



## Pharmacological Potential of *Boerhaavia diffusa*: An Overview

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### ABSTRACT

Ayurveda, the science of life, deals with the holistic view of healthy living. It covers various physiology and pathology of diseases and their therapies. From time immemorial mankind's efforts and ultimate aim have been to seek eternal happiness. And his endeavour has been to overcome and seek appropriate remedies for things that stand in his way. Physical and mental well being is of prime importance towards this end Medical Science while relieving his malady assures him of a healthy life man or living being for that matter is afflicted by grief or disease even since inception. Phytochemical, Pharmacological, experimental and Clinical investigations on various medicinal plants have been conducted by many scientist, researchers etc. It may be seen that the ancient Ayurvedic physicians clearly understood the process of digestion and metabolism. *Boerhaavia diffusa* (Punarnava) is one of the most famous medicinal plants in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. This paper explains the evidence-based information regarding the pharmacological activity of this plant. It has many ethnobotanical uses (the leaves are used as vegetable; the root juice is used to cure asthma, urinary disorders, leukorrhea, rheumatism, and encephalitis), and is medicinally used in the traditional Ayurvedic system.

**Keywords:** Ayurveda, Pharmacological action, *Boerhaavia diffusa*, Medicinal plant.

### INTRODUCTION

*Boerhaavia diffusa* (*Nyctaginaceae*) commonly known as Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, Shilatika [1-2] is a herbaceous plant species growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds. The plant was named in honor of Hermann Boerhaave, a famous Dutch physician of the 18<sup>th</sup> century. [3] The plant is mentioned in the Atharvaveda with the name 'Punarnava', because the top of the plant dries up during the summer season and regenerates again during the rainy season. Thus the plant generally perennates through the roots in the soil. [4] *Boerhaavia diffusa* (*Nyctaginaceae*) is one of the most famous medicinal plants in India; it is a perennial herbaceous plant growing in tropical regions such as the Antilles, South America, India and Africa. [5]

### DESCRIPTION

The roots are very variable diffusely branched low spreading

or creeping herbaceous perennial with an elongated fusiform or tapering tap root. The stems are numerous; 1-2 m long arises from the crown of the root, slender, round, nodose, jointed and often reddish or purplish in color. The leaves are simple, opposite, short petiolate, exstipulate, unequal in each pair, 2.5-5 cm long by 1-4.5 cm wide, oblong or suborbicular, acute, obtuse or rounded at apex, cordate, rounded or truncate at base, entire or wavy along the margin, subfleshy, glabrous or sparingly hairy above, silvery white beneath, petioles 0.7-3 cm long, slender, deeply grooved above. The flowers are small, regular, sessile or subsessile, pale rose to pink, in irregular clusters of 4-10, small umbels on extra axillary peduncles. Perianth-short, tubular about 3mm long and deeply constricted at the middle. The lower tubular part is greenish, persistent and is covered with glandular hairs. The upper limb is light rode, funnel shaped and 5 lobed. The stamens are 2 or 3, Filaments united in the ovary at the base and not exerted outside of the perianth. The anthers are small and 2 celled. The ovaries are small completely covered by the perianth. The fruits are very small, one seeded and enclosed in persistent lower half the perianth. The perianth is covered with sticky glandular hairs. [6]

### CHEMICAL CONSTITUENTS

Many rotenoids have been isolated from the roots of the *Boerhaavia diffusa*. [7-10] Plant also includes a series of

