

# Anti cataract potential of phyllanthus niruri in galactose induced cataractogenesis of rat

Suresh Kumar Gupta<sup>1</sup>, Vivekanandhan Kalaiselvan<sup>2</sup>, Ashish Sharma<sup>1</sup>, Sushma Srivastava<sup>1</sup>, Shyam Sunder Agrawal<sup>1</sup>

<sup>1</sup> Department of Ocular Pharmacology, Delhi Institute of Pharmaceutical Sciences and Research, Sector-III, Pushp Vihar, M. B Road, New Delhi-17, India

<sup>2</sup> Kovai Medical Center and Hospital, College of Pharmacy Coimbatore-35, India

**Correspondence to:** Vivekanandhan Kalaiselvan. Kovai Medical Center and Hospital, College of Pharmacy Coimbatore-35, India. vivekarts@rediffmail.com

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## Abstract

• **AIM:** To evaluate anti cataract effect of phyllanthus niruri (PN) both *in vitro* and *in vivo* galactose induced cataract.

• **METHODS:** Aqueous extract of PN was evaluated against galactose-induced cataract both *in vitro* and *in vivo*. Galactosaemic cataract was induced in rats by feeding 300g/L galactose diet. PN was administered orally at three-dose levels 75, 150 and 300mg/kg of body weight. Rat lenses were subjected to osmotic stress *in vitro* by incorporating galactose (30mmol/L) in the culture medium. The effect of PN (720 and 880 $\mu$ g/mL) on the glutathione (GSH) and polyols levels was studied.

• **RESULTS:** PN 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 PN 880 $\mu$ g/mL concentration showed higher levels of GSH and decreased levels of polyols *in vitro*. *In vivo*, 75mg/kg significantly delayed the onset and progression of cataract as compared to control.

• **CONCLUSION:** Phyllanthus niruri delays the process of cataractogenesis in the experimental models. However, further study is required to extrapolate the use in human beings for the prevention of cataract.

• **KEYWORDS:** galactose cataract; glutathione; polyols; phyllanthus niruri

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## INTRODUCTION

Cataract, the opacification of the lens of the eye, is the leading cause of blindness, it accounts for approximately 50% of all blindness worldwide. World Health Organization launched Vision 2020, to eliminate cataract as priority diseases [1]. In India, cataract is responsible for almost 80% of blindness [2,3]. 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 [4-7].

Diabetes has been considered to be one of the major risk factors of cataract. Many *in vitro* and *in vivo* experimental studies support the view that diabetes is one of the causes of cataract [8,9]. 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 [2]. Ingestion of excessive 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 [10,11]. 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. Another pathophysiological mechanism of cataract formation includes deficient glutathione levels contributing to a faulty antioxidant defense system within the lens of the eye [12]. Normally the lens contains significant levels of reduced glutathione (GSH), which keeps the proteins in their reduced form. However, there are significantly lower levels of GSH in cataractous lenses. Therefore, prevention of polyol accumulation and maintaining GSH level to prevent cataract and diabetes has received considerable attention.

A great number of medicinal plants are reported to possess anti diabetic and offer protection in various pathological conditions such as cardiovascular diseases, neurodegeneration *etc* [13-15]. A large number of plants/species are now well recognized to possess hypoglycemic potential [16-18]. Many of these hypoglycemic agents have not been investigated for their beneficial effects on secondary complications of diabetes such as cataract. It would be of great importance to evaluate both pharmacologically and biochemically, which might be helpful in the better management of secondary complications of diabetes.

Phyllanthus niruri (Euphorbiaceae) commonly known as bhui-amla', has been used in Ayurvedic medicine for over 2000 years and has a wide number of traditional uses including diabetes [19-21]. The current study was undertaken with the aim of investigating the anti cataract potential of aqueous extract of phyllanthus niruri (PN) whole plant against galactose cataract in rats as well as against galactose induced morphological and biochemical changes *in vitro*. Together these results imply that PN may be explored as an anticataractogenic agent for diabetic cataract.

