

## Anticataract potential of *Boerhavia diffusa* roots on galactose induced cataractogenesis

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Cataract has been a major cause of bilateral blindness worldwide, and it is responsible for 50-80% of the bilaterally blindness in India. Oxidative stress is the major risk factor causing cataract. *Boerhavia diffusa*, commonly known as spreading hogweed or tarvine and locally, punarnava, is known for its antioxidant potential. Here, we evaluated anticataract potential of alcoholic extract of *B. diffusa* roots using galactose induced cataract model on Wistar albino rats. The rats were divided into five groups. The first group was taken as control, the second as disease control group and all other three groups test groups, given three doses of plant extracts i.e. 100, 200 and 400 mg/kg body wt., respectively for 28 days. Periodically slit lamp photographs were taken to know the percentage incidence and progression of cataract. The plant extract significantly delayed the onset and maturation of galactose induced cataract. Biochemical analyses were performed at the end of the study. The analyses revealed the plant extract at highest dose exhibited an efficient antioxidant effect. It has shown 69% inhibition on galactitol accumulation. Aldose reductase (AR) inhibitory activity was also performed on isolated rat lenses. The significant percentage inhibition of AR was shown at a dose of 70 µg/mL. In conclusion, our results demonstrated that the alcoholic extract of *Boerhavia diffusa* roots delay the process of cataractogenesis in galactose induced cataract.

**Keywords:** Aldose reductase, Antioxidant potential, Cataract, Galactitol, Punarnava, Oxidative stress, Spreading hogweed, Tarvine

Cataract, a visual impairment causing disturbance in lens transparency occurs mainly due to opacification or optical dysfunction of the crystalline lens. It reduces the amount of incoming light and results in deterioration of the vision<sup>1</sup>. Apart from senile cataract, various other factors, such as oxidative stress, diabetes, excessive exposure to ionising radiation, inflammatory diseases of the eye increase the risk of cataract. In all these factors, oxidative damage plays a most important role in causing cataract<sup>2</sup>. Surgically, cataractous lens can be replaced with artificial lens; however, epidemiologically the problem persist owing to the cost and post operational complications of surgery<sup>3</sup>. There are no plants which are proven clinically but more work is now going on curcumin, though at pre-clinical stage only<sup>4,5</sup>. Surgery is the alternative for treating cataract.

Aldose reductase (AR), also known as aldehyde reductase, is NADPH dependant oxidoreductase

which catalyses the reduction of galactose into galactitol. Galactitol can't be further metabolised. When the concentration of galactitol increases it causes pronounced increase in lens hydration. It leads to osmotic imbalance and formation of vacuoles in the eye<sup>6</sup>.

Few plants have been reported in the ancient literature for ophthalmic use, but most of them have no scientific data. *Boerhavia diffusa* (Fam.: Nyctaginaceae), commonly called Tarvine or Spreading hogweed, locally known as 'punarnava', is a herbaceous perennial plant well distributed all over India and has been shown to have a wide range of biological activities such as anthelmintic<sup>7</sup>, anticancer<sup>8</sup>, antidiabetic<sup>9</sup>, antidepressant<sup>10,11</sup>, antihaemolytic<sup>12</sup>, antifungal<sup>13</sup>, antileishmanial<sup>14</sup>, antimetastatic<sup>15</sup>, antimicrobial<sup>16</sup>, antioxidant<sup>17</sup>, free radical scavenger<sup>18</sup>, hepatoprotective<sup>19</sup>, immunomodulatory<sup>20</sup> and neuroprotective<sup>21</sup> activities. It is also having many folklore uses, including eye problems<sup>22-24</sup>. However, scientific information on the use of this plant in treating eye diseases is not available yet. Hence, we

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explored *Boerhavia diffusa* for anticataract activity, if any.