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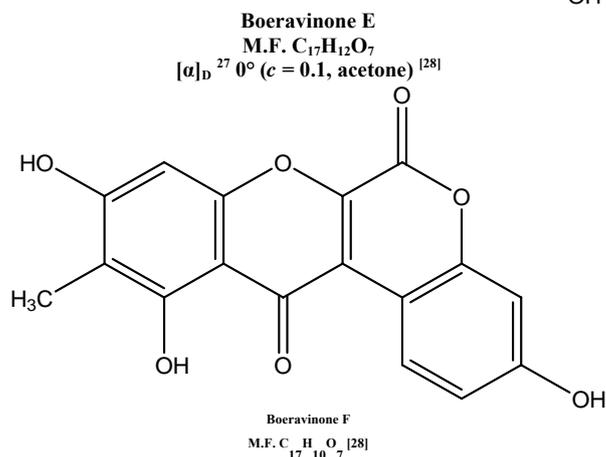
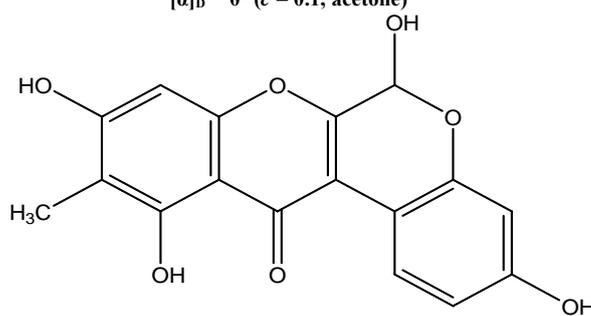
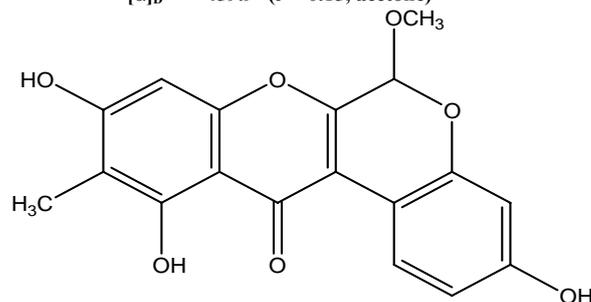
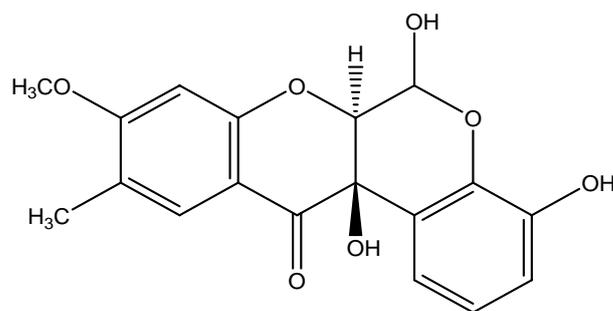
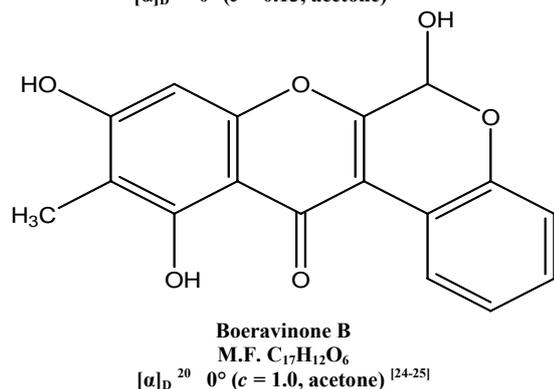
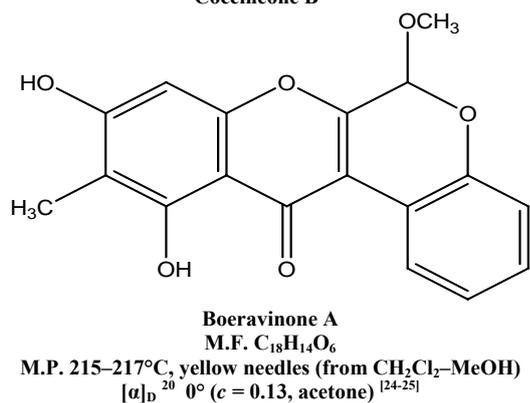
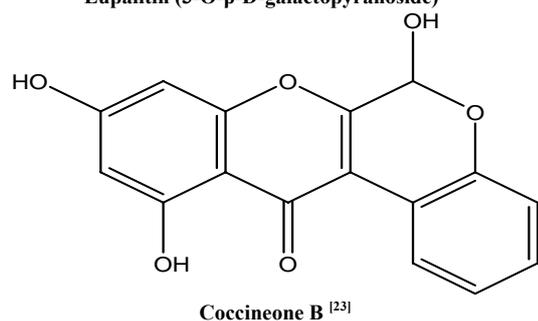
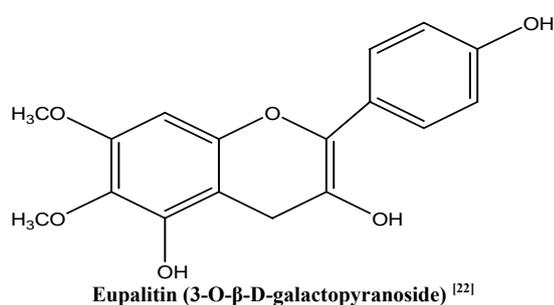
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boeravinones viz., boeravinone A, boeravinone B, boeravinone C, boeravinone D, boeravinone E and boeravinone F. Punarnavoside, a phenolic glycoside, is reportedly present in roots [11-12]. C-methyl flavone also has been isolated from *Boerhaavia diffusa* roots [13]. Two known lignans viz., liriodendrin and syringaresinol mono- $\beta$ -D-glycoside have been isolated [14]. Presence of a purine nucleoside hypoxanthine 9-L-arabinose [15], dihydroisofuroxanthone-boerhavin [16], phytosterols [17-18] have been isolated from the plant.

