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HEPATOPROTECTIVE POTENTIAL OF *IN VITRO BACOPA MONNIERI* L AGAINST CARBON TETRACHLORIDE - INDUCED HEPATOTOXICITY IN ALBINO MICE

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ABSTRACT

Bacopa monnieri L plants raised through micropropagation were evaluated for the production of major secondary metabolite, 'Bacoside-A' and its pharmacological properties in comparison to the conventional source i.e. , natural (*in vivo*) plants. The hepatoprotective function of the ethanolic extract containing Bacoside-A of *in vitro* grown *B. mannieri* was evaluated in carbon tetrachloride (CCl_4) - intoxicated albino mice. Analysis of ethanolic extract of *in vitro* raised Bacopa plants by HPTLC confirmed presence of Bacoside-A identical to that of seed borne Bacopa. Administration of *Bacopa* ethanolic extract from either source (*in vitro* or natural) markedly prevented CCl_4-induced hepatic damage in albino mice model as indicated by the levels of serum markers (SGPT, SGOT and Bilirubin) of hepatic damage. The present study confirmed that *in vitro* generated *Bacopa monnieri* produces 'Bacoside-A' with pharmacological function identical to that generated from natural plantlet. Thus, *in vitro Bacopa* cultures serve as efficient alternate source of 'Bacoside-A' for pharmacological use.

KEY WORDS: **SGPT, SGOT, *Bacopa,* Bacoside-A, Hepatotoxicity, Bilirubin, CCl_4**

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INTRODUCTION

Bacopa monnieri L. commonly known as Brahmi is an important nootropic plant used in Indian Ayurvedic preparations. In Ayurveda, the herb has been extensively used for the treatment of insanity, epilepsy and hysteria. The various medicinal properties of Brahmi includes anticonvulsive, memory enhancer[1], analgesic, cardiotonic, sedative, spasmodic, anti-cancerous activity, anti-anxiety agent, relieves and prevents stress[2], anti ulcerogenic activity, digestive aid as well as improve respiratory function in case of bronchoconstriction. Further, neuroprotective [3], antioxidant[1], [4, 5] and hepatoprotective [6, 7, 8], anti lipid peroxidation[9] activities of *Bacopa* extract are well documented. The pharmacological properties of *Bacopa monnieri* are extensively studied and the activities are attributed mainly to the presence of characteristic saponin called "Bacosides". 'Bacoside- A' is the principal bioactive compound of the plant [1].

According to the NMPB report (2007), the annual market demand for Brahmi is around 1,000 tones (in year 2000) and is expected to be more in the coming years due to its potential applications in ayurvedic system of medicine. As the requirement of *Bacopa* is met exclusively from the wild, there is a rapid depletion of this plant from the wild and the plant is listed as 'endangered species' by the International Union for Conservation of Nature and National Resources [10]. On the basis of ayurvedic and pharmaceutical importance and potential for further research and development in the field of herbal biotechnology, *B. monnieri* is now placed second in a priority list of the most important Indian medicinal plants [11]. It is one among 32 medicinal plants identified for cultivation and conservation by the NMPB (National Medicinal Plants Board 2004), Government of India and one among the 7 important medicinal plants recommended for immediate attention and included in the list as a highly endangered medicinal plants in India by NMPB and Technology Information Forecasting and Assessment Council (TIFAC), (http://www.nmpb.nic.in/prioritisemedicinalplants.htm).

Encouraging reports are available in the literature utilizing organ cultures for exploitation of secondary metabolite production in various medicinal plants [12]. Hence, tissue cultures could be considered as a viable alternative for production of bacoside to meet the demands of present market. Although, *in vitro* regeneration studies in *B. monnieri* have been attempted [13- 18] limited information on the production of bacosides in cell, organ culture is available. Further, the pharmacological validations of the bioactive molecules produced *in vitro* are not available. Present study, therefore, aimed to analyze nature and hepatoprotective function of 'Bacoside –A' present in *in vitro* grown *Bacopa monnieri* L.

MATERIALS AND METHODS

In the present study, we tested the hepatoprotective, and antioxidant activity of the i*n vitro* regenerated plants ethanolic extracts in comparison to the natural *(in vivo*) extract using Swiss albino mice as experimental system (approved by IAEC) and SGPT (Serum Glutamate Phosphate Transaminase), SGOT (Serum Glutamate Oxaloacetate Transaminase), and Bilirubin as biochemical markers.

