

# Inhibitory Effect of *Trigonella Foenum-Graecum* on Galactose Induced Cataracts in a Rat Model; *in vitro* and *in vivo* Studies

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**Purpose:** To evaluate the *in vitro* and *in vivo* anti-cataract potential of *Trigonella foenum-graecum* (TF) on galactose induced cataracts in an animal model.

**Methods:** In the *in vitro* group, enucleated rat lenses were maintained in organ culture containing Dulbecco's Modified Eagles Medium alone (normal group), or with the addition of 30 mM galactose (control group). The medium in the test group was supplemented with both galactose and TF. All lenses were incubated at 37°C for 24 hours and then processed for determination of levels of reduced glutathione and malondialdehyde. In the *in vivo* group, cataracts were induced in rats by a 30% galactose diet alone (control) or with the addition of TF (treated group).

**Results:** Reduction (26%) in glutathione level and elevation (31%) in malondialdehyde content were observed in controls as compared to normal lenses. TF significantly ( $P < 0.01$ ) restored glutathione and reduced malondialdehyde levels as compared to controls. A significant delay in the onset and progression of cataract was observed with 2.5% TF diet; after 30 days none of the treated eyes developed mature cataracts as compared to 100% of control eyes.

**Conclusions:** TF can delay the onset and progression of cataracts in an experimental rat model of galactose induced cataracts both *in vitro* and *in vivo*.

**Key words:** Cataract; *Trigonella foenum-graecum*; Antioxidant

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## INTRODUCTION

Cataract is the leading cause of blindness worldwide and is likely to present an increasing burden to health care systems as the world's population ages.<sup>1-3</sup> The total number of persons with cataracts is estimated to rise to 30.1 million by 2020.<sup>4</sup> In Australia, it is esti-

mated that by the year 2021, the number of people affected by cataracts will increase by 63% due to an aging population.<sup>5</sup> The number of Americans affected by cataracts requiring surgery will also dramatically increase over the next 20 years as the US population ages.<sup>4</sup> Cataract surgery is the only remedy, however due to postoperative complications, the disease

still remains a major cause of blindness.<sup>6</sup> Therefore there is an ongoing search for pharmacological intervention to maintain transparency of the crystalline lens. A large number of herbal drugs and their formulations such as *Camellia sinensis*, *Ocimum sanctum*, soybean and lycopene are reported to possess antioxidant properties and offer protection against cataracts.<sup>7-10</sup>

The present study evaluates the anti-cataract potential of *Trigonella foenum-graecum* (TF) aqueous extract on galactose induced cataracts in a rat model *in vitro* and *in vivo*. TF, commonly known as fenugreek, is native to India and southern Europe. It has been shown to lower serum low-density lipoprotein cholesterol and to possess anti-diabetic effects.<sup>11</sup> In recent research, fenugreek seeds were experimentally shown to protect against breast and colon cancers.<sup>12,13</sup> The hepatoprotective property of fenugreek seeds has also been demonstrated in experimental models.<sup>14</sup> However, information on its anti-cataract potential is limited. This study was conducted to evaluate the anti-cataract potential of TF in galactose induced cataracts in an experimental rat model both *in vitro* and *in vivo*.

## METHODS

Chemicals required for the enzyme assay were obtained from Sigma Chemical Co., St. Louis, USA. The oxidative stress inducing agent, sodium selenite was purchased from Central Drug House (P) Ltd., New Delhi, India. Wistar rats of either sex (60-80 g) were procured from the animal house, Delhi Institute of Pharmaceutical Sciences and Research, after getting approval from the Institutional Animal Ethical Committee. The animals were treated in accordance with institutional guidelines and Association for Research in Vision and Ophthalmology statement for the use of animals in research. The mother and suckling pups were left undisturbed to acclimate for 4 days prior to the experiment.

