

Evaluation of Anticataract Activity of Methanolic Extract of *Trianthema Decandra* Leaves against Galactose Induced Cataractogenesis



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Abstract

Aim: To evaluate anticataract activity of methanolic extract of *Trianthema decandra* leaves against Galactose induced Cataract.

Methods: Methanolic extract of *Trianthema decandra* (METD) leaves against Galactose induced Cataractogenesis both in vitro and in vivo was evaluated. Cataract was induced in rats by feeding 300g/L galactose diet. METD was administered orally at three different dose levels 75, 150 and 300mg/kg of body weight. Rat lenses were subjected to osmotic stress by incorporating galactose (30mmol/L) in the culture medium. The effect of METD (720 and 880µg/mL) on the glutathione (GSH) and polyols levels was studied.

Results: METD significantly delayed the onset and progression of cataract in vivo. In addition to the delay in reaching various stages of development of cataract, stage IV did not develop with lower doses till the completion of experimental period. Lenses treated with METD 880µg/ML concentration showed higher levels of GSH and decreased levels of polyols in vitro. In vivo 75 mg/kg significantly delayed the onset and progression of cataract as compared to control.

Conclusion: *Trianthema decandra* delays the progression of cataractogenesis in the experimental models. However, further work is required to extrapolate clinical use in human beings for the avoidance of cataract.

Keywords: Cataract; Galactose; Glutathione; Polyols; *Trianthema decandra*

Introduction

Opacification of the lens of the eye, cataract is the foremost cause of sightlessness it accounts for approximately 50% of all blindness worldwide. World Health Organization launched Vision 2020, to get rid of cataract as priority diseases [1,2]. In India, cataract is responsible for almost 80% of blindness [3,4]. Apart from senile cataract various risk factors such as nutritional deficiency, sunlight, smoking environmental factors, lack of consumption of antioxidants and diabetes are known to increase the risk of cataract [5-8].

Diabetes has been painstaking to be one of the chief threats for cataract. Numbers of in vitro and in vivo research studies hold up the scrutiny that diabetes is one of the causes of cataract [9]. Cataract is considered a major cause of visual impairment in diabetic patients. The incidence and progression of cataract is elevated in patients with diabetes mellitus. During hyperglycemia extra

cellular glucose diffuses into the lens uncontrolled by the hormone insulin, the lens is one of the body parts most affected in diabetes mellitus. The proteins of the lens are extremely long-lived, and there is virtually no protein turnover, which can lead to posttranslational modification [3]. Intake of unwarranted galactose has been shown to induce the formation of cataracts in several species of experimental animals. The cataractogenic effect is primarily related to the synthesis and accumulation of excessive sorbitol (polyols) in the lens fibers and consequent osmotic stress [10]. Sorbitol is synthesized by aldose reductase utilizing NADPH and does not easily cross cell membranes; it can accumulate in cells and cause damage by disturbing osmotic homeostasis.

Second Pathophysiological mechanism of cataract formation includes undersupplied glutathione levels contributing to a defec-

tive antioxidant defense system within the lens of the eye [11]. Normally the lens contains significant levels of reduced glutathione (GSH), which keeps the proteins in their reduced form. However, there are significantly decreased levels of GSH in cataractous lenses. Therefore, prevention of polyol accumulation and maintaining GSH level to prevent cataract and diabetes has received extensive interest.

A variety of therapeutic plants are reported to possess anti diabetic and offer protection in various pathological conditions such as cardiovascular diseases, neurodegeneration [12]. A large number of plants/species are now well recognized to possess hypoglycemic potential [13,14]. Number of these hypoglycemic agents has not been investigated for their favorable effects on secondary complications of diabetes such as cataract. It would be of huge magnitude to evaluate both pharmacologically and biochemically, which might be helpful in the better management of secondary complications of diabetes.

