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

T. Sumathi* and S. Ramakrishnan

Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600113, Tamilnadu, India

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 alcholic 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 tranquillizing 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 Polymorpho nuclear 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 hepato-toxicity in rats.

Experimental

Chemicals – D-Galactosamine hydrochloride, 2-thio-barbituric 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 Srinivasamurti Drug Research Institute for Ayurveda, Arumbakkam, Chennai. The shade dried and coarsely powdered whole plant material (1 kg) was extracted with

*Author for correspondence
Fax: +91-44-24540709; E-mail: sumathi_doctor@yahoo.co.in

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 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 light ether anaesthesia. Immediately, after blood withdrawal all the groups were sacrificed. Liver samples were also collected for biochemical estimations. The 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 was 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 phosphatase (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 nmoles of phosphate liberated/mg protein/min and LDH activity was expressed as nmoles 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 pyrogallol auto oxidation. Catalase was assayed according to Sinha (1972) and was expressed as µmoles of H_2O_2 consumed/min/mg protein. Glutathione peroxidase was performed by Rotruck *et al.* (1973) and expressed as nmoles of glutathione oxidized/min/mg protein. Glutathione reductase was assayed by Pinto and Bartley (1969) and was expressed as nmoles of oxidized glutathione (GSSG) utilized/min/mg protein. Lipid peroxidation measured by the method of Ohkawa *et al.* (1979) and expressed as nmoles 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 1. Effect of BME on tissue defence systems of the rats intoxicated with D-galactosamine

Parameters	Group I control	Group II D-GalN	Group III BME + D-GalN	Group IV BME
GSH	6.05 ± 0.45	3.34 ± 0.40 $a^{\$}$	5.68 ± 0.63 $b^{\$}$	6.15 ± 0.41a^{NS}
SOD	7.38 ± 0.38	3.97 ± 0.16 $a^{\$}$	6.79 ± 0.21 $b^{\$}$	6.99 ± 0.46 a^{NS}
Catalase	73.95 ± 8.07	44.47 ± 4.4 $a^{\$}$	69.73 ± 5.77 $b^{\$}$	73.29 ± 7.56 a^{NS}
GPX	168.24 ± 12.74	86.19 ± 5.59 $a^{\$}$	162.33 ± 5.75 $b^{\$}$	163.47 ± 8.58 a^{NS}
GR	43.24 ± 4.88	31.46 ± 4.35 $a^{\#}$	41.56 ± 4.41 $b^{\#}$	42.52 ± 3.93 a^{NS}
LPO	1.67 ± 0.12	3.19 ± 0.19 $a^{\$}$	1.73 ± 0.13 $b^{\$}$	1.67 ± 0.11 a^{NS}

Each value represents mean ± SD; n = 6.
a-as compared with group I; b-as compared with group II.
$^{\$}P < 0.001$; $^{\#}P < 0.01$; $^{@}P < 0.05$; NS-Not statistically significant.
GSH (µg/mg protein), SOD (unit (50% inhibition of pyrogallol auto oxidation)/mg protein), Catalase (µmoles of H_2O_2 consumed/min/mg protein), GPX (nmoles of glutathione oxidized/min/mg protein), GR (nmoles of oxidized glutathione (GSSG) utilized/ min/mg protein), LPO (nmoles of MDA formed/min/mg protein).

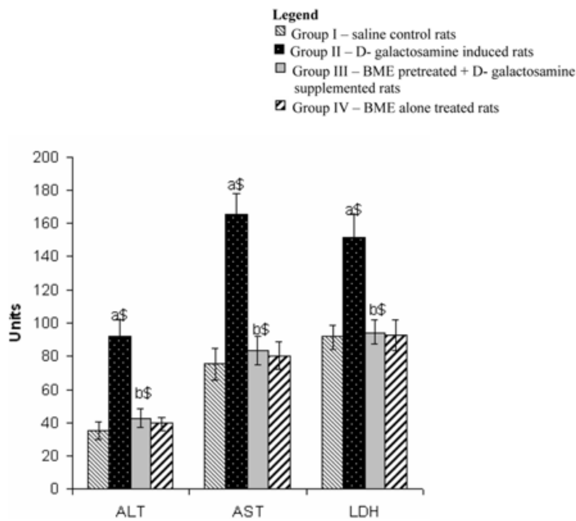

Fig. 1. Effect of BME on marker enzymes in serum of control and experimental rats.
Each value represents mean ± SD; n = 6.
a-compared with group I; b-compared with group II.
$^{\$}P < 0.001$; $^{\#}P < 0.01$; $^{@}P < 0.05$; NS-Not statistically significant.
Alanine transaminase (ALT),Aspartate transaminase (AST) (IU/L), Lactate dehydrogenase (LDH) (nmoles of pyruvate liberated/mg protein/min).

