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To Evaluation of Anti-cataract Potential of Kojic Acid by using in vitro goat lens model.

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ABSTRACT:

This study includes Kojic Acid has four Oxygen molecules, six Hydrogen molecule, and six Carbon molecule. Goat eyeballs were used in the present study. They were obtained from the slaughterhouse. Glucose at a concentration of 55 mM was used to induce cataracts; glucose (5.5 mM) concentration served as a normal control, and Ascorbic Acid served as a standard control. Incubation of lenses with glucose 55 mM showed opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 72 hrs. Positive control (Ascorbic acid 40µg/ml) - lenses exhibit small opacity; the transparent lens was not discovered. Test 1 (Kojic Acid 2.0 µg/ml) - There is a small amount of opacity in the lenses; no clear lens was discovered. Test 2 (Kojic Acid 2.4 µg/ml) - There is a small amount of opacity in the lenses; no clear lens was discovered. Test 3 (Kojic Acid 2.6 µg/ml) - Zero-degree opacity occurs, and a clear lens is obtained. Test drug inhibits cataractogenesis. Test drug inhibits cataract genesis. This study showed that Kojic Acid-treated groups have been shown to increase the total protein content, retarding the process of cataractogenesis initiated by high glucose concentration. Kojic Acid possesses antioxidant activity; thus, it should have anticataract activity. Therefore, the Kojic Acid drug shows antioxidant activity and the drug shows anticataract activity.

KEYWORDS: Anti-cataract, Kojic Acid, Artificial aqueous humour, In-vitro, goat lens.

INTRODUCTION :

A cataract is a major contributing factor to blindness. It is defined as a clouding of the natural lens, a part of the eye responsible for focusing and producing a clear, sharp image. It is called a “peril of sight” because cataracts have blinded more people throughout the ages than any other affliction of the eye, and opacity forms in the centre of the lens.

A cataract is derived from the Latin word “cataract”, meaning waterfall. (ARN) Age-Related Nuclear Cataract is the most common form of cataract found in people over 45 years and forms in the centre of the lens [(1)].

Cataract is the main cause of loss of vision worldwide. In 2020, an estimated 15 million people older than 50 years were classified as blind (presenting visual acuity <3/60 or less than 10° visual field around the central fixation), and an estimated 79 million people older than 50 years were classified as having moderate to severe visual impairment (presenting visual acuity <6/18 to 3/60) due to cataract worldwide. These estimates show a 30% increase in cataract blindness [(2)].

Signs and symptoms vary depending on the type of cataract, though considerable overlap occurs. People with nuclear sclerotic or brunescent cataracts often notice a reduction in vision. Minor lens opacities are extremely common and rarely interfere substantially with vision. More extensive lens opacities may absorb or deflect the light rays entering the eye, and thus produce a distorted image on the retina. About 85% of cataracts are classified as senile or mature, and a substantial proportion of these are associated with diabetes. Other kinds of cataracts include congenital cataracts, metabolic cataracts such as those associated with galactosemia, endocrinological cataracts associated with hypothyroidism and hypercalcemia, cataracts associated with certain skin diseases such as atopic dermatitis, toxic cataract resulting from medication, traumatic cataract, and after cataract, which is a recurrent growth of lens material after partial removal. Surgery is the only treatment for cataracts.

Various treatments like phacoemulsification, aldose reductase inhibitors, certain antioxidant treatments, and protection against non-enzymatic glycation have been used, but there has been no definite solution for the same [(2)]. Drugs developed to delay or prevent lens opacification have failed to give convincing positive results in clinical trials. This stimulates the research towards the experimental work on cataract to understand all possible pathways and mechanisms that are responsible for the generation of cataract. While the main treatment for cataract is surgical intervention, it is associated with certain risks and subsequent suboptimal outcomes [(1)]. Drugs developed to delay or prevent lens opacification have failed to give convincing positive results in clinical trials. This stimulates the research toward the experimental work on cataract to understand all possible pathways and mechanisms that are responsible for the generation of cataract. While the main treatment for cataract is surgical intervention, it is associated with certain risks and subsequent suboptimal outcomes [3]. The formation of superoxide radicals in the aqueous humour in the lens and its derivatisation to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to opacification. The aldose reductase enzyme also plays an important role in the pathogenesis of cataract [(4)]. Upon literature review, we came across the in vitro safety profile and antioxidant activity of KDP-loaded MEs, which were evaluated. The developed MEs showed a Dnm of approximately 1 micrometre and a ZP of −13mV, and no change was observed in Dnm or ZP of the system with the addition of KDP. KDP-unloaded MEs exhibited “shear thinning” flow behaviour, whereas KDP-loaded MEs exhibited Newtonian behaviour, which is characteristic of antithixotropic materials. The Kojic acid molecule is characterised by the presence of a hydroxypyrone ring, which is responsible for its antioxidant and anti-inflammatory properties. Kojic Acid has been shown to improve oxidative stress and chronic inflammation associated with severe chronic diseases. Kojic Acid is able to effectively counteract oxidative stress and the inflammatory pathways, and these therapeutic targets may be relevant for its impact on patient quality of life. [(5)]. Thus, the present study is planned to evaluate the antioxidant and anti-inflammatory activity of Kojic Acid.