It contains about 0.04 % of alkaloids known as punarnavine and punarnavoside, an antifibrinolytic agent. It also contains about 6 % of potassium nitrate, an oily substance, and ursolic acid. [19] The seeds of this plant contain fatty acids and allantoin and the roots contain alkaloids. [20] The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid. [21]



## ADULTERANTS AND SUBSTITUTES

Market samples of Raktapunarnava (*Boerhaavia diffusa* Linn.) are often adulterated with *Trianthema portulacastrum* Linn. Two plants are the sources of two different Ayurvedic drugs punarnava and Varshabhu possibly with similar therapeutic effects. The two species differ widely in their stomatal indices and palisade ratios, *Trianthema portulacastrum* possessing higher values. [29]

## PHARMACOLOGICAL AND BIOLOGICAL ACTIVITY

The plant has drawn lot of attention due to following biological activities

**Immunomodulatory effects**

The alkaloidal fraction of *Boerhaavia diffusa* was studied for its effect on cellular and humoral functions in mice. Orally administration is significantly inhibited SRBC-induced delayed hypersensitivity reactions in mice. However, the inhibition was observed only during post-immunisation drug treatment, while no effect during pre-immunisation drug treatment was observed.<sup>[30]</sup>

**Immunosuppressive activity**

*B. diffusa* hexane, chloroform and ethanol extracts, and two pure compounds Bd-I (eupalitin-3-O-h-Dgalactopyranoside) and Bd-II (eupalitin) were evaluated in vitro for their effect on T cell mitogen (phytohemagglutinin; PHA) stimulated proliferation of human peripheral blood mononuclear cell (PBMC), mixed lymphocyte culture, lipopolysaccharide (LPS) stimulated nitric oxide production by RAW 264.7, PHA and LPS induced IL-2 and TNF- $\alpha$  production, in human PBMCs, superoxide production in neutrophils, human natural killer (NK) cell cytotoxicity and nuclear translocation of nuclear factor- $\kappa$  B and AP-1 in PHA stimulated PBMCs. The chloroform and ethanol extracts inhibited PHA stimulated proliferation of peripheral blood mononuclear cells, two-way MLR, NK cell cytotoxicity as well as LPS induced NO production by RAW 264.7; the hexane extract showed no activity. Bd-I purified from the ethanolic extract at equivalent dose, inhibited PHA-stimulated proliferation of peripheral blood mononuclear cells, two-way MLR and NK cell cytotoxicity as well as LPS induced NO production by RAW 264.7 equally or more effectively than the parent ethanolic extract. Bd-I inhibited production of PHA stimulated IL-2 at the protein and mRNA transcript levels and LPS stimulated TNF- $\alpha$  production in human PBMCs; it also blocked the activation of DNA binding of nuclear factor- $\kappa$  B and AP-1, two major transcription factors centrally involved in expression of the IL-2 and IL-2R gene, which are necessary for T cell activation and proliferation. Our results report selective immunosuppressive activity of *B. diffusa* leaf.<sup>[31]</sup>