## Materials and Methods

### Chemicals

NADPH, dl-glyceraldehyde and galactitol (Sigma Aldrich), Quercetin (Euca enterprises, Pune, India), Galactose, dithiobisnitro benzoic acid (DTNB) reagent, thiobarbituric acid (TBA), hydrogen peroxide, lithium sulphate and mercapto ethanol (SD Fine chemicals).

### Animals

The animals in the present study were treated in accordance with the institutional guidelines (CPCSEA approval no. 1358/ac/10) for the use of animals in research. Wistar albino rats of either sex weighing 100-120 g (aged 6 weeks) were used for the study. An acclimatization period of 15 days was allowed before the start of the experiment.

### Extract preparation

The roots of the punarnava *Boerhavia diffusa* (Linn.) were procured from the local market and the plant material was authenticated (authentication number is 1210) by Dr. K Mahdava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India. Alcoholic extract was prepared by maceration process for 3 days followed by soxhlet extraction for 3 h<sup>25</sup>. The extract was dried in vacuum oven at 60°C. Three doses i.e. 100, 200 and 400 mg/kg body wt. were used for the study.

### Acute toxicity study

An acute toxicity test was performed according to the Organization of Economic Cooperation and Development (OECD) guideline 420 for testing of chemicals. Rats of both sex, aged 6-8 weeks old were used. The plant extract was dissolved in 10% Tween 20 and administered orally (only once) at a single dose of 5000 mg/kg at a rate of 20 mL/kg to both male and female rats ( $n = 12$ ; 6 males and 6 females), whereas the control group received only 10% Tween 20 as a vehicle. After administration of extract, rats were observed for 24 h, with special attention given to the first 4 h and once daily further for a period of 14 days. Observations, such as sedation, convulsions, tremors, salivation, lethargy, death, etc. were systematically recorded with individual records of each animal<sup>26,27</sup>. No mortality was seen at this dose level.

### Determination of Aldose reductase inhibitory activity by *in vitro* method

Aldose reductase (AR) inhibitory activity was performed according to the method described by Hayman and Kinoshita<sup>28</sup>. Eyeballs were removed from 9 wk old rats. Lenses were removed by extra capsular extraction by posterior approach, washed with saline and their fresh weights were recorded. Lenses were homogenized in phosphate buffer (pH 7.4), centrifuged and supernatant was collected and used as a source of aldose reductase. Four doses @ 10, 30, 50 and 70 µg/mL of AR were taken for the study, and Quercetin as standard. The percent inhibition with test compound was calculated considering the AR activity in the absence of inhibitor as 100%.

### Experimental methodology

The rats were divided into five groups, each with seven animals ( $n=7$ ). Group I served as normal control; Group II was disease control group; and groups III-V were given BD extract through oral route @ 100, 200 and 400 mg/kg body wt., respectively. All the animals were caged separately with similar ambient environment and the diurnal rhythm was maintained throughout the course of the study. The animals were fed with a balanced diet in the form of pellets and free access to 30% galactose solution except control group was provided throughout the experimental period<sup>29,30</sup>. Treatment was followed for 28 days.

The eyes were examined every week using a slit lamp biomicroscope (NeuMicromed International Pvt. Ltd.) after dilating the pupil with a topical ophthalmic solution containing 1% tropicamide. Initiation and progression of lenticular opacity were graded according to Sippel's classification (Table 1)<sup>31-33</sup>. The incidence of cataract appearance was expressed as the percentage of total lenses in each group.

At the end of the experiment, animals were anaesthetized mildly with low doses of ketamine and lenses were extracted, washed and each lens

Table 1 — Sippel's classification of lenticular opacity

|           |   |
|-----------|---|
| Stage 0   | No vacuoles present or clear lens.  |
| Stage I   | Vacuoles of less than one third of the lens radius.   |
| Stage II  | Vacuoles located at the periphery of the lens occupying an area of between one-third and two thirds of the radius from the periphery. |
| Stage III | Vacuoles extending up to two-thirds of the radius from the periphery. Nuclear opacity may be seen.                                    |
| Stage IV  | Vacuoles cover the entire lens, which appears white to the naked eye (mature cataract) <sup>32-34</sup> .                             |

was homogenized in 1 mL of 0.1 M phosphate buffer (pH 7). The homogenate was used for the estimation of reduced glutathione (GSH), catalase, malondialdehyde (MDA), and galactitol.