MATERIALS AND METHODS:

Kojic Acid was received as a gift sample from Dolphine Pharmacy Instrument PVT LTD., Mumbai, Maharashtra, India. All other chemicals were of analytical grade purchased from local suppliers.

Experimental procedure:

a) Collection of eyeballs:

Goat eyeballs used for the study were collected from the local slaughterhouse and stored at 0-40^{0}C [6].

b) Lens Culture:

Artificial aqueous humour is used for anti-cataract activity. Aqueous humour contains (NaCl 140 mM, KCl 5mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, $NaHPO_4$ 0.5mM, $CaCl_2$ 0.4 mM, and glucose 5.5 mM) at room temperature and maintains pH 7.4 by the addition of $NaHCO_3$. Penicillin G and streptomycin 250 mg were added for the prevention of bacterial growth [7].

c) Cataract formation:

Glucose in a concentration of 55 mM was used to induce cataracts. Glucose in the lens is metabolised through the sorbitol pathway and accumulation of polyols (sugar alcohols), causing overhydration and oxidative stress. This led to cataractogenesis. A total of 24 lenses were used for the study. These lenses were incubated in artificial aqueous humour with different concentrations of glucose (5.5 mM served as normal control, and 55 mM served as toxic control) for 72 hours [8].

d) Study design and groups:

Goat lenses were divided into six groups of six lenses each, following the Table.

Table 1. Treatment group.

Group No	Group name	Treatment	Drug dose
1	Negative Control	aqueous humor + glucose 55Mm	-
2	Normal Control	aqueous humor + glucose 5.5mM	-
3	Standard Control	aqueous humor + glucose 55mM +Ascorbic acid.	40 µg/ml)
4	Test Group 1	aqueous humor + glucose 55mM + Kojic Acid	2.0 µg/ml
5	Test Group 2	aqueous humor + glucose 55mM + Kojic Acid	2.4 µg/ml
6	Test Group 3	aqueous humor + glucose 55mM + Kojic Acid	2.6 µg/ml

e) Homogenate preparation:

After 72 hours of incubation, the homogenate of the lens was prepared in Tris buffer (0.23 M, pH 7.8) containing 0.25×10^{-3} M EDTA, and the homogenate was adjusted to 10% w/v, which was centrifuged at 10,000 rpm at 4ºC for 1 hour, and the supernatant was used for the estimation of biochemical parameters [8].

f) Biochemical Parameters:

1. Estimation of total Protein Content:

The amount of total protein present in the tissue homogenate was estimated by the method. To 0.1 ml of tissue homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly, mixed quickly, and incubated at room temperature for 30 min for colour development. Reading was taken against a blank prepared with distilled water at 610 nm in UV- a UV-visible spectrophotometer. The protein content was calculated and expressed as µg/mg lens tissue [(9)].

2. Statistical Analysis [(9)]:

All data were expressed as Mean ± SD. All data were analysed with Prism Pad software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Dennett's test. The values are expressed as Mean ± S.D., and results were considered significantly different if $P<0.05$. Statistical variations are compared as follows: Normal Goat lens vs. Goat lens + Glucose 55mM, Goat lens + Glucose 55mM vs. Goat lens + Glucose 55mM + Kojic Acid.