A research is also carried out to evaluate the immunomodulatory properties of this plant extract on various in vitro tests such as human natural killer (NK) cell cytotoxicity, production of nitric oxide (NO) in mouse macrophage cells, RAW 264.7, interleukin-2 (IL-2), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), intracytoplasmic interferon- $\gamma$  (IFN- $\gamma$ ) and expression of various cell surface markers on human peripheral blood mononuclear cells (PBMCs). Ethanolic extracts of *B. diffusa* roots inhibited human NK cell cytotoxicity in vitro, production of NO in mouse macrophage cells, IL-2 and TNF- $\alpha$  in human PBMCs. Intracytoplasmic IFN- $\gamma$  and cell surface markers such as CD16, CD25, and HLA-DR did not get affected on treatment with *B. diffusa* extract. Hence, it demonstrates immunosuppressive potential of ethanolic extract of *B. diffusa*.<sup>[32]</sup>

**Antidiabetic activity**

A study was carried out to investigate the effects of daily oral administration of aqueous solution of *Boerhaavia diffusa* L. leaf extract (BLEt) (200 mg/kg) for 4 weeks on blood glucose concentration and hepatic enzymes in normal and alloxan induced diabetic rats. A significant decrease in blood glucose and significant increase in plasma insulin levels were observed in normal and diabetic rats treated with BLEt.<sup>[33]</sup> Chloroform extract of *B. diffusa* leaf produced dose-dependent reduction in blood glucose in streptozotocin-

induced NIDDM rats comparable to that of glibenclamide. The results indicate that the reduction in blood glucose produced by the extract is probably through rejuvenation of pancreatic beta-cells or through extra pancreatic action.<sup>[34]</sup>

**Anti-metastatic activity**

Administration of Punarnavine (40 mg/kg body weight) prophylactically (95.25 %), simultaneously (93.9 %) and 10 days after tumor inoculation (80.1 %) could inhibit the metastatic colony formation of melano main lungs. Survival rate of the metastatic tumor – bearing animals were increased significantly by the administration of Punarnavine in all the modalities compared to the metastasis bearing untreated control. These results correlated with the biochemical parameters such as lung collagen hydroxylproline, uronic acid, hexosamine, serum sialic acid, serum  $\gamma$  glutamyl transpeptidase and serum vascular endothelial growth factor (VEGF) level and histopathological studies. Punarnavine administration could suppress or down regulate the expression of MMP-2, MMP-signal- regulated kinase) and VEGF in the lung tissue of metastasis-induced animals. Punarnavine could inhibit MMP-2 and MMP-9 protein expression in gelatin zymographic analysis of B16F-10 cells. These results indicate Punarnavine could inhibit the metastatic progression of B16F-10 melanoma cells in mice.<sup>[35]</sup> Prophylactic administration of the methanolic extract (0.5 mg/dose) inhibited the metastases formation by about 95 % as compared to untreated control animals. There was 87 % of inhibition in the lung metastases formation in syngenic C57BL/6 mice, when the extract was administered simultaneously with tumour challenge.<sup>[36]</sup> The total WBC count prior to irradiation was 7500 $\pm$ 500 cells/mm<sup>3</sup>, which was reduced to 1500 $\pm$ 500 cells/mm<sup>3</sup> in the irradiated control group on day 9 after radiation exposure. But in the *B. diffusa* treated group, irradiated animals showed the lowest count on day 3 after irradiation (4000  $\pm$  400 cells/mm<sup>3</sup>), where the count for irradiated control animals was 2100  $\pm$ 440 cells/mm<sup>3</sup>. By day 9, the level reached 6250 $\pm$ 470 cells/mm<sup>3</sup> in *B. diffusa*-treated irradiated animals.<sup>[37]</sup>