Trianthema decandra has been used in various parts of Asia, Africa, Australia and South America for curing various diseases. In some African countries the plant has been popular use for skin diseases, wound healing, fever and tooth aches. In India it is used in the treatment of ophthalmic [15]. The root applied to the eye cures corneal ulcers, itching, dimness of sight and night blindness. The current study was undertaken with the aim of investigating the anticataract potential of methanolic extract of *Trianthema decandra* leaves against galactose cataract in rats as well as against galactose induced morphological and biochemical changes in vitro. Together these results entail that METD may be explored as an anti cataractogenic agent for diabetic cataract.

Materials and Methods

Plant materials and extraction [16]

Trianthema decandra plant was collected from fields of Kanchna Garipally, Kamalapuram, Kadapa, Andhrapradesh, India. The plant was identified and authenticated by Dr. M. Madhusudhan Reddy of Yogi Vemana University, Kadapa. Fresh *Trianthema decandra* leaves allowed drying (air-dry) for one week. The dried parts of plant was cleaned and ground to coarse powder, using commercial mill. They were sieved through No.20 mesh sieve and stored in an airtight container until the time of use. Approximately 500gm of the ground plant powder was macerated in 1000 ml methanol at room temperature for 72h with occasional shaking. It was filtered through a Whatman grade 1 filter paper in a Buchner funnel under vacuum. The filtrate was evaporated to dryness under controlled temperature between 40-50°C to obtain a crude methanolic extract of *Trianthema decandra* (METD).

Drugs and chemicals

Dulbecco modified Eagle's medium (DMEM) was procured from Hi Media Laboratories (Mumbai, India). Galactose was purchased from SD Fine-Chem Limited (Mumbai). Streptomycin and

penicillin were obtained from Hindustan Antibiotics Ltd. (Pune, India). All other chemicals and reagents used were of analytical grade.

Animals

The animals in the current study were treated in accordance with the institutional guidelines and Association for Research in Vision and Ophthalmology statement for the use of animals in research. For in vivo study, Wistar rats of either sex, weighing 260-280g were divided into control and treated groups and for in vitro study, the lenses were enucleated from the Wistar rats of either sex belonging to the normal group (without any treatment) for cardiovascular and anti-fertility studies being conducted in the institute were used.

Lens organ culture

The lenses were carefully enucleated from eyes with a posterior approach. Each isolated lens was placed in a Falcon plastic culture plate (24-well) containing 2mL of DMEM supplemented with 200mL/L fetal bovine serum, 100g/mL of streptomycin, and 100IU/mL penicillin. The lenses were incubated at 37°C under 900g/L moisture, 950mL/L air, and 50mL/L CO₂ gas atmosphere for 2 hours. The damaged lenses that developed artificial opacities were discarded and only transparent lenses were taken for the subsequent in vitro experiment.

Galactose-induced Osmotic Stress Transparent

In vitro cultured lenses were randomly divided into normal, galactose only and two treatment groups each comprising six lenses. Normal lenses were incubated in DMEM alone, whereas control group lenses were incubated in DMEM supplemented with 30mmol/L of galactose. Medium in the treated groups was additionally supplemented with two different concentrations of METD (720 and 880µg/mL) along with galactose. All the lenses in different groups were maintained for 24 hours at the above-mentioned experimental conditions of incubation. Post incubation, the lenses were examined for the presence of any opacity, and photo documentation was done. Thereafter, lenses were washed, weighed, and processed for the estimation of biochemical parameters. Each lens was homogenized in 1mL of 0.1mol/L-phosphate buffer (pH 7.0). The homogenate was divided into two equal parts. One part was used for the estimation of GSH and the other for polyols.

Estimation of GSH

The GSH content was estimated by the method of Parmar et al. [17]. The homogenate was centrifuged at 5000r/min for 15 minutes at 4°C To the supernatant; 0.5mL of 100g/L trichloroacetic acid was added and recentrifuged. The protein-free supernatant thus obtained was reacted with 4mL of 0.3mol/L of Na₂HPO₄ (pH 8.0) and 0.5mL of 0.4g/L (w/v) 5, 5'-dithiobis-2-nitrobenzoic acid. The absorbance of the resulting yellow color was read spectrophotometrically at 412nm. A parallel standard was also maintained.