## MATERIALS AND METHODS

**Materials** Lyophilized aqueous extract of PN was obtained from Promed Exports Pvt Ltd. (New Delhi, India). 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).

**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 60-80g were divided into control and treated groups and for *in vitro* study, the lenses were enucleated from the Wistar rats (60-80g) 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 CO<sub>2</sub> 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 *in vitro*** Transparent 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 PN (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 Moron *et al* [22]. The homogenate was centrifuged at 5 000r/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 Na<sub>2</sub>HPO<sub>4</sub> (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 and Rapoport [23]. 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*** Wistar rats of either sex, weighing 60-80g was divided into control and treated groups (in each group *n*=8). 300g/L galactose was fed to all groups ad libitum induced cataract.

Seven days prior to start of galactose diet 75, 150 and 300mg/kg body weight dose of PN 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<sup>[24]</sup>.

**Statistical Analysis** All data were expressed as mean±SD. The groups were compared by one-way ANOVA using post-hoc Dunnett's test, with a  $P < 0.05$  considered as significant.

**RESULTS**

**Food Intake and Body Weights** There was no significant effect of feeding of galactose or galactose plus PN extract (75,150 and 300mg/kg body weight) on food intake and body weight of the animals during the entire course of the study.

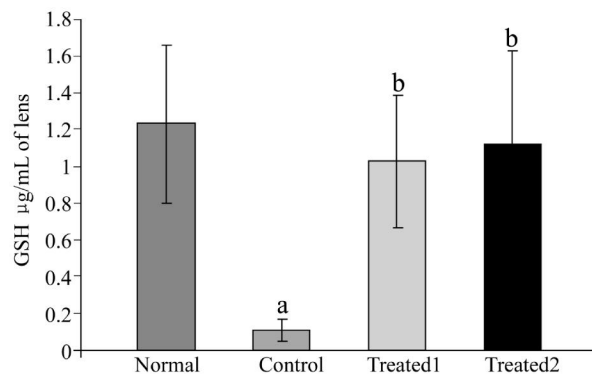
**Lens Morphology** All the lenses in DMEM alone were transparent. However, lenses after 24 hours of incubation in the presence of galactose developed dense opacity. Incorporation of PN (720 and 880µg/mL) in the culture medium prevented the development of opacity to different extent. Sixty-four percent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

**Effect of PN on GSH and Polyols** To investigate the possible mechanisms of differential effects of phyllanthus niruri at different doses on galactose-induced cataract, levels of GSH and polyols were estimated which are related to the oxidative stress and polyol pathway.

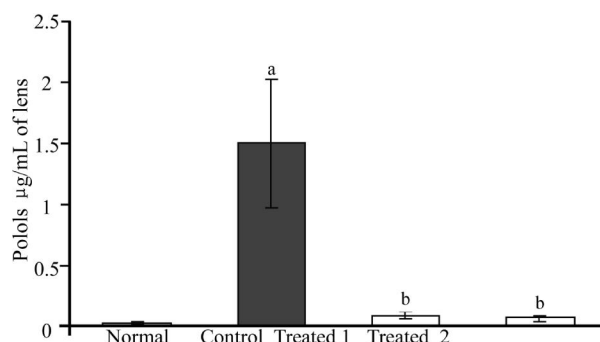
Galactose produced a significant difference in GSH in the galactose-only group lenses in comparison with normal lenses. Treatment with PN (720 and 880µg/mL) significantly restored the GSH concentration (Figure 1).

Effect of PN 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, PN at 720 and 880µg/mL concentration was found to inhibit polyol accumulation in the treated group. Treatment with PN 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 PN on Galactose Cataract in Rat** The rate of progression of cataract in the control and the treatment