**Antioxidant activity**

Leaves revealed stronger antioxidant activity than roots, the first analysis of volatile compounds of a widely used medicinal plant, *B. diffusa*, using a HS-SPME-GC-MS technique directly into the headspace of the aqueous extract of the leaves and roots. In addition to phenolics (determined by HPLC-DAD), the organic acids (HPLC-UV) profile and in vitro antioxidant and anti acetylcholinesterase activities are described for the first time, providing further knowledge on this species' chemistry and biological potential.<sup>[38]</sup>

Ethanol and methanol extracts were prepared and screened for in-vitro antioxidant activities using Ferric reducing power and Hydrogen peroxide scavenging activity. The activity was compared to standard antioxidant like ascorbic acid. Both the extract showed strong antioxidant activity in both the methods. Between these two extracts, ethanolic extract has shown better antioxidant activity as compared to methanolic extract in both the activities.<sup>[39]</sup>

**Antiproliferative and Antiestrogenic activity**

Treatment with varying concentrations of BME (20-320  $\mu$ g/mL) resulted in moderate to very strong growth inhibition in MCF-7 cell lines. BME competed with [3H]-estradiol for binding to ER with IC<sub>50</sub> value of 320 $\pm$ 25  $\mu$ g/mL. RT-PCR analysis revealed that BME reduced the mRNA expression of pS2 indicating the antiestrogenic action of BME. BME

treatment for 48 h resulted in a remarkable increase in the number of MCF-7 cells in the G0-G1 fraction from 69.1% to 75.8 %, with a reciprocal decrease of cells in all other phases indicating cell cycle arrest at G0-G1 phase. Hence, it demonstrates that *Boerhaavia diffusa* possess antiproliferative and Antiestrogenic properties and suggest that it may have therapeutic potential in estrogen dependent breast cancers. [40]

#### **Analgesic and Anti-inflammatory activity**

The analgesic property of aqueous extracts obtained from *B. diffusa*, mainly from the leaf juice of the plant. The data also confirmed the traditional indications. The mechanism underlying this analgesic effect remains unknown, but the aqueous extract obtained from leaf juice is endowed with an apparently morphinomimetic central analgesic property. [41]

#### **Antilymphoproliferative Activity**

It inhibited T cell mitogen phytohemagglutinin and concanavalin A-stimulated proliferation of human peripheral blood mononuclear cells (PBMC). It also inhibited purified protein derivative antigen-stimulated PBMC proliferation and human mixed lymphocyte culture. In addition, *B. diffusa* extract inhibited the growth of several cell lines of mouse and human origin, such as mouse macrophage cells (RAW 264.7), human macrophage cells (U937), human monocytic cells (THP-1), mouse fibroblast cells (L929), human embryonic kidney cells (HEK293), mouse liver cells (BNLCL.2), African green monkey kidney cells (COS-1), mouse lymphoma cells (EL-4), human erythroleukemic cells (K562), and human T cells (Jurkat). [42]

#### **Anti-viral activity**

The *Boerhaavia diffusa* plant is reported to possess many pharmacological, clinical, and antimicrobial properties. Recently, it is observed potent antiviral efficacy of this plant against phytopathogenic viruses. The antiviral agent isolated from this plant was found to be a glycoprotein with a molecular weight of 16–20 kDa. Administered by foliar spraying in the field, this antiviral agent could protect some economically important crops against natural infection by plant viruses. [43]

Root of *Boerhaavia diffusa* contains basal proteins which show high virus inhibitory activity against plant viruses. Root extract of this plant induce strong systemic resistance in susceptible host plant. In the study, we found that the BD-SRIP induces the resistance against the TMV infection. [44]