### *In vitro* Studies with the Lowest Effective Concentration of TF

Rats were anesthetized with ether. The anterior

segment of both eyes of each rat was removed by cutting just posterior to the limbus under magnification provided by a coaxial operating microscope using stainless-steel surgical equipment. The lens was gently removed without disturbing capsular integrity after cutting the suspensory ligaments; care was taken to avoid contamination from neighboring tissues and environmental sources. Freshly dissected lenses were rolled in filter paper to remove adherent vitreous. Each isolated lens was placed in a Falcon plastic culture plate (24-well) containing 2 ml of Dulbecco's Modified Eagles Medium (DMEM) supplemented with 20% fetal bovine serum, 100 µg/ml streptomycin, and 100 IU/ml penicillin. Crystalline lenses were incubated at 37°C under 90% moisture, 95% air, and 5% CO<sub>2</sub> gas atmosphere for 2 hours. Damaged lenses that developed artifactual opacities were discarded and only transparent lenses were used for the subsequent *in vitro* experiment.

Transparent cultured lenses were randomly divided into normal, galactose only (control) and three treatment groups each comprising of six lenses. Normal lenses were incubated in DMEM alone, whereas control lenses were incubated in DMEM supplemented with 30 mM galactose. Medium in the treated groups was additionally supplemented with different concentrations of TF (25, 50 and 100 µg/ml) along with galactose. All lenses in the different study groups were incubated for 24 hours in the above-mentioned experimental conditions followed by examination for presence of any opacity and photo-documentation. Thereafter, lenses were washed, weighed and processed for determination of biochemical parameters. Each lens was homogenized in 1 ml of 0.1 M phosphate buffer (pH 7). The homogenate was divided into two equal parts; one part was used for the estimation of reduced glutathione (GSH) and the other for polyols.

GSH content was estimated using the method described by Moron et al.<sup>15</sup> The homogenate was centrifuged at 5000 rpm for 15 minutes at 4°C. The supernatant was isolated and 0.5 ml of 10% trichloroacetic acid was added followed by repeat centrifugation to obtain a protein-free supernatant. The supernatant was then reacted with 4 ml of 0.3 M of

$\text{Na}_2\text{HPO}_4$  (pH 8.0) and 0.5 ml of 0.04% (wt/vol) 5,5'-dithiobis-2-nitrobenzoic acid. The absorbance of the resulting yellow color was measured by spectrophotometry at 412 nm. A parallel standard was also maintained.

Polyol estimation was performed as described by West and Rapoport.<sup>16</sup> The homogenate was reacted with 0.6 M perchloric acid. The precipitate was removed by centrifugation and the supernatant was neutralized with a 2 Normal solution of NaOH. The precipitate was removed again by centrifugation and the clear supernatant was reacted with freshly prepared 0.125 M stannous chloride and 0.2% chromotropic acid. The absorbance of the purple colored complex was measured spectrophotometrically at 570 nm. A parallel standard specimen was subjected to the above-mentioned steps for measurement of polyols in the samples.

### Galactose Cataract *in vivo*

Wistar rats of either sex, weighing 60-80 g were divided into control and treated groups of 8 rats each. Cataract was induced in all groups by feeding 30% of galactose diet *ad libitum*. TF, 1, 2.5 and 5% diet was prepared from the aqueous extract and fed to treated groups 7 days prior to initiating the galactose diet and was continued until the end of the experiment. Control animals received only distilled water and the galactose diet. Eyes of the rats were examined using a slitlamp after dilating the pupil with 1% tropicamide (Tropicacyl, Sunways [India] Pvt. Ltd, Mumbai). Cataracts were graded according to Sippel's classification<sup>17</sup> as follows: stage 0, normal; stage I, faint peripheral opacities; stage II, irregular peripheral opacities with slight involvement of the center of the lens; stage III, pronounced opacity readily visible as white spots; and stage IV, mature cataract.

### Statistical Analysis

All data were expressed in mean  $\pm$  standard deviation. The groups were compared by one-way ANOVA using post-hoc Dunnett's test, with significance level set at  $P < 0.05$ .

## RESULTS

### Lens Morphology *in vitro*

All lenses incubated in DMEM alone remained transparent. However, after 24 hours of incubation with galactose, all control lenses developed dense opacities. Incorporation of TF (25, 50 and 100  $\mu\text{g}/\text{ml}$ ) in the culture medium prevented the development of opacity to different extents such that at the lowest dose, 64% of lenses remained transparent while using the 50  $\mu\text{g}/\text{ml}$  dose, 84% of the lenses remained transparent as compared to controls. The same 84% of the lenses remained clear with 100  $\mu\text{g}/\text{ml}$  concentration. The lowest effective concentration (50  $\mu\text{g}/\text{ml}$ ) was chosen for further biochemical studies