1. Plant Material

The seeds of *Bacopa monnieri* (Brahmi) were collected from Shivpuri town 103 Km away from Gwalior) and were grown in earthern pots in the green house. Aerial parts of the plants (leaves and stems) were collected from 3 month old plants and used for the present study.

2. In vitro studies

The callus was raised from leaf explants on Murashige and Skoog's [19] (MS) (1962) + 4.52 µM/l 2, 4-D + 2% sucrose medium and suspension cell cultures were developed from the established callus cultures. Liquid cultures were then maintained for two weeks on orbital shaker at 25°C temperature, 24hr illumination and 150rpm shaking. Cell suspension cultures were then transferred to petri plates containing MS medium with BAP for multiple shoots and plantlet regeneration. Finally, plantlets were transferred to root trainers and then to field [18]. The *in vitro* regenerated plants were grown separately in earthen pots.

3. Preparation of plant extracts

Aerial parts from 3 months old *in vitro* generated as well as seed generated plants were collected in bulk from the green house. The aerial parts were washed, shade dried and then milled into coarse powder using a mortar and pestle. Ethanolic extract of the plant material was prepared from 1g powdered plant material using standard protocol [9]. The ethanolic extract was dried and stored in desiccators for further use.

4. Analysis of Bacopa extract

The ethanolic extracts were subjected to HPTLC according to the procedure described by Gupta *et al*.[20].

5. Animals

Male Swiss albino mice of 60-65g weight (obtained from DRDE, Gwalior) were used in this experiment. They were maintained in a 12h light/dark cycle at 25˚C. Animals were provided with standard dry diet and normal sterile water. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC).

6. Experimental design

Animals were divided into four Groups (I-IV), each with 6 mice: Group I (Normal control), Group II (CCl_4 treated) toxicant control, Group III (seed based plant extract + toxicant) and Group IV (*in vitro* raised plant extracts+ toxicant). Group I animals were fed with normal saline water at a single dose per day by oral route. Group II. III and IV were fed with 0.5ml of 5% CCl_4 at a single dose per day by oral route for induction of hepatic injury (HI). Serum markers of hepatic damage viz., SGPT, SGOT and Bilirubin (total and direct) were estimated on every alternate day. The animals in Group II were continued on CCl_4 till the end of the experiment. Group III animals were fed with seed based plant extract (400 mg/kg body weight) along with 5% CCl_4 at a single dose per day. Similarly animals of Group IV were fed with *in vitro* grown plant extract (400 mg/kg body weight) along with 5% CCl_4 at a single dose per day.

7. Serum Analysis

At the end of one week feeding of different plant extracts to Group III and IV animals, blood was drawn (by ocular bleeding) from animals of all groups (I –IV), sera were separated and used for the assay of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Bilirubin (total and direct), using the ERBA Mannheim kits (Transasia bio-medicals LTD, Baddi, India).

8. Data Analysis

The experiment was repeated twice and the data were pooled and standard error (SE) was calculated

RESULTS AND DISCUSSION

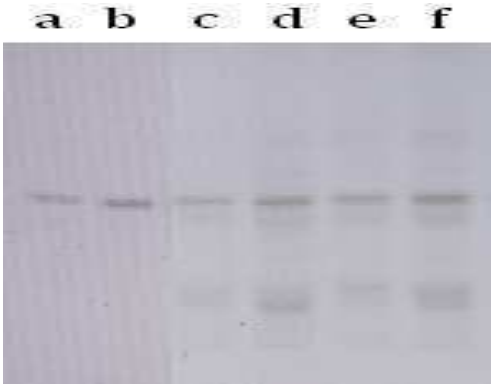
Plant callus cultures are found to be potent sources of plant secondary metabolites. Production of medicinally important secondary metabolites in micropropagated plants as well as *in vitro* cultures of several plants has been achieved earlier[21-26]. The present study compared natural *Bacopa* plants and those raised by tissue culture means as sources of the medicinally important plant metabolite 'Bacoside-A'. Multiple shoots and plantlets were raised from suspension cell cultures of

Bacopa (Figure-1) and he regenerated plants were then sequentially transferred to root trainers, thermacol cups and then finally to pots (Figure-2). *In vitro* generated plants were successfully maintained in the pots for prolonged period. HPTLC analysis of ethanolic extracts of *Bacopa* raised by either *in vitro* means or seed borne confirmed the presence of Bacoside-A indicating the identical nature of the 'Bacoside-A ' from both the Figure 3.

Figure 1
Multiple shoots and plantlets raised from suspension cell cultures of Bacopa

Figure 2
In vitro raised plants successfully maintained in the thermacol cups and pots.

