



## Therapeutic properties of *in vitro* plant aqueous extract of *Boerhaavia diffusa* L. against carbon tetrachloride induced hepatotoxicity in male and female swiss albino mice

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**ABSTRACT:** In the present study, multiple shoots were raised from *Boerhaavia diffusa* L. nodal segments on MS + BAP (6-benzylaminopurine) medium. Root induction and plant regeneration was achieved on MS + NAA (alpha naphthalene acetic acid) medium. Regenerated plants were transferred to field successfully and used for the analysis of their therapeutic potential. Albino female and male mice were separately intoxicated with Carbon tetrachloride (CCl<sub>4</sub>) for hepatic injury. Recovery of the mice with hepatic injury was noticed when concurrently administered with *in vitro* plants ethanolic extract (Group III) and also in Group IV (fed with natural plant extract) mice proved that the *in vitro* regenerated *Boerhaavia* plants produced bioactive molecules that show hepatoprotective activity similar to that of the natural ones.

**Key words:** In vitro, *Boerhaavia diffusa* L, hepatoprotective, CCl<sub>4</sub>, Intoxication.

### INTRODUCTION

Plants are important source of natural products used in pharmaceutical industries. Increase in demand for natural medicines has resulted in the production of these products by alternative approaches such as biotechnological approaches especially plant tissue cultures. Scientific approaches for the pharmacovigilance of these herbal bioactive molecules produced in *ex situ*, however, needs to be analysed.

*Boerhaavia diffusa* L. (Nyctaginaceae), commonly known as “Punarnava” in the Indian system of medicine, is a perennial creeping herb found throughout the waste lands of India.

A large number of compounds have been isolated from the roots of *Boerhaavia diffusa* L., namely punarnavine, -sitosterol, -D-glucoside, tetracosamine, hexacosanoic, ursolic acid and punarnavoside (Jain and Khanna, 1989). The major active principle present in the roots is an alkaloid known as Punarnavine. The alkaloid is responsible for the therapeutic and pharmacological properties of *Boerhaavia diffusa*. (Mishra and Tiwari 1971). *Boerhaavia* is known to possess many pharmacological properties and used to cure asthma, urinary disorders, leucorrhoea, rheumatism

and encephalitis (Chakroborty and Handa, 1989). This medicinal plant shows an excellent hepatoprotective activity (Rawat, 1997) and widely used in number of herbal drugs. A significant work has been done to exploit tissue culture for conservation and the production of active metabolites in various medicinal plants. Reports on *in vitro* regeneration of *Boerhaavia* are limited (Chaudary *et al.*, 2011, Chaudhary and Dantu 2011, 2011). However, very few reports on the evaluation of the therapeutic potential of these *in vitro* produced bioactive molecules are available (Tejovathi *et al.* 2012.).

In the present study, *Boerhaavia* plants were raised in the tissue cultures using nodal explants. The *in vitro* raised plants were successfully transferred to field. The hepatoprotective activity of the active principle produced in the *in vitro* plants was analysed and compared to natural (*in situ*) plants, using in CCl<sub>4</sub> intoxicated Swiss albino mice as model system, using Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) level as biochemical markers for assess the hepatoprotective potential of the herb.

## MATERIAL AND METHODS

**Collection of the Plant Material:** Plant material was collected locally from Gwalior city. Plants were micropropagated in the tissue culture lab to obtain the regenerated plant material. Both natural as well as regenerated whole plant material was used for the testing the hepatoprotective activity.

**Tissue culture studies:** The shoots collected from healthy plants were defoliated and were dipped in soap solution and then washed with running tap water for 10-12 min. Surface sterilization was done by following the procedure given by Tejovathi *et al* 1996. Finally, the nodal explants of 0.5-1.0 cm. were inoculated on Murashige and Skoog's (MS) medium supplemented with four different concentrations of BAP (4.44 to 21.5  $\mu$ M).

Multiple shoots of above 3 cm. length were harvested and sub cultured on the MS medium containing four different concentrations of NAA (5.4 -21.5 $\mu$ M) for the induction of roots.

Healthy plantlets were transferred onto filter paper wicks and finally these plantlets were transferred to soil and covered with poly bags. Plants were gradually acclimatized to natural environment for acclimatization and hardening.

All the *in vitro* cultures were maintained at 25 $\pm$ 1 $^{\circ}$ C and continuous fluorescent light.

The experiment was repeated thrice and the data was pooled.

**Preparation of the Drug:** To extract the drug, 5g of finely crushed dry material (regenerated as well as natural plants) was soaked in 100 ml of 70% ethanol and kept on a shaker for 7-8 hours. This extract was filtered and the process was repeated for 3 times. The total filtrate was concentrated at low temperature (40-45 $^{\circ}$ C) till the final volume was approximately 10 ml. This extract was used directly as drug. Each animal of group III and IV was given a treatment dose of 250 mg/Kg body weight.

**Therapeutic studies:** Swiss Albino mice (*Mus musculus*), 5-6 weeks old, 22 $\pm$ 2 g were procured from DRDE (Defense Research Development Establishment, Gwalior, M.P.). The animals were acclimated to temperature 24  $\pm$  2 $^{\circ}$ C and humidity 30-70% with 12 hours light and dark rhythms for a week prior to the start of the experiment. All the animals were allowed free access to standard diet and water *ad libitum*.

