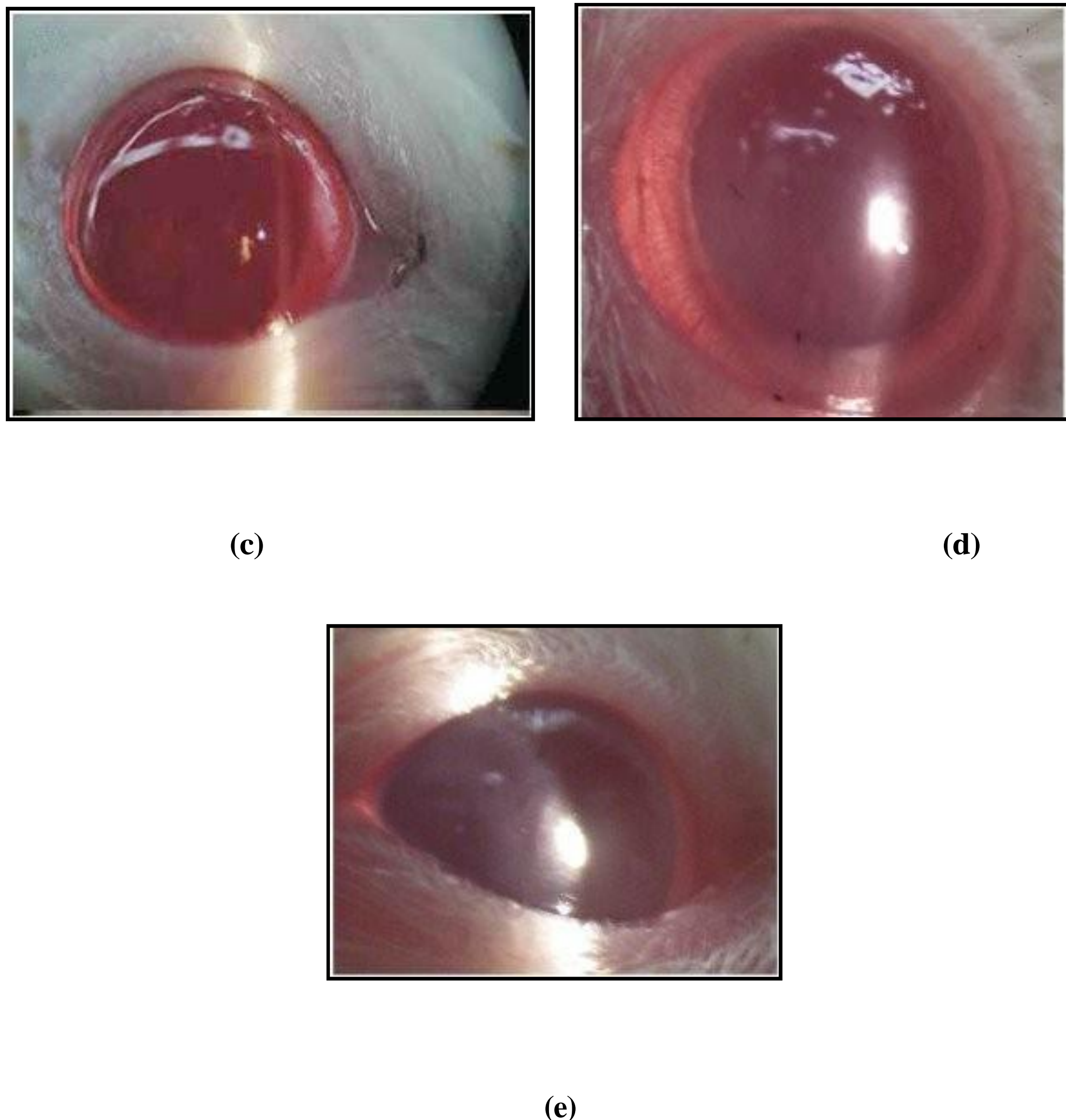


Fig 2: SLIT-LAMP EVALUATION OF CATARAC





(a) **(b)**



(c) **(d)**

(e)

Fig 3: SLIT-LAMP BIOMICROSCPE PHOTOGRAPHY OF RAT EYE

Slit lamp examination of normal control animals that received only vehicle revealed a clear crystalline lens with normal light reflection (Fig 3 a), whereas in group II rats that received naphthalene, slit lamp examination revealed anterior subcapsular, white opacities along with nuclear sclerosis (Fig 3 b), indicating a Stage 3 development of cataract. Concomitant dosing of 200 mg/kg of C. grandis EE with naphthalene significantly improved the observed alterations in group 2. Slit lamp examination showed a clear lens **(Fig 3 c)**. In group IV rats which received 10 ml/kg FSJ of *C. grandis* along with naphthalene showed a clear crystalline lens almost with normal light reflection **(Fig 3 d)**. Treatment with the standard drug, vitamin

E 50 mg/kg showed that there were no diffuse central opacities hence a clear crystalline lens with normal light reflection was observed **(Fig 3 e)**.