Figure 3
HPTLC plate showing the Bacoside-A separation.



a and b- standard and c, d, e and f in Regenerated Plants

Table 1
SGPT, SGOT, and Bilirubin levels in serum samples of mice

S. No.	Groups	SGPT (IU/l) ± S.E	SGOT (IU/l) ± S.E	Bilirubin mg/ml ± S.E	
				Total	Direct
1	Control	218.75±0.64	218.00 ±0.98	5.37 ± 0.42	4.44 ± 0.12
2	Toxicant	306.52± 4.46	314.14 ± 2.10	9.76 ± 0.79	7.68 ± 0.29
3	Toxicant+ *in vivo*	234.97 ± 2.52	231.38 ±3.98	7.31 ± 0.23	5.68 ± 0.06
4	Toxicant +*in vitro*	237.63 ± 0.25	233.49±1.55	7.59 ± 0.36	5.94 ± 0.03

Figure 4
SGPT and SGOT *levels in serum samples of mice*

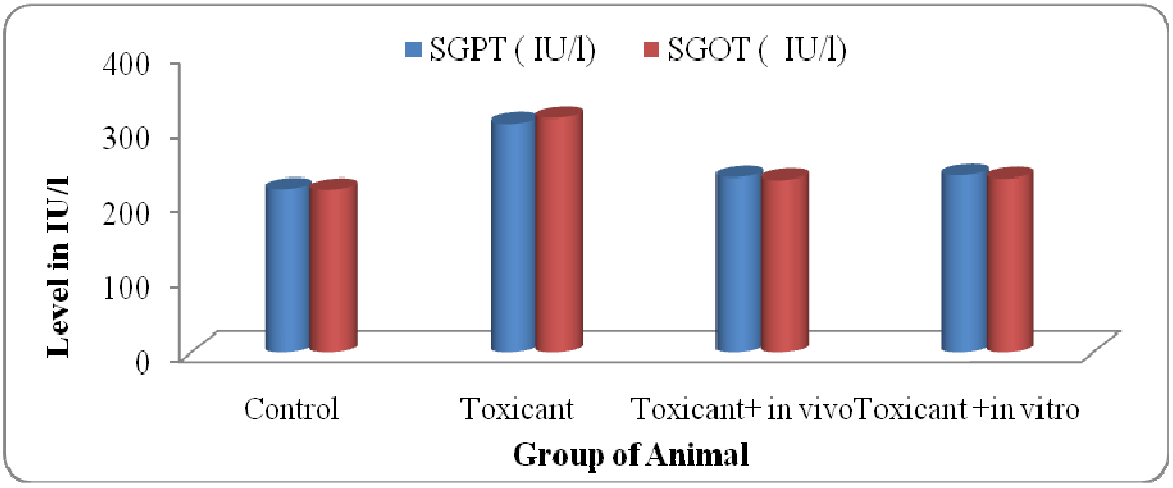
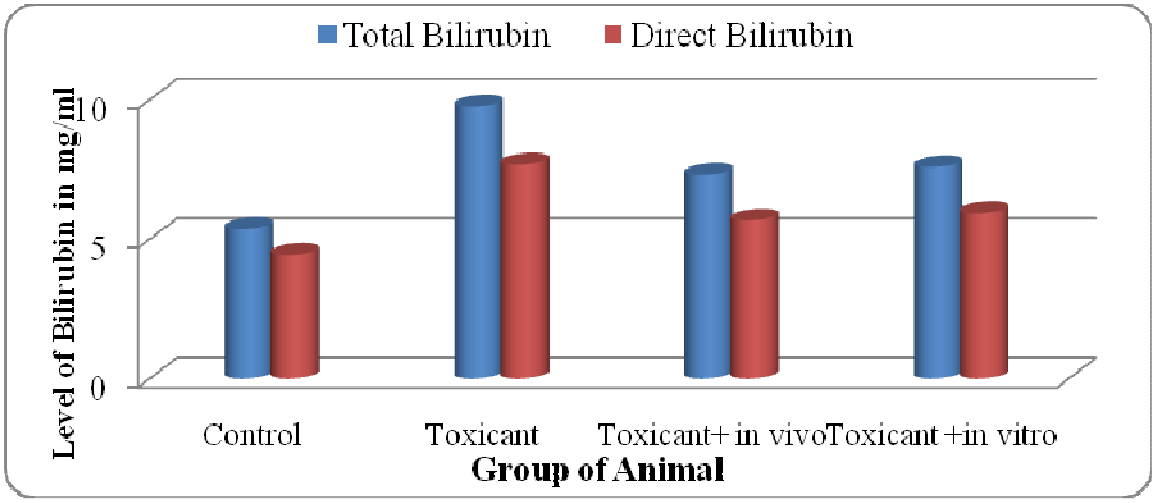