### Effect on GSH and Polyols (Osmotic Stress Markers) *in vitro*

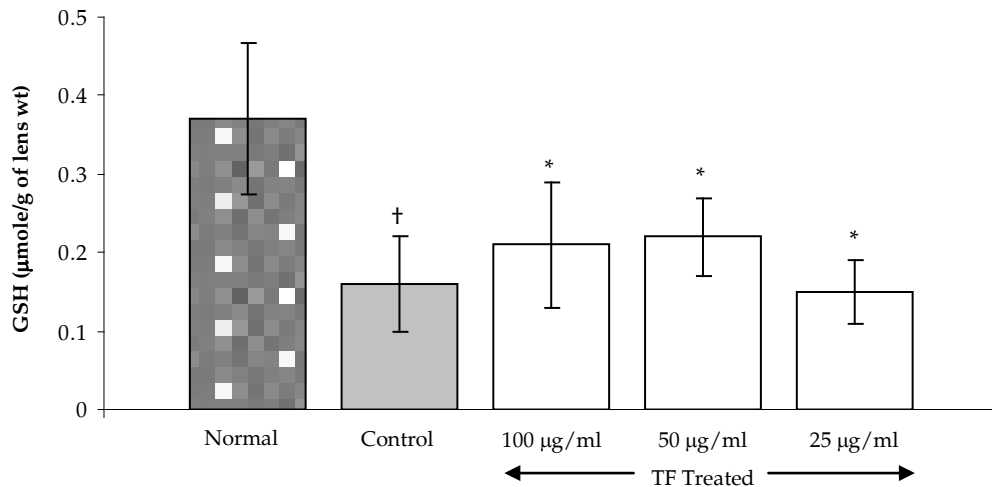
GSH level was  $0.33 \pm 0.10$  and  $0.16 \pm 0.06$   $\mu\text{mol}/\text{g}$  of lens weight in normal and control groups respectively ( $P < 0.05$ ). Corresponding figures in lenses treated with 25, 50 and 100  $\mu\text{g}/\text{ml}$  of TF were  $0.15 \pm 0.04$ ,  $0.22 \pm 0.05$  and  $0.21 \pm 0.08$   $\mu\text{mol}/\text{g}$  of lens weight respectively (Fig. 1) which were significantly different from controls. Polyol level in the normal group (no treatment) was found to be  $0.08 \pm 0.02$   $\mu\text{g}/\text{mg}$ . Polyol level was  $0.71 \pm 0.16$   $\mu\text{g}/\text{mg}$  of lens weight in controls but  $0.38 \pm 0.01$  and  $0.20 \pm 0.13$   $\mu\text{g}/\text{mg}$  of lens weight in lenses treated with 25 and 50  $\mu\text{g}/\text{ml}$  of TF ( $P < 0.005$ ). TF at 50  $\mu\text{g}/\text{ml}$  concentration was found to inhibit polyol accumulation ( $0.40 \pm 0.08$   $\mu\text{g}/\text{mg}$  of lens weight) as compared to controls (Fig. 2).

### Effect on Galactose Cataract Formation *in vivo*

Different stages of cataract formation in control and treated groups are shown in figure 3 and opacity indices in all groups at various time intervals are presented in figure 4. Onset of cataract formation was within 7 days of galactose feeding in both control and treated groups. All control eyes developed lenticular changes. In treated groups receiving 1, 2.5 and 5% TF diet, the onset of cataracts was delayed such