(II) The percentage incidence of cataracts and the opacity index were computed using the formula below.

Table 3: Effect of EE and FSJ of *C. grandis* on incidence (%) of cataracts on the 28th day

GROUPS	CATARACT INCIDENCE (%)				
	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**
Group I (Normal control)	100	0	0	0	0
Group II (Cataract control 1mg/kg)	0	0	0	66.66	33.33
Group III (EE 200mg/kg)	33.33	50	16.66	0	0
Group IV (FSJ-10ml/kg)	0	66.66	33.33	0	0
Group V (Vitamin E 50mg/kg)	66.66	33.33	0	0	0

On the 28th day of therapy, animals treated with naphthalene exhibited varied degrees of cataract genic alterations, with around 66.6% in stage 4 and 33.3% in stage 5 mature cataracts. On the 28th day, none of the animals in the EE or FSJ treatment groups developed mature stage 5 cataracts. The EE treatment revealed 33.33%, 50%, and 16.6% of animals in stage 1, stage 2, and stage 3 cataracts, respectively, whereas the FSJ exhibited 66.6% in stage 2 and 33.33% in stage 3 mature cataracts. Treatment with the conventional treatment, vitamin E, revealed 66.6% of animals in stage 1 and 33.3% of animals in stage 2. Additionally, there were no diffuse center opacities in stage 4 and full cataracts in stage 5 (Table 3).

Table 4: Effect of ethanolic extract and fresh juice of *C. grandis* on opacity index in control and experimental animals on the 28th day.

GROUPS	OPACITY INDEX ON 28th day
Group I (Normal control)	1
Group II (Cataract control 1mg/kg)	4.26
Group III (EE 200mg/kg)	1.83
Group IV (FSJ-10ml/kg)	2.3
Group V (Vitamin E 50mg/kg	1.32

On the 28th day of treatment with naphthalene, the opacity index increased due to full opacification, reaching 4.16. When compared to the naphthalene control group, the groups treated with EE and FSJ of C. grandis had an opacity index drop of 1.83 and 2.3, respectively.

The vitamin E-treated group had a significantly lower opacity index (1.83) than the experimental groups (Table 4).

(III) Preparation of Lens Homogenate:

Table 5: Effect of EE and FSJ of *C. grandis* on *in-vivo* rat lens homogenate SOD, catalase, protein, GSH, and LH in control and experimental groups.

GROUP	Superoxide Dismutase (mmoles/min/mg protein)	Catalase (mmoles/min/mg protein)	Total protein (mg/g tissue)	Reduced Glutathione (mmoles/min/ mg protein)	Lipid peroxidation (mmoles/ min/ mg protein)
Group I (Normal control)	3.8 ± 0.4858 a***	2.105 ± 0.3833 a***	103.4± 0.4622 a***	3.478 ± 0.3394 a***	0.1572 ± 0.03970 a**
Group II (Cataract control 1mg/kg)	1.66 ± 0.3670	0.3923± 0.07930	56.50± 0.6191	1.487 ± 0.3953	1.012 ± 0.09390
Group III	2.333 ± 0.2805	1.148± 0.2069	83.59± 0.4432	2.238 ± 0.2088	0.0865 ±

(EE 200mg/kg)	b**	b***	b***	b**	0.01007 b*
Group IV (FSJ-10ml/kg)	2.1 ± 0.2429 c**	0.9333± 0.08165 c*	60.3± 0.4195 c***	3.312± 0.2569 c***	0.2183 ± 0.06706 c*
Group V (Vitamin E 50mg/kg)	3.317 ± 0.4446 d***	1.271± 0.2325 d***	96.29± 0.4195 d***	3.370± 0.4231 d***	0.06067 ± 0.006532 d**

The values are provided as mean ± SEM from six animals in each group. Group II compared. Group I (a), Group II vs. Group III (c), Group II vs. Group IV (d), and Group II vs. Group V are the comparison groups. Significance: *** p<0.001, ** p<0.01, * p<0.05, ns-not significant.

Table 6: The effect of C. grandis EE and FSJ on blood SOD, catalase, protein, GSH, and LH levels in the control and experimental groups.