Figure 5
Total Bilirubin and Direct Bilirubin levels in serum samples of mice



Chemically induced hepatotoxicity in the animals has been widely used for the screening of hepatoprotective herbal compounds[27]. This mimics both pathogen induced as well as chemical induced liver injury in man. Earlier studies have already shown that CCl_4 induces cirrhosis of the liver similar to human cirrhosis [28, 29]. CCl_4 induces liver damage by the free radical mechanism [30, 31]. SGPT (Serum Glutamate Phosphate Transaminase), SGOT (Serum Glutamate Oxaloacetate Transaminase), and Bilirubin are common biochemical markers for the analysis of liver function [32]. Ethanolic extract of *Bacopa* plant is reported to be rich in saponins and this ethanolic extract has been shown to possess free radical scavenging, antioxidant, hepatoprotective and neuroprotective activities[33]. Active constituent, identified and found to be responsible for the pharmacological benefits of *Bacopa* is Bacoside-A [34]. Extensive work has been done to evaluate the pharmacological properties of *Bacopa monnieri* extract for hepatoprotective, neuroprotective, antioxidant, antidepressant and anticolvulsive activities using rat, mice and human as model systems [35, 36, 37].

In the present study, these biochemical markers were used to validate the pharmacological properties of Bacoside-A present in ethanolic extracts of *in vitro* raised plants. The results obtained from our studies are presented in Table-1 and Figure-4. In Group-I (normal control) animals, the SGPT was recorded as 218.75±0.64 IU/l and SGOT as 218.00±0.98 IU/l. In CCl_4 intoxicated animals (Group-II), the level of SGPT was increased to 306.52±4.46 IU/l and SGOT to 314.14±2.10 IU/l (Table-1) indicating the hepatic damage has been induced. Concurrent administration of CCl_4 and *Bacopa* extract (seed borne) to animals in Group-III prevented elevation of SGPT and SGOT in those animals with activities 234.97±.52 IU/l and 231.38±3.98 IU/l respectively. Similarly concurrent administration of Bacopa extract (*in vitro*) to animals of Group IV, also prevented elevation of both SGPT and SGOT with activities recorded as 237.63±0.25 IU/l and 233.49±1.55 IU/l respectively (Table-1).

Liver damage induced by CCl_4 reflects disturbances of liver cell metabolism leading to changes in the activities of serum enzymes. Elevated serum enzymes of hepatic origin are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver. Hence, significant rise in the transaminase levels could be taken as an index of liver damage. In the present experiment, serum enzymes i.e. SGPT and SGOT were found to be significantly increased in CCl_4 intoxicated mice (Group-II) as compared to control (Group-I) animals (Figure-4). However, elevation of these enzymes of hepatic origin were prevented in animals of Group-III and IV significantly, almost equaling to the control (Table-1), indicating the hepatoprotective activity of both *in vitro* generated and seed borne *Bacopa* extracts. The levels of serum Bilirubin both total and direct, recorded in Groups (I-IV) are presented in Tabel-1 and Figure- 5. In control animals, the serum Bilirubin level was recorded as 5.37±0.42 mg/ml (total) and 4.44±0.12 mg/ml (direct). After one week of intoxication, the serum Bilirubin values of Group II animals increased to 9.76 mg/ml (total) and 7.68 mg/ml (direct) (Table-1). After oral administration of *Bacopa* extract, from both *in vivo,* (Group-III) as well as *in vitro* (Group-IV) sources, the Bilirubin in Group III animals was estimated at 7.31 mg/ml (total) and 5.68 mg/ml (direct). Similarly Group IV animals recorded 7.59 mg/ml of total Bilirubin and 5.94 mg/ml of direct Bilirubin (Table-1), indicating the hepato protective function of *Bacopa* extracts (*in vivo* or *in vitro*). The present study provides strong evidence of hepatoprotective nature of ethanolic extracts of *in vitro* regenerated *Bacopa* plants similar to that of *in vivo* plantlets. The *in vitro* cell cultures as well as *in vitro* plants showed identical nature of the bioactive molecule, 'Bacoside-A' with that of the the natural plants (Figure-3). Thus this study documented potential of in *vitro* regenerated plants with

elevated levels of Bacoside-A (unpublished observations), compared to natural plants, serve as source of Bacoside-A with pharmacological properties similar to those of natural plant sources.

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