#### **Hepatoprotective Activity**

The effect of 50 % ethanolic extract of roots of *Boerhaavia diffusa* on country made liquor (C. M. L.) induced hepatotoxicity was studied in albino rats. *B. diffusa* (100 mg/100 g body weight/day) protected the rats from hepatotoxic action of C. M. L. as evidenced by changes in serum alanine aminotransferase (ALT), Triglycerides (TG), Cholesterol and total lipid levels in both serum and tissues. Histopathological studies showed marked reduction in fat deposits in animals receiving *B. diffusa* along with C. M. L. [45] An alcoholic extract of whole plant *Boerhaavia diffusa* given orally exhibited hepatoprotective activity against experimentally induced carbon tetrachloride hepatotoxicity in rats and mice. The extract also produced an increase in normal bile flow in rats suggesting a strong choleric activity. The extract does not show any signs of toxicity up to an oral dose of 2 g/kg in mice. [46]

The hydro alcoholic extract of roots of *Boerhaavia diffusa* (HEBD) exhibited a significant protective action on liver

evident by a reduction in elevated levels of serum lysosomal enzymes namely Serum Glutamate pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphate (ALP) in both CCl<sub>4</sub> and rifampicin-isoniazid induced hepatotoxicity. Hence HEBD showed a dose dependent hepatoprotective activity. [47]

#### **Antibacterial Activity**

We conclude that the methanol extract of *Boerhaavia diffusa*, leaves had significant *in vitro* antimicrobial activity, hence, further results revealed that among several pathogenic bacteria, only *Staphylococcus aureus* was susceptible for *Boerhaavia diffusa*. In *Boerhaavia diffusa*, maximum inhibition was observed in *Staphylococcus aureus* followed by *Bacillus megaterium* and *Bacillus cereus* respectively at 50 µl concentration. [48]

#### **Antistress & adoptogenic Activity**

The extract improved the stress tolerance by significantly increasing the swim duration & reducing the elevated WBC, blood glucose & plasma cortisol. Immunomodulatory activity was evaluated by carbon clearance & delayed hypersensitivity test. The extract significantly increased carbon clearance, indicating the stimulation of reticuloendothelial system. The extract also produced an increase in DTH response to SRBC in mice. [49]

#### **Nitric Oxide Scavenging Activity**

The extracts of various polyherbal drugs exhibited dose-dependent NO scavenging activities and the potency was in the following order: abana > chyavanaprasha > triphala > geriforte > septilin > mentat > *Gingko biloba*. The present results suggest that the traditional Indian polyherbal crude drugs may be potent and novel therapeutic agents for scavenging of NO, and thereby inhibit the pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite. These findings may also help to explain, at least in part, the pharmacological activities like rejuvenating, adaptogenic, anti infection, anti-inflammatory, cardioprotective and neuroprotective activities of these traditional, clinically used non toxic drugs, because NO is an important bioregulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial and antitumor activity. [50]

#### **Adaptogen Activity**

Adaptogens seem to be useful during both adrenal hyperstress as well as adrenal hypofatigue. By definition, an adaptogen implies the capability for bi directional or normalizing effects. The most important adaptogens for the adrenals include *Panax Ginseng*, Siberian Ginseng, Ashwagandha, Rhodiola, *Boerhaavia diffusa*, and Holybasil Leaf Extract. *Boerhaavia diffusa* (PUNARNAVA) has the ability to support both adrenal over and under activation. In stressful conditions it has demonstrated the ability to buffer the elevations of serum cortisol and prevent the suppression of the immune system that takes place with elevated cortisol. On the other hand, *Boerhaavia diffusa* has also demonstrated the ability to improve cortisol levels with end stage adrenal exhaustion. [51]

#### **Growth Inhibition of Struvite Crystals**

This *in vitro* study had been carried out in the presence of herbal extract of *Boerhaavia diffusa* Linn. by using single diffusion gel growth technique. Sodium metasilicate solution of specific gravity 1.05 and an aqueous solution of ammonium dihydrogen phosphate of 0.5 M concentration

were mixed so that the pH value 7.0 could be set. After the gelation, equal amount of supernatant solution of 1.0 M magnesium acetate prepared with 0.5 and 1 % concentrations of the herbal extract of *B. diffusa* Linn. were gently poured on the set gels in the respective test tubes in the aseptic medium. The growth of crystals without and with herbal extracts was monitored at regular time intervals. As the concentration of *B. diffusa* Linn. increased the inhibition of crystals also increased in the gel media as well as the dissolution of crystals at the gel-liquid interface increases. The de-fragmentation of some grown crystals was also noticed.<sup>[52]</sup>