#### Biochemical estimations of oxidative stress marker and antioxidant enzymes

Reduced glutathione was estimated using the method of Ellman<sup>34</sup>. Catalase was estimated using method of Aebi<sup>35</sup>. MDA was estimated according to the method of Ohkawa<sup>36</sup>.

#### Estimation of galactitol

The homogenate was treated with 0.5 mL of alcohol to precipitate proteins. It was centrifuged at 8000 rpm for 20 min. The supernatant was used for the estimation of galactitol. Quantification of galactitol (dulcitol) in the biological samples carried out on a Shimadzu LC-6A liquid chromatograph pump with a 7725 Rheodyne valve injector 20  $\mu$ L fixed loop (Japan Spectroscopic C., Ltd., Tokyo, Japan) equipped with LC solutions software "class VP series version 5.03" (Shimadzu). A refractive index detector, polymerex column LiChro CART $\text{\AA}$  250-4 HPLC-Cartridge, LiChros- Pher  $\text{\AA}$  100 RP-18 (5  $\mu$ m) and RP-8 (10  $\mu$ m), Merck, Darmstadt, Germany (25 $\times$ 4.6 $\times$ 5  $\mu$ m) with a temperature of 80 $^{\circ}$ C, cell temperature of 60 $^{\circ}$ C. A 20  $\mu$ L of the sample was injected into the chromatograph and eluted with water in the isocratic mode at a flow rate of 1.0 mL per min. The run time is 15 min<sup>37</sup>. Galactitol was quantified on the basis of the area by the use of calibration curve.

#### Statistical analysis

The difference between the toxicant and treated groups were analyzed using one way ANOVA followed by TURKEY'S multiple comparison test. The differences were considered significant if P was at least <0.05.

## Results

#### Aldose reductase inhibitory activity

The percentage inhibition of the plant extract and standard against AR enzyme was measured at four different doses (Fig. 1). The IC<sub>50</sub> values were determined by nonlinear regression analysis with a plot of percent inhibition versus log inhibitor concentration. The significant inhibitory effect was produced at a dose of 70  $\mu$ g/mL in both standard and plant extract. The plant extract and standard inhibited rat lens ALR2 with an IC<sub>50</sub> value of 38 and 39  $\mu$ g, respectively.

#### Lens morphology

Slit lamp photographs (Fig. 2) were taken on 14<sup>th</sup> and 28<sup>th</sup> day of the experiment to observe % incidence and progression of cataract. It was observed by slit lamp biomicroscopy on hour after dilation of pupils. The number of lenses that developed opacity against the total number of lenses was considered for calculating the percentage of incidence of cataract in each group (n=7) (Fig. 3). Results indicate that all the rats in group I appeared to be normal and free of opacity during the experimental period. (Fig. 2A). Animals in group II developed stage II cataract in 71.4 % of the lenses and the rest of the lenses in stage III on 14<sup>th</sup> day of the experiment. At the end of the study, 14.28 % of the lenses were in stage III and

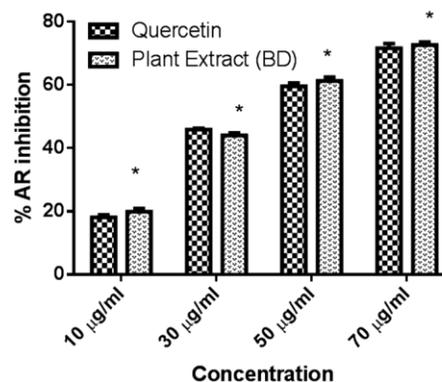


Fig. 1 — Effect of *Boerhavia diffusa* on % inhibition of aldose reductase in galactose induced cataract. [The results are mean $\pm$ S.E. of four parallel measurements.  $P < 0.05$ . The asterisk (\*) denotes that the data are significantly differ from standard group]