**Allocation of Group and Experimental Procedure:** The animals (24 males and 24 females), were randomly divided into 4 groups as below:

**A. Group I - Control:** The animals in this group were maintained as untreated control and not given any toxicant or drug.

**B. Group II - Toxicant:** All the animals were given orally toxicant *i.e.* CCl<sub>4</sub> after every 24 hours.

**C. Group III - Herbal extract from natural isolates:** All the animals were given orally toxicant *i.e.* CCl<sub>4</sub> after every 24 hours. Concurrently ethanolic extract prepared from dried plant material from naturally grown plants in the green house was administered after 6 hours of toxicant.

**D. Group IV- Herbal extract from tissue culture/Regenerated isolates:** The Animals of this group were also given CCl<sub>4</sub> orally after every 24 hours. The extract prepared from tissue culture raised and growing in the field conditions these were dried in shade to prepare the herbal extract. This extract was administered orally after 6 hours of the toxicant *i.e.* CCl<sub>4</sub>.

**Toxicant used:** The chemical used for inducing hepatic injury, Carbon tetrachloride CCl<sub>4</sub>, was purchased from Ranbaxy fine Chemicals Ltd. 10% diluted CCl<sub>4</sub> was injected intraperitoneally at a dose of 2 ml/kg body weight to all the animals of Group II, III and IV. After the confirmation of hepatic injury, analysed through the serum analysis, the Group III and IV were administered with the drug as planned.

**Serum Analysis:** After the drug treatment for the period of 9 days, serum analysis was performed to study the hepatotoxicity and its cure through the drug. Blood was drawn through ocular bleeding and the serum was separated for the assay. Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) were assayed spectrometrically in all the animals sera using ERBA Mannheim Kit (Transasia biomedical Ltd. Baddi, India). The activity was calculated International Units (IU/l) using the formula-

$$\text{IU/l} = \frac{(\text{A/min.}) \times \text{T.V.} \times 10^3}{\text{S.V.} \times \text{Absorptivity} \times \text{P}}$$

Where

T.V. = Total reaction volume in liter

S.V. = Sample volume in liter

Absorptivity = Millimolar absorptivity of NADH at 340 nm = 6.22

P = Cuvette lightpath = 1 cm

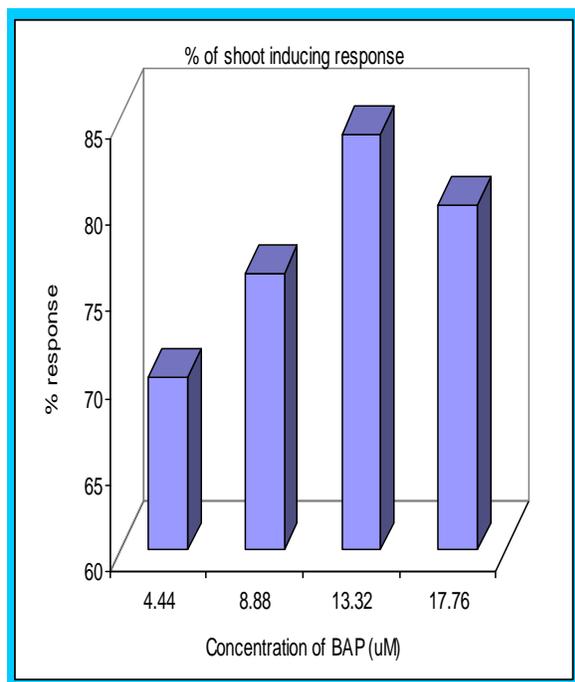
## RESULT AND DISCUSSION

Liver is the central site for the biotransformation of xenobiotic chemicals and therefore is involved in the detoxifying mechanism of the body. Liver is responsible for detoxifying the chemical substances in the blood and in this process it is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury (Glaister, 1986).

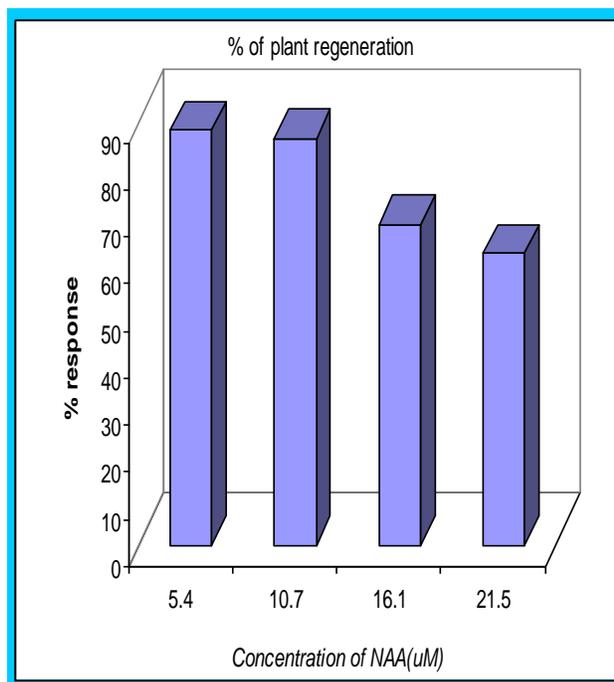
Chemically induced hepatotoxicity in animals has been widely used for the screening of hepatoprotective properties of herbal remedies (Liu *et al.*, 1994).

