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EVALUATION OF ANTI -CATARACT ACTIVITY OF METHANOLIC EXTRACT OF *ZIZIPHUSXYLO PYRUS* FRUIT USING IN-VITRO MODEL ON GOAT LENS AND CHICK LENS

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Abstract

A cataract is a condition where clouding of the eye's natural lens occurs. It acts a major complication in case of diabetes mellitus. The present study investigates the effect ofmethanolic extract ofZiziphusxylopyrus fruiton cataract induced by glucose. Ziziphusxylo pyrus, a traditional folklore plant has antioxidant, antiulcer properties. Goat eye lens were divided into four groups. Group I lenses were incubated in artificial aqueous humor with glucose concentration of 5.5 mM (normal control). Group II lenses were incubated with glucose concentration of 55 mM (toxic control). Group III and IV lenses were incubated with glucose concentration of 55 mMalong with methanolic extract of Ziziphusxylo pyrus fruit(50µg/ml)and(100µg/ml). The entire lenses weresubjected to photographic evaluation for opacity. The same grouping was done for chick lens too. The grades of opacity was 0, +++, +, ++ in each group respectively. The extract at 100µg/mlshowed potent prevention on in vitro glucose induced cataract. Thus, the goat lens and chick lenses could be used for testing of various anti-cataract agents.

Key word: *Ziziphus xylopyrus*, Cataract, Artificial aqueous humor, lens.

Introduction

Cataract is the opacification of lens often associated with old age and is a major complication of diabetes mellitus due to elevated glycosylated hemoglobin levels, which are significantly associated with increased risk of cataract [1]. Although many cataractogenic factors have been identified, the biochemical background of cataractogenesis is still unknown. It is a multifactorial disease which occurs mainly due to formation of large protein aggregates in the lens. The lens Na^+-K^+-ATPase activity plays an important role in maintaining the lens transparency, and its impairment causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibres leading to cataractogenesis [2]. Although a number of agents have been tried for prevention and treatment of cataract, but none have proved to be useful [3]. Aldose reductase is an enzyme of lens probably involved in the development of this eye problem (cataract) [4]. Aldose reductase acts on sugars like glucose, galactose and xylose and converts them into their respective alcohols. These alcohols, also known as polyols accumulate within the lens there by producing osmotic effects. Since polyols are not capable of diffusing out easily nor metabolizes rapidly and causes hypertonicity which is responsible for formation of cataract [5]. Oxidative mechanism plays an important role in biological phenomena including cataract formation. The formation of superoxide radicals in the aqueous humor and in lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to formation of cataract[6].Vision loss due to cataract is related to risk factors including malnutrition, sunlight, smoking, hypertension, aging and diabetes. Progression of cataracts results in opaque eye lens leading to poor or complete vision loss**.** Decrease in antioxidant enzyme activities in the cataractous lens points to the importance of antioxidant enzymes in the prevention of oxidative damage to the lens and the subsequent development of cataract [7].

*Zizyphusxylopyrus*belonging to the family Rhamnaceae is a large, straggling shrub or a small three, armed withspines, up to 4-7 meters in height, which is found throughout North-Western India, Pakistan and China. This plant is widely used in turkish folk medicines as a potent sedative. The leavesare chewed for 15 days as well as the fruit is used in urinary troubles. The methanolic extract shows analgesic andanti-inflammatory activities in animal model. It has been reported that the chloroform extract ofbark shows anthelmintic activity. *Ziziphusxylopyrus* fruit is used in treatment of tuberculosis, bronchitis, fever, dysentery, healing of flash wounds and also for the treatment of hypoglycemic condition. It is also used as an antimicrobial and antioxidant [8]. In the present study, anti cataract activity of methanolic extract of the *Ziziphusxylopyrus* fruit was evaluated using various animal lens.

