



## Effect of the Aqueous Extract of Boerhavia Difusa Roots and Leaves of Hypoglycemic as well as Immuno Support Activity

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### Abstract

*Boerhavia diffusa* (Family Nyctaginaceae) is a herbal plant, which is common in the tropics in both dry and rainy seasons. It is found in India and many other countries. In India it is found especially in swampy areas and commonly used as rabbit food by the local population. The herbalists, however, use the aqueous leaf extract to treat diabetes in man (unpublished data). The plant exhibits a somewhat periodic efficacy, with its maximum activity being noticed in the month of May. *diffusa* is used in traditional medicine for its anti-inflammatory, antibacterial and cardiogenic properties. It is used in the treatment of elephantiasis, night blindness and corneal ulcers.

**Keywords:** Boerhaavia diffusa L, physicochemical, phytochemical, microscopy, TLC.

### Introduction

Boerhaavia diffusa is used in Ayurvedic medicine system to treat various health problems. Present work deals with analysis of the antiangiogenic properties of *B. diffusa* extracts by chicken chorioallantoic membrane (CAM) assay in vivo. The extracts of *B. diffusa* prepared in acetone, alcohol and benzene were tested administering at 48, 72 and 96 hrs of incubation to observe angiogenesis of the CAM at 144 hrs of development. These extracts of *B. diffusa* reduced neovascularization. Acetone- extract showed highest inhibitory activity in angiogenic response; followed by benzene and alcohol extracts. The quantitative and microscopic analysis indicate that these results indicated inhibition elongation and proliferation of both secondary and tertiary vessels. It seem to be consequences of interference of extracts in a) signaling of angiogenic agents from epithelial cells or b) cellular apoptosis, which in its absence results in normal CAM angiogenesis. This observations support strong claims of antiangiogenic ethnomedical properties of this plant.

Boerhavia diffusa the plant used in present investigation (Family; Nyctaginaceae) is mainly an herbaceous creeping weed commonly known as punarnava and is widely distributed in the tropical and temperate region of the world<sup>1</sup>. It exhibits a wide range of medicinal properties as per Ayurvedic claims. The whole plant of *B. diffusa* has been employed for the treatment of various disorders like liver disorders, gastrointestinal disorders, and heart diseases. In earlier studies of some groups it has shown to have laxative, diuretic, antiurethritis, anticonvulsant, antifibrinolytic, antinematodal and antibacterial properties<sup>2-6</sup>. The plant has also been screened for anti-inflammatory, antimicrobial, immunosuppressive, hepatoprotective, antitumorogenic, antileprotic and antiasthmatic activities<sup>7-11</sup>.

The whole plant analysis of *B. diffusa* is known to contain numerous phytochemicals constituents that include flavonoids, alkaloids, triterpenoids, steroids, lipids, lignins, tannins, phlobaphenes and ursolic acid<sup>12-15</sup>. Studies on its different extracts i.e. hexane, chloroform and ethanol extracts of *B. diffusa* had shown to block the activation of DNA binding of nuclear factor-KB and AP-1, two major transcription factors centrally involved in expression of the IL-2 and IL-2R gene, that are necessary for T cell activation and proliferation<sup>16-17</sup>. *B. diffusa* extracts were also able to attenuate the proliferation, migration and differentiation of endothelial cells. Besides, *B. diffusa* plant showed much higher inhibition of O<sub>2</sub> - production<sup>18</sup>. Thus though plant has been screened for bioactivities its angiogenic potential remained to be studied. Our present studies as a part of our search for natural product-based antiangiogenic agents, include the antiangiogenic activity of acetone, alcohol and benzene extracts of *B. diffusa* (1mg/ml) by chicken chorioallantoic membrane (CAM) in vivo. The detailed evaluations of quantitative and histological analysis of the alterations in angiogenesis influenced by the extracts have been provided in following results. In earlier work from this laboratory in the mortality studies of these extracts safe doses have been already noted<sup>19</sup>. Which have been used for present studies.

Phytochemical research has demonstrated the presence of alkaloids and amino acids in *B. diffusa*. Two known lignans, liriodendrin and syringaresinol mono- $\beta$ -D-glucoside, have been isolated from a methanol extract the roots of Boerhaavia diffusa and the former compound was found to exhibit a significant calcium channel antagonistic effect<sup>10</sup>. The seeds of this plant contain fatty acids and allantoin and the roots contain alkaloids. The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid<sup>11</sup>. TLC and HPTLC are methods commonly applied for the identification, the assay and the

testing for purity, stability, dissolution or content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmetics, nutriment)<sup>12</sup>. These flexible and cost-effective techniques present the advantage of the simultaneous processing of standards and samples with versatile detection possibilities, including a great variety of post-chromatographic derivatization reagents. The validation of analytical methods is largely recognized as the best safeguard against the generation of unreliable data and is becoming an absolute requirement in many fields.