Carbon tetrachloride ( $\text{CCl}_4$ ) induces liver damage by the free radical mechanism and consists of two stages. Initially, liver injury is caused by the free radical of  $\text{CCl}_4$  (trichloromethyl radical  $\text{CCl}_3^\cdot$ ) followed by cascade of events in the metabolism of trichloromethyl radical  $\text{CCl}_3^\cdot$  which leads to lipid peroxidation (Timbrell, 1991), hence can be used for predicting the mode of action of the hepatoprotective agent.

*In vitro* studies: The response of nodal segments on BAP and NAA supplemented media is presented as bar diagram in Fig. 1 and 2 respectively.



**Fig. 1.** Effect of BAP concentration on Multiple shoot induction from the node explants *Boerhaavia*.



**Fig. 2.** Effect of NAA concentration on rooting and plant regeneration in *Boerhaavia*.

Multiple shoot induction response was highest on MS + 13.32 $\mu\text{M}$  BAP medium. While, lowest NAA concentration tested i.e. 5.4 $\mu\text{M}$  induced highest rooting percentage and plant regeneration (Fig. 2). Regenerated plants were successfully transferred to field and were maintained in the green house for their establishment. Extinction of medicinal plant resources from the nature has forced to look for alternative means of conservation of these plants and for their bioactive molecules production. *In vitro* techniques are the most suitable and potential methods to meet the industrial demands. Number of important medicinal plants are conserved and used for bioactive molecular extraction (Pandey

1993). However, studies are limited on the validation of the efficacy and the therapeutic potential of the extracts.

*Therapeutic studies:* Herbal compound are tested for their medicinal value on animal systems by chemically inducing the injury. Carbon tetrachloride is the most commonly used compound for inducing the hepatic injury, which induces cirrhosis of the liver by free radical mechanism. In the present study, SGPT (Serum Glutamate Phosphate Transaminase) and SGOT (Serum Glutamate Oxaloacetate Transaminase) are the two common biochemical markers that are widely used for the detection of liver injury.

The results obtained from our animal studies are given in the Table 1. Healthy albino mice (Group- I) showed 99.04 – 103.8IU/l of SGOT and about 42.13- 42.91 IU/l of SGPT activity in male and females respectively. On intoxication, the enzyme activities increased to 2780.4, 2176.7 (SGOT) and 2048.55±0.53, 1696. ± 0.33 (SGPT), in male and female mice respectively. When the natural extract was given sequentially (Group III), the enzyme SGOT and SGPT levels were reduced to 112.28 ± 0.77 IU/l, 64.79±0.87 IU/l in males, 129.41 ± 0.78 IU/l and 45.27±0.92 IU/l in females respectively. Similarly, when *in vitro* plant extract was administered concurrently, in male and females of Group IV, SGOT level was 163.76 ± 0.65 IU/l and 115.65±0.61 IU/l and SGPT level was 151.32± 0.72 IU/l and 112.93±0.66 IU/l (Table 1). When CCL<sub>4</sub> damages the liver it is reflected as disturbances in the serum enzymes activity. Elevated serum enzymes of hepatic origin are markers

for the cellular leakage and loss of liver function. A significant increase in the Transaminase enzymes could be taken as index for liver damage. In the present study, serum enzymes SGOT and SGPT, both in male and female mice in Groups II, showed 50-100 fold increase, indicating the intoxication by CCl<sub>4</sub> as compared to Group I (control) animals. However, increase in these enzymes activity was prevented when natural boerrravia plant extract (Group-III) was given (Table 1). Similarly when the *in vitro* regenerated plant extract was given along with intoxicant (Group IV) it inhibited increase in the levels of SGOT and SGPT in both male and female animals indicating the protective activity of *in vitro* extract. Our recent studies with *Bacopa in vitro* plant extract also showed likewise results, thus, proving the therapeutic effect of *in vitro* plants bioactive molecules function similar to that of the natural ones.

**Table 1: Effect of natural and regenerated herb on mice.**

S. No.	Group	Gender	SGOT (IU/l)	SGPT (IU/l)
1.a	CONTROL	Male	99.09 ± 0.98	42.13 ± 1.08
1.b		Female	103.8 ± 0.78	42.91 ± 0.55
2.a	TOXICANT	Male	2780.4 ± 0.15	2048.55 ± 0.53
2.b		Female	2176.7 ± 0.63	1696.96 ± 0.33
3.a	NATURAL	Male	112.28 ± 0.77	64.79 ± 0.87
3.b		Female	129.41 ± 0.78	45.27 ± 0.92
4.a	REGENERATED	Male	163.76 ± 0.65	215.65 ± 0.61
4.b		Female	151.32 ± 0.72	122.93 ± 0.66

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