Methods

Collection of plant material

The fruits of *Ziziphusxylopyrus*wild were purchased from the local market of Guntur.It was authenticated by Prof A.Amala, Professor, Nagarjuna University, Guntur and a voucher specimen was kept for further reference.

Preparation of plant extract

The fruits were shade dried and pulverized. Powdered material of fruit is taken and extracted with 500ml of methanol by soxhlet apparatus. The solvent was removed under reduced pressure until the volume of extract reaches 20ml. Then the final extract is collected, dried and stored in a suitable container.

Phytochemical Screening

The coarse fraction of powder was subjected for phytochemical studies and qualitative tests were conducted for themethonolic extract of*Ziziphusxylopyrus*fruit to identify the various phytoconstituents [9], [10]. The various tests and reagents used are given below and the observations were recorded in table 1.

Test for alkaloids

The methanolic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent then observed for the presence of turbidity or yellow precipitation.

I. **Mayer's Test:** Filtrate was treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.
II. **Wagner's test:** Filtrate was treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown or reddish brown precipitate indicates the presence of alkaloids.
III. **Dragendroff's test:** Filtrate was treated with Dragendroff's reagent (solution of potassium bismuth iodide).Formation of red precipitate indicates the presence of alkaloids.

IV. **Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow coloured precipitate indicates the presence of alkaloids.

Test for glycosides

To the solution of the extract in glacial acetic acid, a few drops of ferric chloride and concentrated sulphuric acid were added and observed for a reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer. They are also identified using various tests.

I. **Modified Borntrager's Test:** Extract was treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.
II. **Legal's test:** Extract was treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Test for Terpenoids

Salkowski test: Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and a reddish violet colour was observed for terpenoids.

Test for Carbohydrates

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

I. **Molisch's Test:** Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of Carbohydrates.
II. **Benedict's test:** Filtrate was treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugar.
III. **Fehling's test:** Filtrate was hydrolysed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling'sA and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Test forSaponins

Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

Test for fixed oils and fats

Stain Test: Small quantities of extracts were pressed between two filter papers.An oily stain on filter paper indicates the presence of fixed oil.

Test forPhytosterols

I. **Salkowski's Test:** Extracts was treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand.Appearance of golden yellow colour indicates the presence of triterpenes.
II. **Libermann-Burchard's test:** Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added carefully along the sides of the test tube.Formation of brown ring at the junction indicates the presence of phytosterols.
III. **Tshugajeu test:** Extract was treated with chloroform and filtered. Excess of acetyl chloride and a pinch of zinc chloride was added, kept aside for some time till the reaction was complete and then warmed on water bath. Appearance of eosin red colour indicates the presence of triterpenes.

Test for phenols

Ferric Chloride Test: Extract was treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins

Test for flavonoids

I. **Alkaline Reagent Test:** Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
II. **Lead acetate Test:** Extract was treated with few drops of lead acetate solution.Formation of yellow colour precipitate indicates the presence of flavonoids.
III. **Shinoda Test:** To the alcoholic solution of extracts, a few fragments of magnesium ribbon

and concentrated hydrochloric acidwere added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

Invitro anti-cataract activity using goat lens:

***Preparation of Lens Culture*:** Fresh goat eyeballs were obtained from the slaughter house and immediately transported to the laboratory at 0-4°C. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) as per the guidelines of CPCSEA, Department of Animal Welfare, Government of India. The lens were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl-140mM, KCl-5mM, $MgCl_2$-2mM, $NaHCO_3$-0.5mM, NaH_2PO_4-0.5mM, $CaCl_2$-0.4mM and glucose-5.5mM) at room temperature and a pH of 7.8 was maintained by addition of $NaHCO_3$). Ceftriaxone at a dose of 5mg/ml was added to the culture media to prevent bacterial contamination of theextract.

