Hepatoprotective Activity of *Bacopa monniera* on D-galactosamine Induced Hepatotoxicity in Rats

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Abstract – Hepatoprotective action of alcoholic extract of *Bacopa monniera* (BME) was evaluated on D-galactosamine (D-GalN) induced rat liver toxicity. *Bacopa monniera* extract reduced the elevated serum enzyme activities of ALT, AST, ALP, LDH, γ-GT and the formation of hepatic malondialdehyde induced by D-GalN. The alcoholic extract of *Bacopa monniera* also significantly restored the decreased levels of glutathione and the decreased activities of glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and glucose-6-phosphatase. Therefore these results suggest that *Bacopa monniera* has hepatoprotective effect against D-GalN induced hepatotoxicity.

Keywords – *Bacopa monniera*, D-galactosamine, Hepatotoxicity.

Introduction

*Bacopa monniera* (Linn) Wettst. (Syn. *Herpestis monniera* (Linn) H.B & K) is a small creeping herb commonly growing in marshy places throughout India ascending to 1320 m, known as brahmi in the Ayurvedic system of medicine. It is a potent nerve tonic used in the treatment of epilepsy, insanity, hysteria and other mental disorders. It is claimed to improve memory and mental functions (Chopra et al., 1956; The wealth of India Raw materials, 1988). The saponin fraction exhibited barbiturate hypnosis potentiation in rats and clinical trials showed it to be an antianxiety agent with an adaptogenic effect (Singh et al., 1979a). The drug was shown to have a tranquilizing effect with an improvement in mental function (Singh et al., 1979b). The saponins designated as bacosides A and B improved the performance of rats in several learning tests as manifested by better acquisition, consolidation and retention of newly acquired behavioural responses (Singh and Dhawan, 1982; Singh et al., 1988). Bacosine, a triterpenoid isolated from the plant showed potent analgesic activity (Vohora et al., 1997). The plant extract also exhibited antiepileptic (Martis et al., 1992), antioxidant (Tripathi et al., 1996), adrenergic (Khanna and Ahmed, 1992) and anticancer (Elangovan et al., 1995) activities. The facilitatory effects of bacoside on the hippocampus, hypothalamus and cerebral cortex have been demonstrated, together with their safety in clinical trials (Singh and Dhawan, 1997). Also *Bacopa monniera* exhibited its antioxidant activity on rat brain regions (Bhattacharya et al., 2000), inhibitory effect on superoxide released from Polymorphnuclear cells (Pawars et al., 2001), protective role on oxidative DNA damage (Russo et al., 2003), antiaddictive properties (Sumathy et al., 2002), protective role on morphine induced hepatotoxicity (Sumathy et al., 2001). This study was undertaken to evaluate the hepatoprotective effect of *Bacopa monniera* extract (BME) against D-Galactosamine induced hepatotoxicity in rats.

Experimental

Chemicals – D-Galactosamine hydrochloride, 2-thiobarbituric acid (TBA) and 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from SRL, India. Reduced glutathione (GSH) and NADPH were purchased from sigma chemicals (St. Louis, USA). All other reagents used were analytical grade.

Preparation of plant extract – The plant material was collected at Chennai, Tamilnadu, India and was authenticated by Dr. P. Brindha, Botonist, Captain Srinivasasamriti Drug Research Institute for Ayurveda, Anumbakkam, Chennai. The shade dried and coarsely powdered whole plant material (1 kg) was extracted with...
90% ethanol in the room temperature (48 hrs). The extract was filtered and distilled on a water bath to obtain a dark green syrupy mass. It was finally dried in vacuo (yield 52 g). It was dissolved in water and given orally as an aqueous suspension.

**Animals** – Adult male albino rats of Wister strain weighing 150 - 200 g were purchased from Tamilnadu Veterinary and Animal Science University, Chennai, Tamilnadu, India. They were housed in an acrylic fiber cage in a controlled room (Temperature 22 ± 2°C) and were maintained on a 12 h light/dark cycle. They were given a solid diet and water ad libitum.

**Experimental design** – Rats were randomly divided into four groups of six animals each. Group I served as normal control and received normal saline for 7 days. Group II served as toxic control and received normal saline for 7 days. Group III and IV were treated with BME (40 mg/kg, p.o.) for 7 days. Group III and IV were also given a solid diet and water ad libitum.