RESULT:

1. Photographic evaluation:

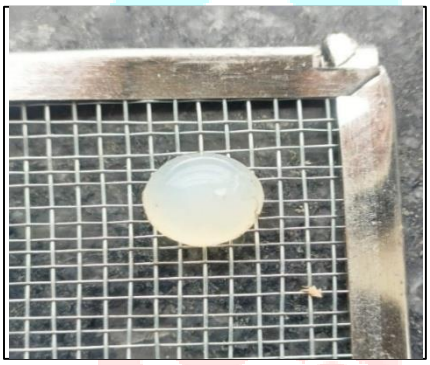

Figure 1. Group I- Normal control

Figure 2. Group II- Negative control

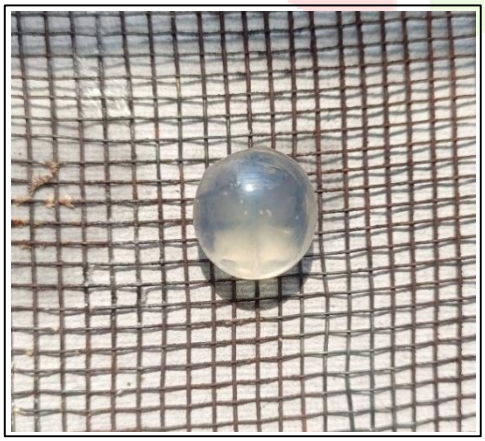

Figure 3. Group III- Positive control

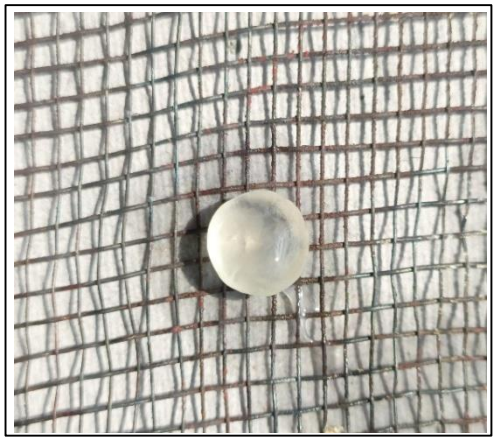

Figure 4. Group IV- Test Group 1

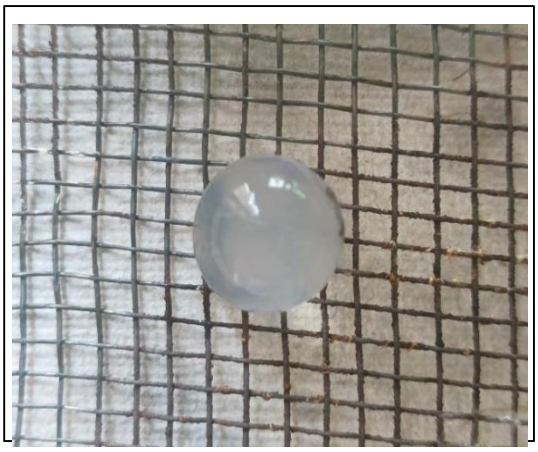

Figure 5. Group V – Test Group 2

Figure 6. Group VI- Test Group 3

Table 2. Effect of Kojic Acid on the degree of opacity of the lens by glucose-induced cataract.

Sr. No.	Compound	Degree of opacity
I	Negative Control (Glucose 55 mM)	1
II	Normal control (Glucose 5.5 mM)	0
III	Positive control (Ascorbic acid 40µg/ml)	1
IV	Test Group 1 (Kojic Acid 2.0µg/ml)	2
V	Test Group 2 (Kojic Acid 2.4µg/ml)	1
VI	Test Group 3 (Kojic Acid 2.6µg/ml)	0

Normal control - There is zero-degree opacity; a clear lens is produced.
Negative control – A large area of thick opacity due to a high concentration of glucose-induced cataract development.
Positive control (Ascorbic acid 40µg/ml) – Lenses exhibit small opacity; the transparent lens was not discovered.
Test Group 1 (Kojic Acid 2.0 µg/ml) – There is a small amount of opacity in the lenses; no clear lens was discovered.
Test Group 2 (Kojic Acid 2.4 µg/ml) – There is a small amount of opacity in the lenses; no clear lens was discovered.
Test Group 3 (Kojic Acid 2.6 µg/ml) – Zero-degree opacity occurs, and a clear lens is obtained. Test drug inhibits cataractogenesis.

2. Estimation of Protein:

Table 3. Total Protein Content.