Estimation of Polyols

Polyol estimation was done by the method described by West & Rapoport [18]. The homogenate was reacted with 0.6mol/L perchloric acid. Precipitate was removed by centrifugation and the supernatant was neutralized with 2mol/L NaOH. Again, the precipitate was removed by centrifugation and clear supernatant was reacted with, freshly prepared 0.125mol/L stannous chloride and 2g/L chromotropic acid. The absorbance of purple colored complex was measured spectrophotometrically at 570nm. Parallel standard was subjected to the above-mentioned steps for the calculation of polyol in the samples.

Galactose cataract in vivo model [19]

In vivo Wistar rats of either sex, weighing 260-280g was divided into control and treated groups (n =6). 300g/L galactose was fed to all group's ad libitum induced cataract. Seven days prior to start of galactose diet 75, 150 and 300mg/kg body weight dose of METD in distilled water (as a vehicle) were given orally once a day to the treated group and continued till the end of the experiment. In control group only distilled water and the galactose diet were given. Eyes were examined through a slit lamp after dilating the rat pupil with 10g/L tropicamide. The stages of cataract were graded according to Sippel's classification.

Statistical analysis

All data were expressed as mean±SD. The groups were compared by one-way ANOVA using post-hoc Tukey test, with a p<0.05 considered as significant.

Results

Effect on food intake and body weights

There were no significant changes on feeding of galactose or galactose plus METD extract (75,150 and 300mg/kg body weight) on food intake and body weight of the animals during the entire course of the study.

Morphology of lens

All the lenses in DMEM alone were transparent. However, lenses after 24 hours of incubation in the presence of galactose developed dense opacity. Incorporation of PN (720 and 880µg/mL) in the culture medium prevented the development of opacity to different extent. Sixty-four percent of lens remained transparent with the supplementation of METD extract at the concentration of 720µg/mL and rest of the lenses developed faint opacity. At the dose of 880µg/mL, METD was more effective. Only 16 percent of lenses showed faint opacity while 84% were transparent.

Effect of METD on GSH and polyols

To investigate the possible mechanisms of differential effects of *Trianthema decandra* at different doses on galactose-induced cataract, levels of GSH and polyols were estimated which are related to the oxidative stress and polyol pathway. Galactose produced a significant difference in GSH in the galactose-only group lenses in comparison with normal lenses. Treatment with METD (720 and 880µg/mL) significantly restored the GSH concentration (Figure 1).

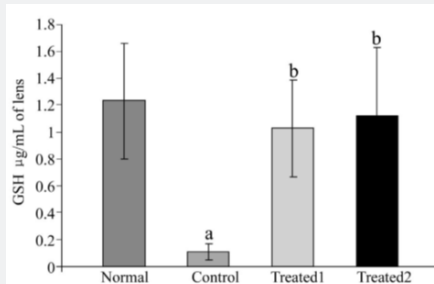


Figure 1: Effect of *Trianthema decandra* on GSH levels in galactose induced cataract rat lens Normal, DMEM, Control: DMEM+30mmol/L galactose, Treated 1: DMEM+30mmol/L galactose+720µg/mL of METD. Treated 2: DMEM+30mmol/L galactose+880µg/mL of METD. Values are mean±SD. a<0.05, control normal; b<0.01, control treated=6.