Group II and III also received D-GalN (400 mg/kg, i.p.) on day 7 after final supplementation of BME. After 24 h of D-GalN administration blood was collected from tail vein under supplementation of BME. Blood samples were allowed to clot for 30 - 40 min. Serum was separated by centrifugation at 3000 rpm for 15 min at 37°C and were used for various biochemical parameters. Liver samples collected were washed with chilled normal saline, weighed and 10% (w/v) liver homogenates were made in ice cold 0.15 M KCl solution using motor driven Teflon pestle. All the procedures carried out on animals were approved by institutional ethical committee.

**Assays** – Alanine transaminase (ALT), Aspartate transaminase (AST) (Reitman and Frankel, 1957), alkaline phosphatase, glucose 6 phosphate (G6P) (King, 1965 a), lactate dehydrogenase (LDH) (King, 1965b) and γ glutamyl transferase (γGT) (Rasalki and Rau, 1972) activities in the serum were assayed by reported procedure. The enzyme activity of ALT, AST, ALP, γGT were expressed as IU/L, G6P as nmole of phosphate liberated/mg protein/min and LDH activity was expressed as nmole of pyruvate liberated/mg protein/min.

**Antioxidant status** – Total reduced glutathione was estimated by 5, 5 dithiobis-2-nitrobenzoic acid (DTNB) Moron et al. (1979) and expressed as µg/mg protein. Superoxide dismutase was assayed according to the method of Marklund and Marklund (1974) and expressed as unit/mg protein. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogalol auto oxidation. Catalase was assayed according to Sinha (1972) and was expressed as µmole of H2O2 consumed/min/mg protein. Glutathione peroxidase was performed by Rotruck et al. (1973) and expressed as nmole of glutathione oxidized/min/mg protein. Glutathione reductase was assayed by Pinto and Bartley (1969) and was expressed as nmole of oxidized glutathione (GSSG) utilized/min/mg protein. Lipid peroxidation measured by the method of Ohkawa et al. (1979) and expressed as nmole of MDA formed/min/mg protein. Total protein in tissue homogenate was estimated by Lowry et al. (1951).

**Statistical analysis** – Data are expressed as mean ± SD. Significance of difference was evaluated using Student’s t-test and P < 0.05 were considered as significant.

### Results and Discussion

The level of total GSH was decreased (P < 0.001) significantly, the activities of SOD (P < 0.001), catalase (P < 0.001), GPX (P < 0.001), and GR (P < 0.01) were also found to be decreased in D-GalN induced rats (group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td></td>
<td>control</td>
<td>D-GalN</td>
<td>BME + D-GalN</td>
<td>BME</td>
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<tr>
<td>GSH</td>
<td>6.05 ± 0.45</td>
<td>3.34 ± 0.40 a,b</td>
<td>5.68 ± 0.63 b</td>
<td>6.15 ± 0.41a NS</td>
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<td>SOD</td>
<td>7.38 ± 0.38</td>
<td>3.97 ± 0.16 a,b</td>
<td>6.79 ± 0.21 b</td>
<td>6.99 ± 0.46 a NS</td>
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<td>Catalase</td>
<td>73.95 ± 8.07</td>
<td>44.47 ± 4.4 a,b</td>
<td>69.73 ± 5.77 b</td>
<td>73.29 ± 7.56 a NS</td>
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<tr>
<td>GPX</td>
<td>168.24 ± 12.74</td>
<td>86.19 ± 5.99 a,b</td>
<td>162.33 ± 5.75 b</td>
<td>163.47 ± 8.58 a NS</td>
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<tr>
<td>GR</td>
<td>43.24 ± 4.88</td>
<td>31.46 ± 4.35 a,b</td>
<td>41.56 ± 4.41 b</td>
<td>42.52 ± 3.93 a NS</td>
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<tr>
<td>LPO</td>
<td>1.67 ± 0.12</td>
<td>3.19 ± 0.19 a,b</td>
<td>1.73 ± 0.13 b</td>
<td>1.67 ± 0.11 a NS</td>
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Each value represents mean ± SD; n = 6. 
1-a as compared with group I; b as compared with group II.
2-a,b as compared with group I.
3-P < 0.001; 4-P < 0.01; 5-P < 0.05; NS-Not statistically significant.

GSH (µg/mg protein), SOD (unit (50% inhibition of pyrogalol auto oxidation)/mg protein), Catalase (µmole of H2O2 consumed/min/mg protein), GPX (nmole of glutathione oxidized/min/mg protein), GR (nmole of oxidized glutathione (GSSG) utilized/min/mg protein), LPO (nmole of MDA formed/min/mg protein).