GROUP	Total protein (mg/g tissue)	Lipid peroxidation (mmoles/min/mg protein)
Group I (Normal control)	86.56 ± 1.635a***	0.3193 ± 0.07328 a*
Group II (Cataract	50.45 ± 0.3722	1.287 ± 0.1337

control 1mg/kg)		
Group III (EE 200mg/kg)	80.59 ± 0.6318 b***	0.07183 ± 0.00775 b**
Group IV (FSJ-10ml/kg)	60.37 ± 0.3931 c***	0.8778 ± 0.1109 c*
Group V (Vitamin E 50mg/kg)	81.29 ± 0.7392 d***	0.02633 ± 0.0116 d**

The values are provided as mean ± SEM from six animals in each group. Group II compared. Group I (a), Group II vs. Group III (c), Group II vs. Group IV (d), and Group II vs. Group V are the comparison groups. Significance: *** $p<0.001$, ** $p<0.01$, *$p<0.05$, ns-Not significant.

- **Antioxidant characteristics, both enzymatic and non-enzymatic**

When compared to the normal control, administering 1mg/kg/day of naphthalene to group II animals for 28 days lowered the amounts of enzymatic antioxidants such as superoxide dismutase and catalase, as well as the non-enzymatic antioxidant glutathione in lenses homogenate. Combining C. grandis EE and FSH at 200 mg/kg and Vitamin E at 50 mg/kg with naphthalene resulted in considerable ($P<0.01$) restoration of both enzymatic and nonenzymatic antioxidant enzymes, equivalent to the control group (Table 5). Among the groups studied, the conventional vitamin E produced the highest level of activity.

- **Biochemical parameter**

Naphthalene-treated animals (Group II) had significantly lower lens protein levels ($P<0.001$) compared to the control group (Group I). Administration of C. grandis EE at a dosage of 200 mg/kg/day p.o. (Group III) and FSJ at a dose of 10 ml/kg/day p.o. resulted in a substantial increase ($P<0.001$) in lens protein compared to the toxic control. Vitamin E at a dosage of 50mg/kg/day p.o. (Group V) produced protein levels comparable to the control (Table 5). LH levels were observed to be higher in naphthalene-treated animal lenses than in group I,

which received just vehicle. Lenses treated with C. grandis EE and FSJ showed considerably lower LH levels ($P < 0.05$) than the negative control group. While the Vitamin E-treated group recovered LH levels to those of the normal control group (Table 5).

- **Serum parameter**

Naphthalene treatment significantly decreased total protein levels and increased lipid peroxidation in the lens compared to the control group ($p<0.01$). Using EE, FSJ of C. grandis, and vitamin E at doses of 200 mg/kg, 10 ml/kg, and 50 mg/kg with naphthalene for 28 days resulted in a substantial decrease ($p<0.01$) in lipid peroxidation and an increase in overall protein content. (Tab 6).

4. DISCUSSION

There is currently no definite pharmaceutical treatment for cataracts, and surgery is the only option for patients with severe cataracts. The limits of cataract surgery have prompted experimental cataract research, mostly aimed at cataract prevention. Plant and plant products are being studied more frequently due to their organic origins and relative safety. It is expected that delaying cataract development by ten years might cut the requirement for cataract surgery in half.As a result, using C.grandis to prevent cataract development is worth investigating further. In the current study, cataract was caused by incubation with 55mM glucose in goat lens and oral administration of naphthalene to rats for 28 days. Naphthalene-induced cataracts are commonly used as models for human senile cataract. Prolonged exposure to high glucose levels induces both acute reversible alterations in cellular metabolism and long-term irreversible changes in stable macromolecules.High glucose concentrations may cause and accelerate lens opacification, resulting to cataract development, via non-enzymatic glycation, oxidative stress, or the polyol pathway. High glucose concentrations contribute to oxidative stress by increasing intracellular glucose metabolism, which produces more reactive oxygen compounds at the mitochondria level.High reactive oxygen species activates the polyol pathway by boosting aldose reductase, resulting in high sorbitol levels. Sorbitol cannot easily pass through membranes and develops in cells, causing harm by disrupting osmotic balance. This intralenticular polyol buildup is a significant contributor in acute sugar cataract models.

The present study investigated lens opacity to distinguish between control and experiment lenses. Incubation with high concentrations of glucose causes a variety of metabolic alterations

that ultimately contribute to cataract development. However, the groups incubation with EE and FSJ reverted the lens opacity to a degree similar to the standard medication, vitamin E.