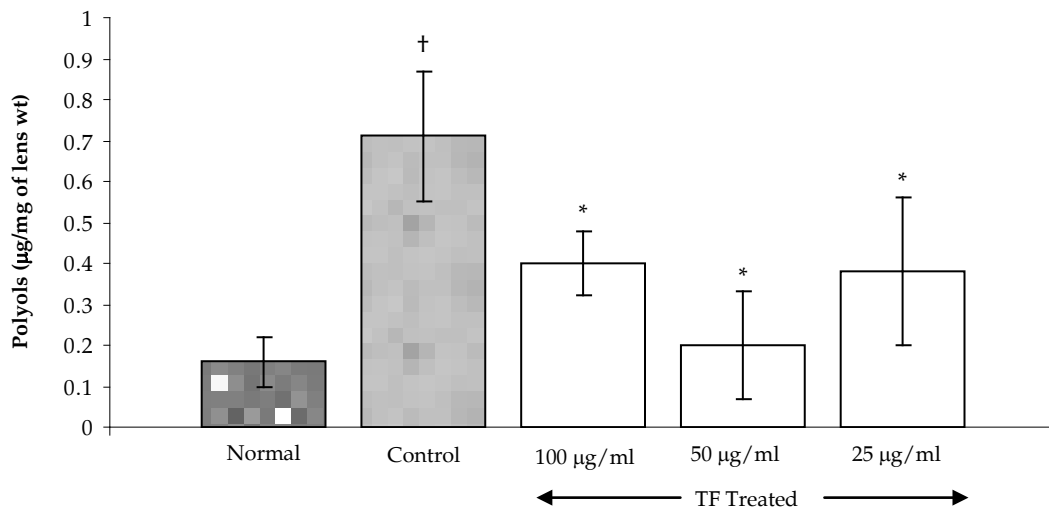
that among 2.5% TF diet fed animals, 12.5% of eyes were normal on the seventh day. On the 14th day, all eyes were in stage II in the control group whereas no eye in the 2.5% TF treated group was in stage II. On the 21st day, none of the eyes in the 2.5% TF treated group were in

stage III while 85.7% of eyes in the control group had stage III cataracts. After 30 days, 100% of control eyes developed mature cataracts, whereas none of the eyes in the 2.5% TF diet fed rats were found to develop mature cataracts.



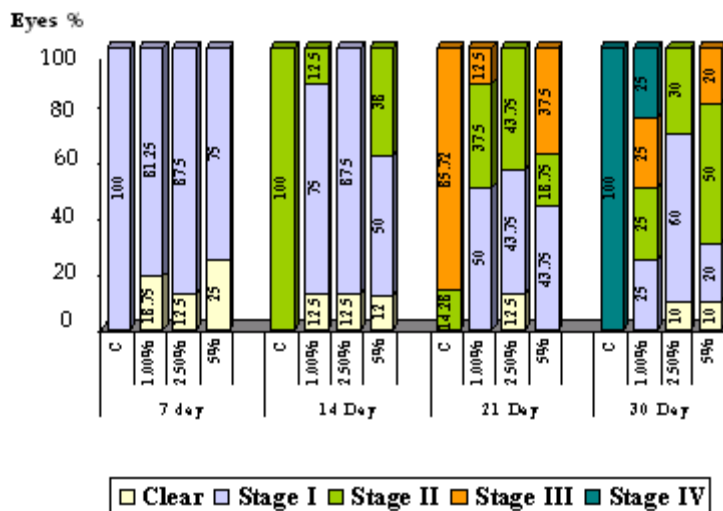
Values are mean ± standard deviation; wt, weight; †P<0.05 (control vs normal); \*P<0.005 (treated vs control); n=6.

**Figure 1** Effect of *Trigonella foenum-graecum* (TF) on glutathione (GSH) levels in galactose induced osmotic stress *in vitro*.



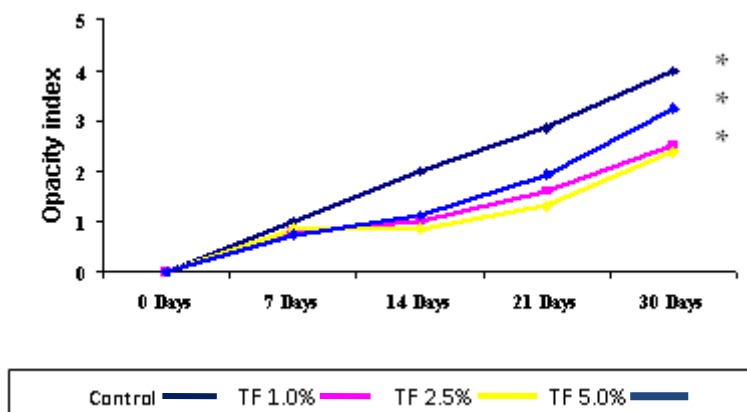
Values are mean ± Standard deviation; wt, weight; †P<0.005 (controls vs normal); \*P<0.01 (treated vs controls); n=6.

**Figure 2** Effect of *Trigonella foenum-graecum* (TF) on polyol levels in galactose induced osmotic stress *in vitro*.



C, control; n=20.