Group	Dose	Total Protein Content
Normal lens Control Glucose 55mM	------	94.9±8.69
Negative control Glucose 5.5 mM	------	10.22±3.07
Glucose 55mM +Ascorbic acid 40 µg/ml	40 µg/ml	50.20±3.09
Glucose 55mM+Kojic Acid 2.0 µg/ml	2.0 µg/ml	65.14±3.62
Glucose 55mM+Kojic Acid 2.4 µg/ml	2.4 µg/ml	55.23±3.69
Glucose 55mM+Kojic Acid 2.6 µg/ml	2.6 µg/ml	30.26±3.11

Values are expressed as Mean ± SEM, N=6. Values are compared over 72 hours using one-way ANOVA and the Student t-test. Comparisons were made as follows: # $p<0.05$, **$p< 0.01$, ***$p< 0.001$ compared to the Negative control. Where, N.S.- non-significant.

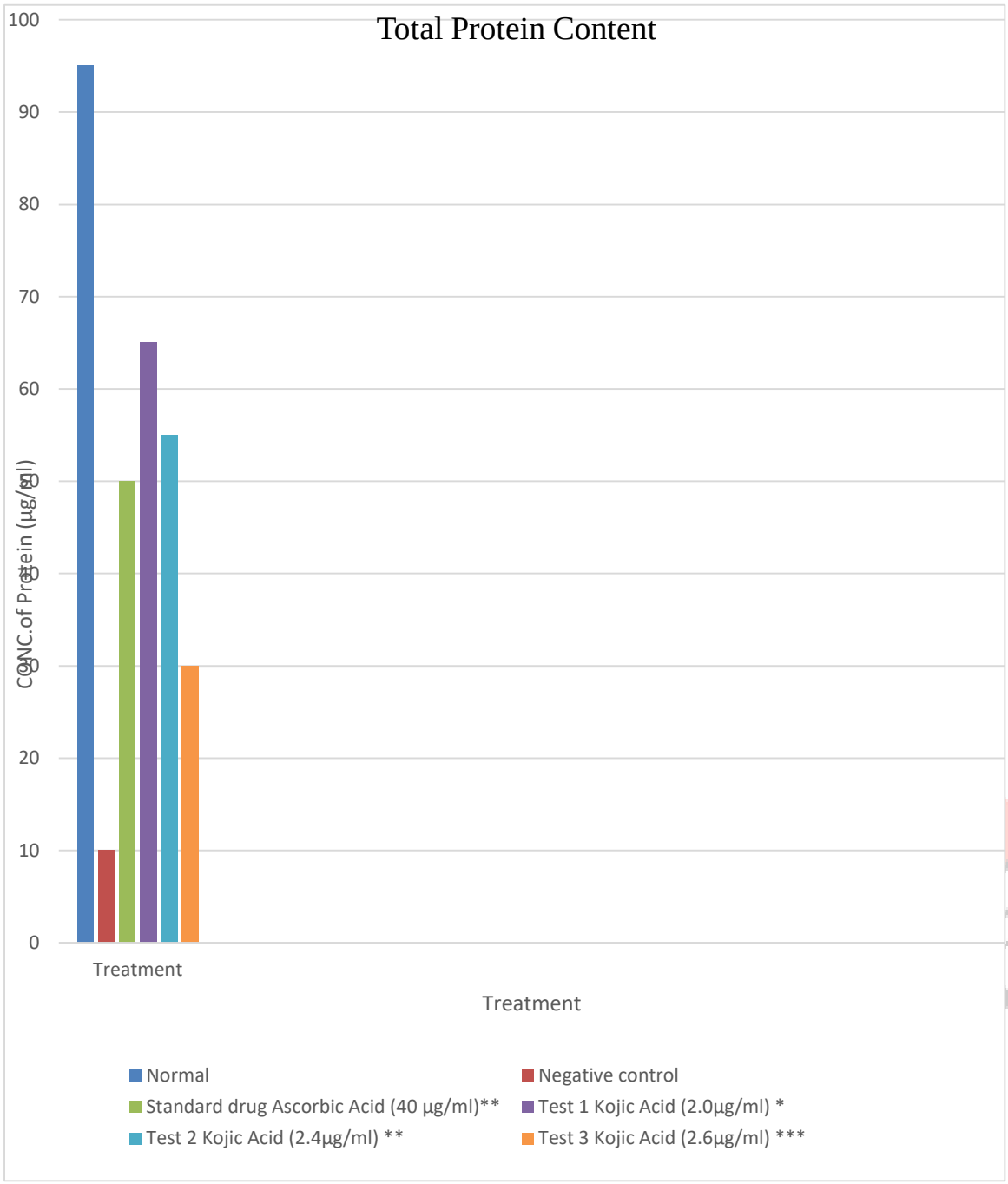

Figure 7. Effect of Kojic Acid on Total Protein Levels in Goat Lens in Glucose-Induced Cataract.