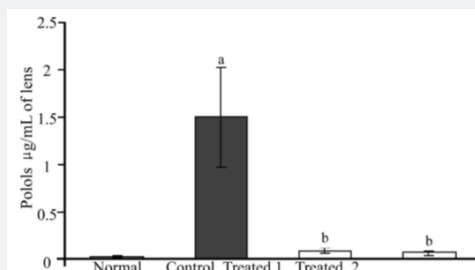


Figure 2: Effect of *Trianthema decandra* on polyols levels in galactose induced cataract rat lens Normal: DMEM, Control: DMEM+30mmol/L galactose, Treated 1: DMEM+30mmol/L galactose+72µg/mL of METD. Treated 2: DMEM+30mmol/L galactose+880µg/mL of METD. Values are mean±SD. a<0.05, control normal; b<0.01, control treated=6.

Effect of METD was studied on the polyol levels in the lenses incubated in medium with galactose. Our results showed a gradual increase in polyol level in control group. However, METD at 720 and 880µg/mL concentration was found to inhibit polyol accumulation in the treated group. Treatment with METD 880µg/mL in the medium significantly inhibited the accumulation of polyol in comparison to that of the normal lenses and with no significant statistical difference in between the treatment groups (Figure 2).

Effect of METD on galactose cataract in rat

The rate of progression of cataract in the control and the treatment group was compared, an overall grade point average referred as opacity index (OI) was calculated for each group on different days, based on slit lamp examination. To calculate OI, normal eyes were given no point, stage I, II, III and IV were given 1, 2, 3 and 4 points respectively.

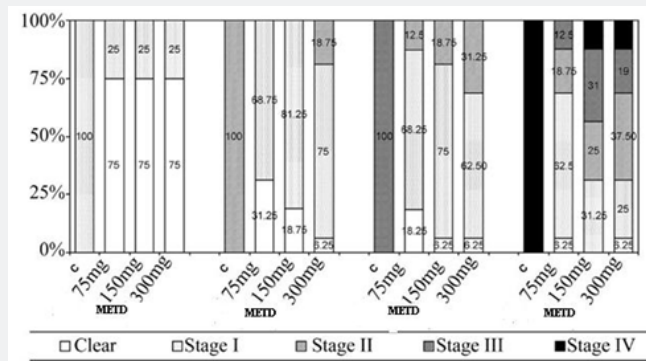


Figure 3: Effect of *Trianthema decandra* on the progression of galactose cataract C: Control, Rats were fed with 300g/L galactose diet and vehicle. Test: Rats were fed METS (75, 150 and 300mg/kg body weight) orally with galactose in the diet. Figure shows the percentage of eyes with different grades of opacity on 7, 14, 21 and 30 days.

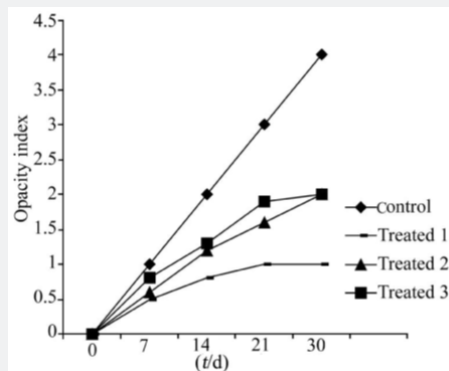


Figure 4: Effect of *Trianthema decandra* on progression pattern of galactose cataract Treated 1: METD 75mg/kg; Treated 2: METD 150mg/kg; Treated 3: METD 300mg/kg.

The different stages of cataract in both control and treated groups on various days are shown in Figure 3. The results of the present study showed that 100% eyes in the control group had opacity by the 30th day of the experiment. On comparing the rates of progression of cataract in the treatment groups, onset was found to be significantly delayed as compared to the control group. It was observed on the end of the 30th day not a single eye was found in stage IV cataract in the dose of 75mg/kg body weight. The same group showed 6.25% eyes were in normal; 62.50% eyes were in stage I, 18.75% eyes were in stage II and 12.50% eyes were in stage III (Figure 4).