Induction of *in-vitro Cataract in goat lens* [11],[12]

Glucose at a concentration of 55 mM was used to induce cataracts. At higher concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration and oxidative stress. This generates cataract. A total of 30 lenses were used for the study. These lenses were incubated in artificial aqueous humor with different concentrations of glucose(5.5 mMserved as normal control and55 mM served as toxic control) for 72 hours.

Study Design and Groups

Goat lenses were divided into five groups of six lenses each and incubated as follows:
Group I: Glucose 5.5 mM (normal control).
Group II: Glucose 55 mM (toxic control).
Group III: Glucose 55 mM +methanolic extract 50µg/ml.
Group IV: Glucose 55 mM +methanolic extract 100 µg/ml.

Induction of in-vitro Cataract in chick lens

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 cataracto genesis. A total of 30 lenses were used for the study. These lenses were incubated in artificial aqueous humor with different concentrations of glucose(5.5 mM served as normal control and 55 mM served as toxic control) for 72 hours.

Study Design and Groups

Chick lenses were divided into five groups of six lenses each and incubated as follows:
Group I: Glucose 5.5 mM (normal control).
Group II: Glucose 55 mM (toxic control).
Group III: Glucose 55 mM +methanolic extract 50µg/ml.
Group IV: Glucose 55 mM +methanolic extract 100 µg/ml.

Results

Morphological and Photographic Evaluation of goat lens

The Lenses from different groups 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
+: Slight degree
++: Presence of diffuse opacity
+++: Presence of moderate diffuse opacity.

Morphological and Photographic Evaluation of Chick Lens

The Lenses from different groups 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
+: Slight degree
++: Presence of diffuse opacity
+++: Presence of moderate diffuse opacity.

Discussion

Oxidative stress is an important factor in the development of cataracts and the use of antioxidants may be advocated in patients to delay or prevent the formation of cataract [13]. Incubation in the media containing high glucose concentration (55Mm) has shown to cause considerable drop in Na^+-K^+-ATPase activity, with progression of opacity [14]. Na^+-K^+-ATPase plays a vital role in maintenance of ionic equilibrium in lens, and thus its impairment leads to accumulation of water and thus swelling of fibres occurs, which leads to cataractogenesis [15]. Earlier studies shown that the methanolic extract of *Ziziphusxylopyrus*fruit possess significant anti oxidative effect [16].

The phytochemical screening of methanolic extract of *Ziziphusxylopyrus*fruit supports the antioxidant activity with the presence of terpenoids and tannins. The goat lenses procured for the study were morphologically evaluated for the degree of opacity and photographic representation was done. The goat lenses were divided into 5 groups. The group I which served as a control received only 5.5mM of glucose showed no opacity. The group II lenses received 55 mM concentration of glucose served as toxic control and showed maximum degree of opacity. There is no standard group for cataract study as there is no standard drug available for the treatment of cataract in the market. Group III which received 55mM concentration of glucose and 50 µg/ml of methanolic extract of *Ziziphusxylopyrus* fruit showed presence of diffuse opacity. Whereas group IV which received 55mM concentration of glucose and 100 µg/ml of methanolic extract of *Ziziphusxylopyrus* fruit showed presence of moderate diffused opacity.

The chick lenses procured for the study were morphologically evaluated for the degree of opacity and photographic representation was done. The chick lenses were divided into 5 groups, each group consists of six lens. The group I which served as a control received only 5.5mM of glucose showed no opacity. The group II lenses received 55mM concentration of glucose served as toxic control and showed maximum degree of opacity. There is no standard group for cataract study as there is no standard drug available for the treatment of cataract in the market. Group III which received 55mM concentration of glucose and 50 µg/ml of methanolic extract of *Ziziphusxylopyrus* fruit showed presence of diffuse opacity. Whereas group IV which received 55mM concentration of glucose and 100 µg/ml of methanolic extract of *Ziziphusxylopyrus* fruit showed presence of moderate diffused opacity.