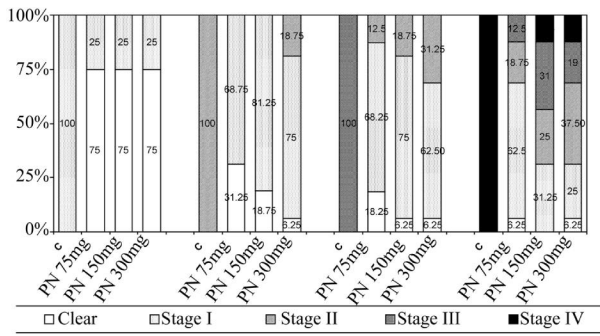
**Figure 1** Effect of phyllanthus niruri on GSH levels in galactose induced cataract rat lens Normal: DMEM, Control: DMEM+30mmol/L galactose, Treated 1: DMEM+30mmol/L galactose+720µg/mL of PN. Treated 2: DMEM+30mmol/L galactose+880µg/mL of PN. Values are mean ±SD. <sup>a</sup> $P < 0.05$ , control  $\nu$ s normal; <sup>b</sup> $P < 0.01$ , control  $\nu$ s treated  $n=6$



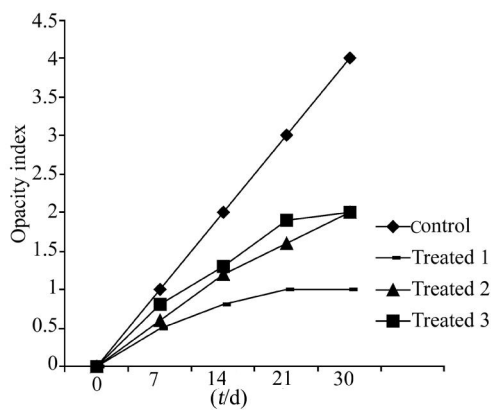
**Figure 2** Effect of phyllanthus niruri on polyols levels in galactose induced cataract rat lens Normal: DMEM, Control: DMEM+30mmol/L galactose, Treated 1: DMEM+30mmol/L galactose+720µg/mL of PN. Treated 2: DMEM+30mmol/L galactose+880µg/mL of PN. Values are mean ±SD. <sup>a</sup> $P < 0.05$ , control  $\nu$ s normal; <sup>b</sup> $P < 0.01$ , control  $\nu$ s treated  $n=6$

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.

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 30<sup>th</sup> 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 30<sup>th</sup> 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).



**Figure 3 Effect of phyllanthus niruri on the progression of galactose cataract** C: Control, Rats were fed with 300g/L galactose diet and vehicle. Test: Rats were fed PN (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 phyllanthus niruri on progression pattern of galactose cataract *in vivo*** Treated 1: PN 75mg/kg; Treated 2: PN 150mg/kg; Treated 3: PN 300mg/kg

## 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 [25]. 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 [26]. 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 [27].

Phyllanthus niruri (PN) is an herb found in central and southern India, all parts of the plant are used medicinally. Its beneficial effects for liver especially in the treatment of jaundice have been proved in clinical studies [28]. It possesses excellent hypoglycemic, antioxidant and diuretic properties [20,29]. Therefore, we have investigated PN for its anti-cataract potential against galactose-induced cataract in rats. The amount of GSH in the lens decreases almost in every type of cataract. The role of GSH in the maintenance of lens clarity is of considerable interest; which serves the major antioxidant function in the lens and keeps the proteins in their reduced form [30,31]. We found that the levels of GSH increased in the lenses of the rats treated with PN, which may directly/indirectly inhibit the consumption of GSH. Alkaloids, flavonoids saponins and phenolic compounds were found to be present in phyllanthus niruri the anticataract activity associated with extract of this plant may be attributed to the presence of these constituents [32]. 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 [33]. 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 [34]. Evidence has shown that there was a significant raise in polyols in galactosemic rats. In the present investigation polyol level was significantly decreased in PN treated rat lenses and we have also found that the PN extract was more effective in lower concentration.

The anticataractogenic effect of PN was confirmed from the results of *in vivo* study. In the present *in vivo* study, oral administration of PN showed significant protection against cataract formation in treated rats. The anticataract potential of PN seems to be related to its antidiabetic property as evident from the results of *in vitro* organ culture studies.

In conclusion, PN 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 PN in human beings for the prophylaxis or the treatment of human cataractogenesis.

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