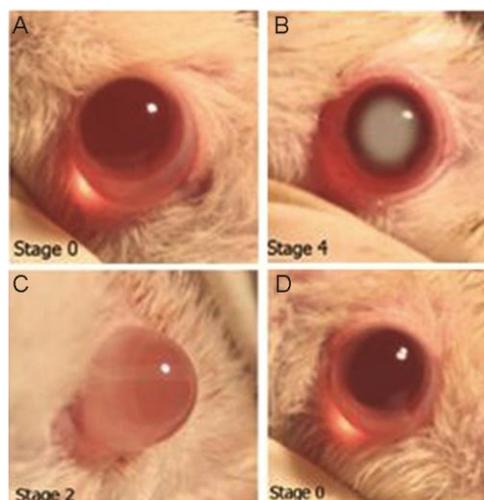


Fig. 2 — Representative photographs of lens from each group at the end of 28 days. (A) Group I (normal control); (B) Group II (disease control group); (C & D) Group IV & V (*Boerhavia diffusa* root extract @ 200 and 400 mg/kg body wt., respectively).

remaining all rats developed mature cataract (stage IV) (Fig. 2B). Rats in the group III slightly delayed the onset of cataract marginally on the 14<sup>th</sup> day of the study. At the end of the study, maturation was not affected as 28.5 % of the lenses were in stage I, II and stage III and only 14.85 % in stage IV in group III rats. Maturation of cataract was also not shown in group IV rats, as only each of 28.5% of the lenses were in stage I and II (Fig. 2C), 14.28 % lenses were in stage III and IV and 14.85% of the lenses had not developed any lenticular opacity on the 28<sup>th</sup> day of the study. Treating with higher doses of BD i.e. 400 mg/kg (group V) shown significant effect on the incidence of cataract as there were 28.5% of the lenses were in stage I, 42.85% were in stage II and no opacity was

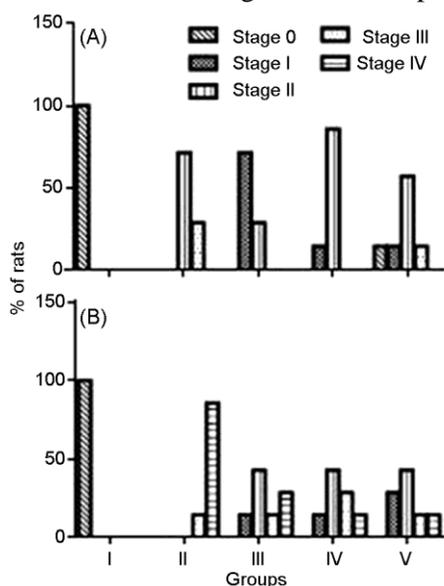


Fig. 3 — Effect of *Boerhavia diffusa* on (A) onset of galactose induced cataract in rats. Cataract progression in rats was observed on 14<sup>th</sup> day of the experiment; and (B) maturation of galactose induced cataract in rats. Cataract progression in rats was observed on 28<sup>th</sup> day of the experiment