#### Anti fibrinolytic activity

A study that evaluates the effect of anti-fibrinolytic agents;  $\alpha$ -aminocaproic acid ( $\alpha$ -ACA), tranexamic acid (AMCA); anti-inflammatory drugs (indomethacin, ibuprofen, naproxen); and plant extract (root extract of *Boerhaavia diffusa*) on endometrial histology of IUD-fitted menstruating monkeys. It is effective in reducing stromal edema, inflammation, & tortuosity of glands, & in increasing the degree of deposition of fibrin & platelets in the vessel lumen.<sup>[53]</sup>

#### Chemopreventive action

In the present study, cancer chemopreventive property of *B. diffusa* was evaluated on 7,12-dimethyl benz(a)anthracene (DMBA) induced skin papillomagenesis in male Swiss albino mice (6-7 weeks old). This leads to the supposition that the inhibition of tumorigenesis by the plant extract might have been executed either by preventing the formation of active carcinogens from their precursors or by augmenting detoxification process, preventing promotional events in the mouse skin through free radical scavenging mechanism.<sup>[54]</sup>

#### Genetic diversity analysis

*Boerhaavia diffusa* is extensively used in herbal medicines as well as in the Ayurvedic system, because it contains a set of clinically important compounds. In the present study, the genetic variability in *Boerhaavia diffusa* between accessions of different geographical origin within the Indian Territory is assessed through random amplified polymorphic DNA (RAPD) markers. Twenty-eight accessions of *Boerhaavia* were screened with eighteen primers of which nine were found to be the most informative. The degree of polymorphism was found to be high in accessions collected from different places of Uttar Pradesh (Set II) in comparison to other states of India (Set I). A relatively lower level of polymorphism was recorded in accessions collected from diverse locations around Lucknow (Set III). Accessions from neighboring geographical regions exhibited more similarity than those from distant regions (as revealed by the set I analysis). Certain diagnostic markers may be correlated with morphological character(s) such as plant type. BDL appeared most distinct and divergent from the rest of the accessions and the BDJ plant in set II also showed least similarity estimate. Fragments of 5.62 Kb and 4.47 Kb with primer GN59 was found to be unique for BDP and BD2 having ovate leaf character, whereas ovoid leaf genotype exhibited 0.79 Kb (GN34 primer) fragment. Similarly a unique band type (0.35 Kb) with primer GN83 was present in BDL and BD1 that share light pink flower. Jaccard's and Nei and Li similarity coefficient values amongst the accessions were in the range of 0.22 to 0.89 and 0.33 to 0.93, respectively. Association of RAPD markers with the leaf characteristics, flower colour as well as with geographical locations has been

made. This shows that RAPD markers are also useful for the study of genetic structure of *Boerhaavia* populations.<sup>[55]</sup>

#### Bronchial asthma

Dried leaves are used in dhoomapana (smoking) in treatment of bronchial asthma. The leaf decoction is an excellent expectorant when decocted with punarnava (*Boerhaavia diffusa*) and then combined with ginger juice and black pepper.<sup>[56]</sup>

#### TOXICITY

Vomiting may be associated with larger doses of Punarnava.<sup>[57]</sup>

Major thrust by whole of the pharmaceutical industry is focused towards design and development of new innovative/indigenous plant based drugs through investigation of leads from traditional system of medicine<sup>[58]</sup> recent years, ethno-botanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. It is best classical approach in the search of new molecules for management of various diseases. Thorough screening of literature available on *Boerhaavia diffusa* depicted the fact that it is a popular remedy among the various ethnic groups, Ayurvedic and traditional practitioners for treatment of ailments. Researchers are exploring the therapeutic potential of this plant as it has more therapeutic properties which are not known.

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