Figure 3 Effect of *Trigonella foenum-graecum* (TF) on different stages of galactose cataract formation.



\*P<0.01 (as compared to controls)

Figure 4 Effect of *Trigonella foenum-graecum* (TF) on progression of galactose cataract.

## DISCUSSION

Cataract is a multifactorial condition associated with several risk factors and is responsible for 50% of blindness worldwide.<sup>18</sup> Although surgery is currently the only remedy for cataracts, it is unable to reduce the incidence of blindness significantly because of the postoperative complications such as posterior capsular opacification, endophthalmitis and uncorrected residual refractive errors.<sup>6</sup> It has been estimated that delaying cataract onset by 10 years could reduce the need for surgery by as much as half.<sup>19</sup> Therefore any pharmacological intervention

that prevents or slows the progression of cataractogenesis may have a significant health impact. In recent years, great emphasis has been placed on exploring the use of natural substances to delay the onset and progression of cataracts.<sup>20-22</sup> In earlier studies, we reported that *Ocimum sanctum* and *Camellia sinensis* possess antioxidant properties and offer protection against cataracts.<sup>7-10</sup>

Studies are ongoing to explore the potential of herbal drugs against cataractogenesis in various experimental models of cataract. Among these models, the galactose induced cataract is commonly used, as it produces large

amounts of its reduced form, galactitol, than does glucose. Furthermore, galactitol is not subsequently metabolized as compared to sorbitol.<sup>23</sup> In the galactose model it is reasonable to assume that factors initiating galactose cataracts in young rats are similar to those involved in the human galactose cataract model.<sup>24</sup> The three mechanisms possibly involved in galactose cataract formation are the polyol pathway, oxidation, and non-enzymatic glycation.<sup>25</sup> Accumulation of sugar alcohol inside the lens leads to osmotic stress. Non-enzymatic glycation is also involved in cataractous changes; under hyperglycemic conditions, excess glucose reacts non-enzymatically with proteins. Accumulation of advanced glycation end-products in diabetic eyes contributes has been demonstrated to accelerate cataractogenesis in hyperglycemic experimental animals and diabetic humans.<sup>26</sup>

The amount of GSH in the lens decreases almost in any type of cataract. The role of GSH in the maintenance of lens clarity is of considerable interest; it serves as the major anti-oxidant in the lens and keeps proteins in reduced form.<sup>27,28</sup> Phytoconstituents from herbal drugs may indirectly inhibit consumption of GSH through oxidation leaving the -SH groups intact. Alternatively, they may directly stimulate GSH synthesis which may be due to a modulating effect on GSH related enzymes in the lens.<sup>29</sup> We observed GSH levels to increase in rat lenses incubated with 25, 50 and 100 µg/ml of TF, which may directly or indirectly inhibit consumption of GSH.

Accumulation of high concentrations of polyols in the lens leads to excessive hydration, sodium overload and loss of potassium ions due to an increase in intracellular ionic strength.<sup>30</sup> The resulting hyperosmotic stress associated with oxidative insult is postulated to be the primary cause for development of diabetic complications such as cataract.<sup>31</sup> In the present investigation, polyol levels were significantly decreased in TF treated rat lenses.

TF extract is rich in polyphenols, flavonoids and gallic acid, and has been evaluated for its protective effect against hydrogen peroxide induced oxidation in normal and diabetic human erythrocytes. The extract has also been

shown to significantly reduce lipid peroxidation.<sup>32</sup> Our results demonstrated that TF is capable of reducing osmotic stress, an effect which may be due to anti-diabetic action reported by Prasanna.<sup>33</sup> We also found that TF decreased oxidative stress, which may be partly due to the presence of flavonoids and polyphenols. Dietary intervention, particularly the use of traditional foods and medicines derived from natural sources, may play a vital role in the prevention of cataracts.<sup>21,34</sup> The present study revealed that feeding 1%, 2.5% and 5% TF diet offered significant protection against galactose induced cataract in rats and delayed the onset and progression of cataract.

In conclusion, *Trigonella foenum-graecum* showed *in vitro* and *in vivo* activity against galactose cataract in an experimental rat model. This effect may be attributed to maintaining higher levels of GSH as well as inhibiting the accumulation of polyols in the lens. This preliminary study is encouraging, but further studies are required to extrapolate the use of this agent in humans.

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