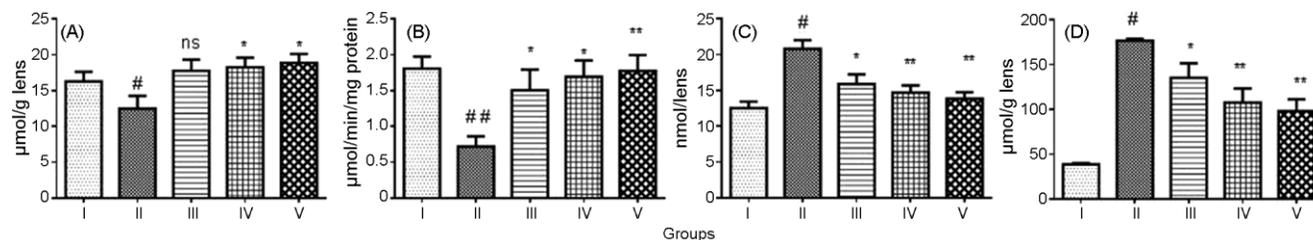


Fig. 4 — Effect of *Boerhavia diffusa* on (A) rat lens glutathione level in galactose induced cataract. One way ANOVA results – F was 3.066 and df was 4 (between columns); (B) rat lens catalase level in galactose induced cataract. One way ANOVA results – F is 4.389 and df is 4 (between columns); (C) Rat lens MDA level in galactose induced cataract. One way ANOVA results – F is 8.440 and df is 4 (between columns); and (D) Rat lens galactitol level in galactose induced cataract. One way ANOVA results – F is 18.54 and df is 4 (between columns). [The results are mean±S.E. of four parallel measurements. p<0.05. The asterisk (\*) denotes that the data are significantly differ from Group II. This (#) indicates that the data is significantly differ from Group I. 'ns' indicates not significant]

observed in 28.5% of lenses on the 14<sup>th</sup> day of the experiment. On the 28<sup>th</sup> day of the study, 28.50% had not developed any opacity (Fig. 2D); 28.50 % of the rats in stage I and II and only 14.28 % in stage III. No rat was observed in stage IV. This higher dose of BD showed promising effects on progression of cataract.

#### Estimation of GSH

A significant decrease in GSH ( $12.47 \pm 1.78$   $\mu$ moles/g lens) was observed in the presence of galactose in group II when compared to the group I ( $16.28 \pm 1.35$   $\mu$ moles/g lens). Treatment with the plant extract significantly increased the GSH ( $17.98 \pm 0.87$  &  $18.28 \pm 1.36$   $\mu$ moles/g lens in groups III & IV, respectively) content in a dose dependent manner when compared with group II which is probably due to inhibition of free radical formation by the plant extract (Fig. 4A)

#### Estimation of catalase

A significant decrease in catalase ( $0.72 \pm 0.14$   $\mu$ moles/min/mg protein) level was observed in the group II as opposed to the group I ( $1.81 \pm 0.17$   $\mu$ moles/min/mg protein). Treatment with the plant extract significantly increased the catalase content in a dose dependent manner ( $1.70 \pm 0.27$  &  $1.77 \pm 0.22$   $\mu$ moles/min/mg protein in groups IV & V, respectively) when compared with toxicant group (Fig. 4B).

#### Estimation of MDA

A significant increase in MDA level was found in group II ( $20.81 \pm 1.21$  nmoles/lens) opposed to the group I ( $12.51 \pm 0.92$  nmoles/lens). The plant extract significantly protected the test group lenses from lipid peroxidation in group III ( $13.91 \pm 1.43$  nmoles/lens) and in group IV ( $13.02 \pm 1.45$  nmoles/lens). The data clearly demonstrated that this plant significantly improves the antioxidant defense mechanisms of the normal lens (Fig. 4C).

### Estimation of Galactitol

Biochemical estimation of galactitol in the lens shows 38.5  $\mu\text{moles/glens}$  was observed in group I lens and 176.8  $\mu\text{moles/glens}$  in group II rats. Treatment with the plant extract shown a reduction in the galactitol content in a dose dependant manner. 60.8  $\mu\text{moles/glens}$  of galactitol was observed in group V rats (Fig. 4D). The % inhibition of galactitol accumulation was also calculated for all the groups. It was 78-fold higher when lenses were incubated in a high-galactose medium, compared with incubation in a galactose-free medium. Group III, IV and V rats inhibited galactitol accumulation in rat lens by 23.35, 55.03 and 65.61%, respectively.