In present study, the methanolic extractof *Ziziphusxylopyrus* fruitat a dose of 100µg/ml has produced maximum protection against cataract in the goat lens and chick lens, when compared to other concentrations.

Conclusion

In the present study, the methanolic extract of *Ziziphusxylopyrus* fruit showed significant reduction of cataract at the dose of 100 µg /ml in both goat and chick lenses. The results 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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Table 1. Phytochemical screening of methanolic extract of *Ziziphusxylopyrus* fruit

PHYTOCHEMICAL CONSTITUENTS	Methanolic extract of *Ziziphusxylopyrus*
Alkaloids	+
Glycosides	+
Flavonoids	+
Terpenoids	+++
Carbohydrates	++
Saponins	_
Phytosterols	_
Fixed oils and fats	_
Phenols	+
Tannins	++

Figure 1. Goat lens –Group I **(Normal control)**

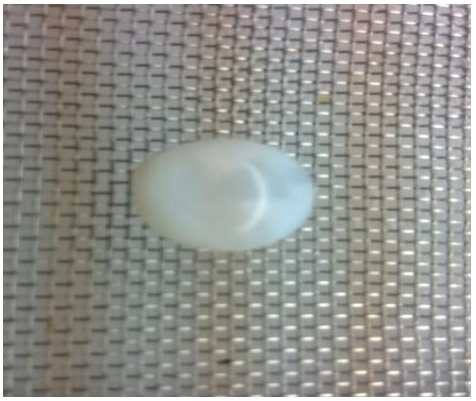

Figure 2. Goat lens –Group II **(Toxic control**)

Figure 3. Goat lens –Group III (Test-I – 50µg/mL)

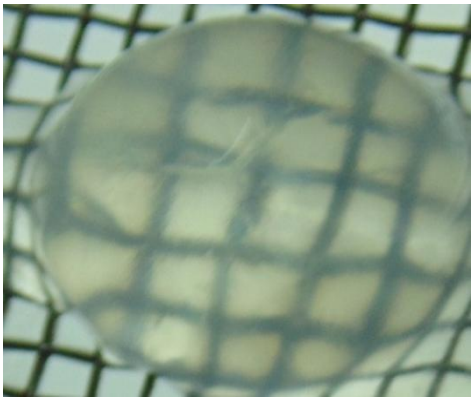

Figure 4. Goat lens –Group IV (Test-II – 100µg/ml)

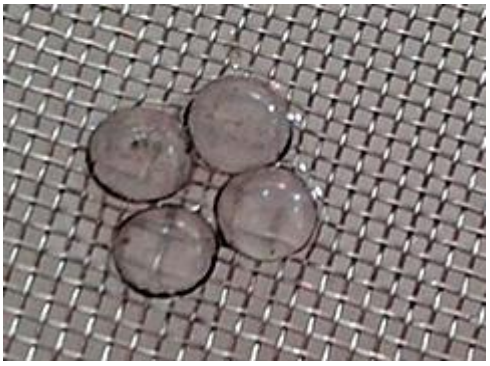

Figure 5. Chick lens –Group I
(Normal control)

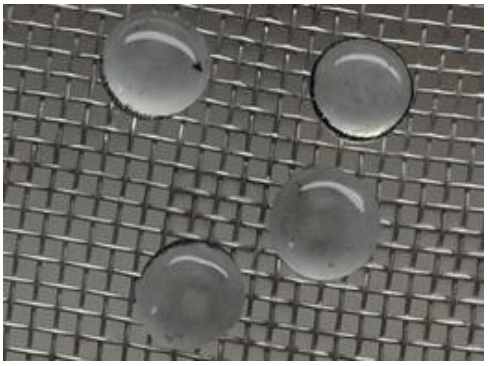

Figure 6. Chick lens –Group II
(Toxic control)

Figure 7. Chick lens –Group III
(Test-I – 50µg/ml)

Figure 8. Chick lens –Group IV
(Test-II – 100µg/ml)