Lipid peroxidation is an autocatalytic reaction that causes cell death. MDA and LH, hazardous chemicals produced by lipid peroxidation, have been linked to cataractogenesis, owing to their cross-linking capabilities. MDA and LH levels were found to be elevated in glucose-induced cataractous goat lenses in this investigation. These effects were negated by incubating extraction and fresh juice with glucose at the same time. This impact was comparable to that of the vitamin E-treated group. Treatment with 55 mM of glucose significantly lowered the amounts of both enzymatic and non-enzymatic antioxidants in goat eye lenses. Incubation with C. grandis extracts and fresh juice, as well as vitamin E, significantly enhanced all of the antioxidant levels evaluated. When compared to FSJ, the results demonstrated that EE considerably raised the levels of antioxidants and biochemical markers. The lens produces several types of harmful oxygen species, including the superoxide anion and lipid hydroperoxides. This rise has been considered a key cause of oxidative damage. Thus, *in vitro*, antioxidant enzymes such as SOD, CAT, and the nonenzymatic antioxidant enzyme GSH were determined. catalyzes the breakdown of H2O2 into water and oxygen, shielding the cell from damage from oxidation caused by H2O2 and hydroxyl radicals. The first antioxidant enzyme is superoxide dismutase, which suppresses the oxygen radical. GSH, a non-enzymatic antioxidant, shields cells from free radicals, peroxides, and many other harmful chemicals. Indeed, GSH depletion enhances cell susceptibility to multiple aggressions while also having metabolic impacts.

In the naphthalene-induced cataract model, ingested naphthalene goes through oxidation in the liver to an epoxide, which is then hydrolysed to ND. The oxidative breakdown of ND to NQ in a lens is thought to be contributing to the development of cataracts in rats given naphthalene. NQ is very reactive and rapidly forms covalent connections with a variety of biological thiols, including glutathione, cysteine, and protein thiols. The creation of NQ in the lens is thought to be the primary mechanism underlying the development of naphthalene-induced cataracts. Lens modification of proteins by NQ is thought to be caused by diverse oxidative stresses.In these research, animals treated with naphthalene had higher amounts of total protein and lipid hydroperoxide in their serum and lenses. The EE and FSJ of C. grandis were treated with naphthalene for 28 days, resulting in a substantial decrease in serum lipid hydroperoxide and an increase in total protein levels. C. grandis' ethanolic fractions and fresh juice dramatically

enhanced catalase levels in naphthalene-induced cataractogenesis. SOD and non-enzymatic antioxidants GSH levels in naphthalene-treated groups were lower than in the normal control group.

The ethanol-based extract and freshly squeezed juice of C. grandis dramatically raised the levels of antioxidant enzymes SOD, Catalse, and LH. It defends the cells from free radicals, peroxides, and other harmful substances. When compared to rats treated with naphthalene, C. grandis EE and FSJ boosted GSH levels considerably. When compared to FSJ, the results demonstrated that EE considerably raised the levels of antioxidants and biochemical markers.In naphthalene-fed rats, cataracts are thought to arise as a result of ND oxidation to NQ in the lens. NQ is very reactive, forming covalent connections with a variety of biological thiols including glutathione, cysteine, and protein thiols. The synthesis of NQ in the lens is thought to be the primary mechanism responsible for naphthalene-induced cataract development. C. grandis administration significantly slowed the formation of cataracts in groups III and IV, and slit lamp photography revealed a clean translucent lens with ordinary light reflection after the trial. In animals, it has been shown to boost detoxifying enzymes, limit DNA damage, promote DNA repair, reduce mutations and tumor development, and have antioxidative properties. The presence of phytoconstituents such as flavonoids is thought to facilitate C. grandis's protective action. Because oxidative stress has been proposed as an ordinary fundamental cause of cataractogenesis, enhancing the ocular lens' antioxidant defenses has been proven to prevent cataractogenesis. C. grandis has been demonstrated to reduce oxidative damage and postpone cataract formation.

5. CONCLUSION:

It was concluded from the results of this work that, the Ethanolic extract and Fresh stem juice of *C. grandis* were found to be effective in both cataract development and pruritus.*C. grandis* holds a defense against glucose-induced cataract genesis by *in-vitro* method and supportive in prevention or reducing the development of cataract. The rat model of naphthalene-induced cataract *in-vivo* method revealed various structural changes of cataract in the crystalline lens and concomitant administration of *C. grandis* ethanolic extract and fresh stem juice to a great extent ameliorated the development of cataract. In addition, ethanolic extract shows significant results when compared to fresh stem juice. Additional investigations of plant *Coccinia grandis* Linn. Voigt. on the separation and recognition of the active elements could lead to a thorough knowledge of the processes of cataract development prevention and pruritus protection.

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