### Discussion

Cataract is produced more rapidly by galactose feeding than by diabetes<sup>38</sup>. It produces a greater increase in its reduced form, galactitol and it does not further metabolise as sorbitol, the reduced form of glucose and it has a lesser affinity to the lens fiber cells. Polyols, being relatively impermeable through biological membranes, once formed may accumulate in the lens fiber cells and cause increased cell hydration, increased electrolyte concentration, membrane stretching, and dysfunction and leads to opacity. This polyol pathway takes place in the presence of the enzyme, aldose reductase.

Aldose reductase (or aldehyde reductase) is a NADPH dependent oxidoreductase enzyme, that catalyses the reduction of a variety of aldehydes and carbonyls including monosaccharides. It is present in the lens, retina, schwann cells of peripheral nerves, placenta and red blood cells. It catalyzes the NADPH-dependent conversion of glucose to sorbitol, the first step in polyol pathway of glucose metabolism. The second and last step in the pathway is catalyzed by sorbitol dehydrogenase, which catalyzes the NAD-linked oxidation of sorbitol to fructose. Thus, the polyol pathway results in conversion of glucose to fructose with stoichiometric utilization of NADPH and production of NADH<sup>38</sup>. Galactose is also a substrate for the polyol pathway, but the corresponding keto sugar is not produced because sorbitol dehydrogenase is incapable of oxidizing galactitol. Nevertheless, aldose reductase can catalyze the reduction of galactose to galactitol.

Aldose reductase is the key enzyme in preventing or delaying the onset of diabetic complications in experimental models provides a strong support to the hypothesis that ALR2 inhibition could be a useful

strategy in the prevention or delay of certain diabetic complications like cataract<sup>40</sup>. The results of the AR inhibitory potential of *B. diffusa* in the form of IC<sub>50</sub> value was found to be highly significant when compared with standard quercetin. Significant percent inhibition was found in 70  $\mu\text{g/mL}$  in both the plant extract and standard given lenses.

In eye lens, reactive oxygen species (ROS) attack biological molecules, including DNA, protein and phospholipids leading to lipid peroxidation and depletion of the antioxidant enzymes, SOD, GST, GPX and catalase, resulting in further oxidative stress. Biochemical estimation showed that the plant extract was observed to exhibit anticataract effect as demonstrated by enhanced activities of antioxidant enzymes, GSH and catalase, and diminished the amount of lipid peroxide against galactose induced oxidative stress. It may suggest that the antioxidative<sup>40-42</sup> property of plant extract contributes to the cataract preventive effect observed in the present study. The level of these enzymes was significantly hampered with galactose treated rats and positively modulated in the presence of plants.

The plant, *Boerhavia diffusa* has reported to contain different secondary metabolites in which flavonoids<sup>43,44</sup> are present in high concentration. Flavonoids are poly phenols which reduce free radical formation and scavenge the free radicals. Due to the presence of flavonoids hence antioxidant activity may be attributed to the inhibition of lipid peroxidation and the increase in antioxidant enzyme concentration.

Galactitol is a sugar alcohol found in the erythrocytes, liver, kidneys and lens. The accumulation of galactitol in the lens causes osmotic imbalance and leads to opacity<sup>45</sup>. The plant extract significantly inhibited the galactitol accumulation when compared to toxicant group. This may be due to the inhibitory potential of the plant on aldose reductase enzyme.

Three possible mechanisms that may affect cataract formation as a result of hyperglycemia or hyper galactosemia are the oxidation, polyol pathway and nonenzymatic glycation<sup>46</sup>. The plant *Boerhavia diffusa* showed significant effect on the polyol pathway since it inhibited the aldose reductase enzyme and galactitol accumulation and also shown an inhibitory effect on oxidative stress induced by galactose feeding.

### Conclusion

In conclusion, *Boerhavia diffusa* root extract exhibited significant anticataract activity against

galactose cataract in experimental animals The effect can be attributed to inhibition of polyol pathway and oxidative stress. Further study may reveal the effect of different active constituents responsible for anticataract activity.

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