DISCUSSION:

Group I (normal control group) showed transparency after 72 hours of incubation [fig. No. 1], but Group II (negative control group) showed no transparency at all [fig. No. 2], suggesting full cataractogenesis. Visible through the lenses of Group III (positive control group) [fig. No. 3] were squares of the graph paper that had been treated with standard ascorbic acid. Groups IV, V, and VI's goat lenses, which had higher dosages of Kojic Acid, showed reduced haze, and the squares on the graph paper could be seen through the lenses, which suggested that the formation of cataracts had been suppressed [Fig. No. 4, 5, and 6]. Group VI [fig 6] showed better suppression of cataract formation than Group IV [fig. 4] and Group V [fig. 5], its higher concentration of 2.6 µg/ml.

In normal control, the lens was incubated for 72 hrs. in aqueous humour and 5.5 mM glucose conc. The low glucose concentration does not affect the lens, and some squares are visible through the lens. Thus, opacity is maintained.

After 72 hr. of lens incubation in aqueous humour and 55 mM glucose, the lens induces a high concentration. Glucose in the lens is metabolised through the sorbitol pathway and polyol accumulation, causing hydration and oxidative stress. This leads to cataractogenesis. The lens showed extensive thick opacity; thus, no squares are visible.

In contrast to Test Drug 3, the number of squares was not visible through the lens after a 72-hour incubation period in aqueous humour, 55 mM glucose, and 40µg/ml ascorbic acid as the Standard Drug. The lens exhibits a modest degree of opacity.

In comparison to the Kojic Acid 80 µg/ml test drug, some squares were not visible through the lens when the lens was incubated for 72 hours in aqueous humour + 55 mM glucose + 2.0 µg/ml and 2.4 µg/ml. The lens exhibits a modest degree of opacity. Upon incubating the lens for 72 hours in a mixture of aqueous humour, 55 mM glucose, and 2.6 µg/ml Kojic Acid test medication, the number of squares became visible through the lens. Because the test medication prevents oxidative stress and cataractogenesis, the lens displayed no opacity.

Using isolated goat lenses, the in vitro model for cataract induction with a glucose concentration of 55 mM offers an efficient model. Goat lens incubation in media with high glucose (55 mM) content has been demonstrated to generate cataracts and to reduce Na^+/K^+ ATPase activity as opacity progresses significantly. The buildup of Na+, loss of K^+, and hydration and thickening of the lens fibres result in cataractogenesis when Na^+/K^+ ATPase is impaired. The protein composition of the lens is altered by this change in the Na^+, K^+ ratio, which lowers the overall number of proteins and results in lens opacification. Kojic Acid, which balances abnormalities in the polyol pathway by lowering intracellular glucose, sorbitol concentration, and aldose reductase activity, avoids the imbalance of Na^+ and K^+.

Kojic Acid's ability to prevent cataracts caused by glucose was assessed in goat eyes. Compared to 100% of the negative control eyes, which had dense nuclear opacity, 50% of the eyes with Kojic Acid-protected lenses had nearly clear lenses. It is clear from the current study that Kojic Acid shields the lens from oxidative damage. The findings of these in vitro investigations on glucose-induced cataracts show that Kojic Acid not only has a preventive impact but also inhibits the development of cataracts through its antioxidant qualities. As a result, Kojic Acid may be helpful in the prevention or treatment of cataracts. In contrast to lenses under normal control, the lens becomes opaque after 72 hours of incubation in glucose at a concentration of 55 mM. Lenses were incubated in both doses of Kojic Acid and ascorbic acid, which appeared to slow down the opacification process compared to lenses incubated in 55 mM glucose (Negative Control). When compared to the Negative Control, the effect of Kojic Acid on the Positive Control group demonstrated a significant delay in the advancement of lens opacification, approaching normal.

Glucose 55 mM treated lenses (Group II) showed significantly lower concentrations of total proteins in the lens homogenate ($P< 0.01$) compared with normal lenses (Group I). Ascorbic acid-treated lenses (Group-III) and Lenses treated with Kojic Acid (Group-IV, V, VI) showed higher concentrations of total proteins ($P< 0.01$) compared with Glucose 55 mM-treated lenses (Group-II).

CONCLUSION:

The Present investigation shows that Kojic Acid-treated groups have been shown to increase the total protein content, retarding the process of cataractogenesis initiated by high glucose concentration. Kojic Acid possesses Anti-cataract activity due to the presence of antioxidant activity, which might help prevent and slow cataract formation.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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