Discussion

Although cataract is the most prevalent disorder leading to visual impairment, pharmacological intervention to inhibit or to

delay the lens Opacification is yet at the experiment stage. Several factors are involved in the induction of this disease process, but exact mechanism of cataract formation is still not very clear. Studies are ongoing to explore the mechanism of cataractogenesis using various models of cataract. Among various experimental models, the galactose model is commonly used, as it produces a greater increase in its reduced form, galactitol, than does glucose and the fact that galactitol does not further metabolize as does sorbitol, the reduced form of glucose [20]. Galactose model is reasonable to assume that the factors initiating the galactose cataract in young rats are very similar to those involved in the human galactose cataract [21]. The lens opacities in rats that are fed galactose, like those in human galactosemic subjects, slowly disappear when rats are placed on diets free of galactose. Three possible mechanisms that may be involved in cataract formation as a result

of hyperglycemia or hypergalactosemia are the polyol pathway, oxidation, and non-enzymatic glycation [22].

Trianthema decandra is a prostrate, glabrous, succulent and annual found almost throughout India as a weed in cultivated and waste land. The plant belongs to the family Aizoaceae. This family is well marked in their characteristics and cannot be confused with any other. The genus *Trianthema* consists of 20 species but only a few species have been phytochemically reported. *Trianthema* is a genus of annual or perennial plant characterized by usual fleshy, opposite, unequal, smooth-margined leaves; prostrate growth form; flowers with five perianth segments; flowers subtended by a pair of bracts; superior fruit a circumscissile capsule with a winged lid; and stamens 5 or 10 [23].

Trianthema decandra has been used in various parts of Asia, Africa, Australia and South America for curing various diseases. In some African countries the plant has been popular use for skin diseases, wound healing, fever and toothaches. In India it is used in the treatment of ophthalmic. The root applied to the eye cures corneal ulcers, itching, dimness of sight and night blindness. The juice of leaves is used to treat the black quarter. The bitter roots are used for curing bacterial infections and it is also given in combination with ginger as a cathartic. The leaves contains huge amount of vitamin C which is used to treat edema. The decoction of the herb is used as a vermifuge and is useful in rheumatitis [24].

In the traditional systems of medicine such as Ayurveda and Unani, *Trianthema decandra* and its species are used for anti-inflammatory, anti-hyperglycemic, hepatoprotective and antioxidant [24]. *Trianthema decandra* has Anti-inflammatory, Analgesic, Wound healing [25], Hepatoprotective [26], Anti-ulcer [27], Antibacterial and antifungal activities [28], Anti-diabetic activity and Anti-cancer property [15,29] have shown significant results without adverse side effects.

Alkaloids, flavonoids saponins and phenolic compounds were found to be present in *Trianthema decandra*, the anticataract activity associated with extract of this plant may be attributed to the presence of these constituents [30]. Sugar cataract formation is associated with diabetes and galactosemia has been linked to the aldose reductase catalyzed production of polyols, sorbitol, and galactitol from glucose and galactose respectively. Accumulation of high concentrations of polyols in the lens leads to excessive hydration, gain of sodium, and loss of potassium ions due to an increase in intracellular ionic strength [31]. Also, there is a loss of membrane permeability and leakage of free amino acids, glutathione, myoinositol, and other small molecular weight substances. The resulting hyper osmotic stress associated oxidative insult is postulated to be the primary cause for the development of diabetic complications such as cataract [32]. Evidence has shown that there was a significant raise in polyols in galactosemic rats. In the present investigation polyol level was significantly decreased in METD treated rat lenses and we have also found that the METD was more effective in lower concentration.

The anticataractogenic effect of METD was confirmed from the results of study. In the present study, oral administration of METD showed significant protection against cataract formation in treated rats. The anticataract potential of METD seems to be related to its antidiabetic property as evident from the results of organ culture studies. In conclusion, METD showed anticataract activity against galactose cataract in experimental animals. The effect is attributed to the maintenance of GSH as well as inhibiting the accumulation of polyols in the lens. This preliminary study is encouraging, but further study is required to extrapolate the use of *Trianthema decandra* in human beings for the prophylaxis or the treatment of human cataractogenesis.

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