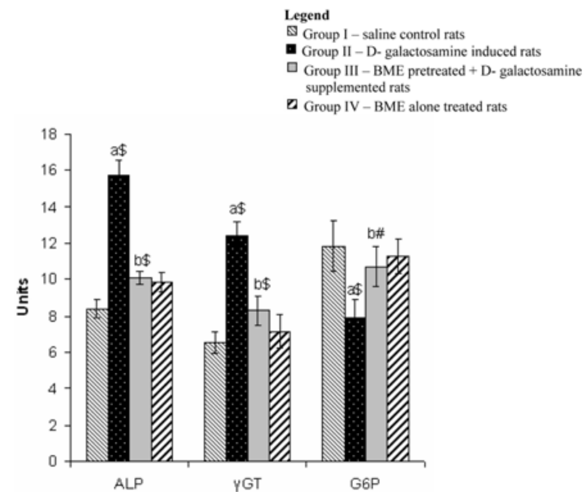

Fig. 2. Effect of BME on marker enzymes in serum of control and experimental rats.
Each value represents mean ± SD; n = 6.
a-compared with group I; b-compared with group II.
$^{\$}P < 0.001$; $^{\#}P < 0.01$; $^{@}P < 0.05$; NS-Not statistically significant.
Alkaline phosphatase (ALP), γ glutamyl transferase (γGT) (IU/L), Glucose 6 phosphatase (G6P) (nmoles of phosphate liberated/mg protein/min).

II) compared with control rats (group I) and a significant increase in the level of LPO ($P < 0.001$) in D-GalN induced rats (group II) compared with control rats (group I) have also been observed (Table 1) Pretreatment with BME (group III) prevented the D-GalN induced change in the levels of GSH, and LPO and also the activities of antioxidant enzymes compared with group II. BME alone treated rats (group IV) had no effect on the activities of these enzymes when compared with control rats (group I).

Fig. 1 illustrates the activities of clinical marker enzymes viz., ALT, AST and LDH in serum of normal and experimental rats. In D-GalN induced rats (group II), the level of marker enzymes viz., ALT ($P < 0.001$), AST ($P < 0.001$) and LDH ($P < 0.001$) were found to be significantly increased when compared with the control (group I) rats. Whereas in BME pretreated (group III) rats, these marker enzymes were maintained at near normal level when compared with the group II rats. Serum marker enzymes were not significantly affected by BME alone treated rats (group IV) when compared with control rats (group I).

Fig. 2 shows the activities of ALP, γ-GT and G6P in serum of normal and experimental rats. In D-GalN induced rats, the level of marker enzymes viz., ALP ($P < 0.001$), γ-GT ($P < 0.001$) and G6P ($P < 0.01$) were significantly altered in serum when compared with the control (group I) rats, whereas in BME pretreated (group III) rats the marker enzymes were maintained at near normal level when compared with the group II rats. BME alone treated rats (group IV) did not show any change in the activities of the above marker enzymes when compared to control rats.

The present study has demonstrated that BME has protective effects against liver damage induced by D-GalN in rats. Liver damage induced by D-GalN generally reflects disturbances of liver cell metabolism, which leads to characteristic change in serum enzyme activities. In our study the rise in AST and ALT level induced by G-GalN was significantly reduced by BME pretreatment shows hepatoprotective activity. However, in recent years, it has been suggested that ROS might be the primary cause in D-GalN-induced liver damage (Stachlewitz *et al*., 1999).

In our study the exposure of D-Galactosamine decreases the liver antioxidant enzymes as compared to control rats. Several previous studies also reported the lowered activities of antioxidant enzyme following D-GalN challenge (Anandan and Devaki, 1998). The BME used in the present study seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane. This is evident from the fact that pretreatment of the rats with BME significantly prevented the toxic effects of D-GalN on the liver as judged by the decreased activities of the marker enzymes of hepatic function studies as compared to the D-GalN

induced rats. Pretreatment with BME attenuate the increased activities of these enzymes, this suggest the possibility of BME able to cause accelerated regeneration of paranchymal cells and protecting against membrane fragility and there by decreasing the leakage of marker enzymes into the circulation. In the rats given BME alone there was no significant change in the activities of these enzymes as compared to the control rats thereby showing the absence of adverse toxic effects of the plant extract. Further studies are required to clarify the hepatoprotective mechanisms of the plant extract against GalN induced hepatotxicity.

The above result clearly showed the hepatoprotective effect of alcoholic extract of Bacopa monniera against D-galactosamine induced liver injury in rats and its antioxidative potential against the oxidative stress induced by the liver damage. In our previous report Sumathy *et al.* (2001) we have shown the hepatoprotective effect of the same extract against morphine induced liver damage. The antioxidant activity of the extract has already been reported by Tripati *et al.* (1996) and Bhattacharya *et al.* (2000). The hepatoprotective activity of BME could be explained by the fact that, it is classified as a rasayana drug in Ayurvedic medicine. Rasayana drugs are general tonics improving health and immunomodulant potential.

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