Validation is the process by which it is established, by laboratory studies, that the performance characteristics of an analytical method meet the requirements for the intended applications<sup>13</sup>. Depending on the objective of the analytical procedure, the typical validation characteristics which can be considered through a statistical approach are accuracy, precision, specificity or selectivity, detection limit, quantification limit, linearity and ruggedness<sup>14</sup>. The mammalian immune system is composed of many cell types like B cells, T cells, NK cells, neutrophils, basophils, eosinophils, macrophages, mast cells, and mediators like cytokines that interact with non-immune cell types and each other in complex and dynamic networks to ensure protection against foreign pathogens. When tissue homeostasis is perturbed, sentinel macrophages and mast cells immediately release soluble mediators, such as proinflammatory cytokines like TNF, and interleukins<sup>2</sup>. Cytokines are elaborated by different types of cells, may act in autocrine, paracrine or endocrine manner, stimulate or regulate the growth and functions of nearby cells through specific receptors, and are highly potent as they are effective in picomolar quantities. They are truly language of communication between cells of the immune system. Some of them produce inflammation and some are chemotactic (chemokines) and these may facilitate innate immune reactions. Due to wide range of effector mechanism possessed by various groups of immune cells and its ability to exert effects with exquisite specificity, immune system provide a good target in cancer therapy. Involvement of the host immune system in the control of cancer progression has been suspected but remained inconclusive for many years. Innate immunity, which according to the immune surveillance theory is responsible for early detection and elimination of malignant cells<sup>3</sup>, may be inefficient in patients who develop malignancy. Immunomodulators are substances, which modify the activity of the immune system. They can enhance or inhibit immunological responsiveness of an organism by interfering with its regulatory mechanisms. This may be antigen independent and may directly induce production of mediators and effector molecules by the immunocompetent cells. This type of antigen independent immunity is thus distinct from one achieved by conventional immunization or by passive immunization using antibodies<sup>6</sup>. Immunomodulators can regulate the cytokine production such as tumor necrosis factor, interleukins and interferons and these cytokines may, in turn activate T-cells or NK cells. Use of plants and plant products as

immunomodulators is still in a developing stage. There are few plants reported with known immunomodulatory activity. *Viscum album* a semiparasitic plant has shown to stimulate both humoral as well as cell mediated immune response<sup>7</sup>. Similarly, an extract from the plant *Withania somnifera* has shown to stimulate the immune system<sup>8</sup> and reduce leukocytopenia during chemotherapy<sup>9</sup> and radiation therapy<sup>10</sup> and inhibit urotoxicity induced by chemotherapeutic drug cyclophosphamide<sup>11</sup>.

## Material and Methods

**Plant material and preparation of extracts:** The properly identified whole plants of *Boerhavia diffusa* were collected from the local areas of Lucknow district. It was shade dried for a week in shadow and blended in fine powder. The powder (10gm) was extracted by routine methods to get acetone, alcohol and benzene extracts. Each of the extracts was concentrated by evaporation by using high-speed vacuum evaporator (Buchi type). The yield of acetone, alcohol and benzene extract was 18.1%, 17.5% & 11.3% respectively. The dried samples of extract were dissolved in hanks balanced salt solution (HBSSHIMEDIA, India) to prepare the stock solutions for use.

The present study aims at investigating the effects of *B. diffusa* aqueous leaf extract on the blood sugar level of rats with a view to elucidating the rationale behind its use in the management of diabetes by herbalists.

The plant was collected from the surrounding of Lucknow University, in the month of May. The leaves were dried, pulverized, finely sieved and subjected to water extraction as used by the herbalists. The percent yield was 11.7. Phytochemical studies of the aqueous extract of the leaves of *B. diffusa*, were carried out by the method of Trease and Evans<sup>4</sup>.

Male Wistar rats weighing 104 g - 214 g were obtained from the Animal Facility Center, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Standard rat cubes (Pfizer Pharmaceutical Plc., Ikeja, Nigeria), and water were provided ad libitum.

The baseline plasma glucose levels were determined prior to administration. All the animals were given 80 mg/kg body weight freshly prepared alloxan monohydrate (Aldrich Chemical Company Ltd) intravenously through the tail vein<sup>5</sup>. They were left for 7 days at the end of which the plasma glucose levels were determined using the Trinder's glucose oxidase method<sup>6</sup>.

The alloxan-induced diabetic rats were divided into four groups of six male rats each. The test groups received 100, 200 and 400 mg/kg aqueous extract, while the control group received appropriate volumes of water orally respectively. At 0, 2, 4, 6, 8 and 24 hours, 0.5 ml of blood from the tail vein of the rats was dropped on the reagent pad of the one touch strip (Life Scan Inc. Milpitas, California, USA). The strip was inserted into a one-

touch brand meter and the reading noted<sup>7</sup>. Data were analysed using the Student 't' test with level of significance as  $p < 0.05$ .

The aqueous extract was found to contain flavonoids, glycosides, tannins, saponins and proteins. The effect of 100, 200 and 400 mg/kg of the aqueous extract of *B. diffusa* leaves is shown on table 1. The extract showed non-dose dependent hypoglycemic activity. The peak activity of the extract was observed at 6h post drug administration. While the 400 mg/kg dose caused a maximum percent reduction of 21.56 in glucose level at 6h, the 100 and 200 mg/kg doses of the extract showed more hypoglycemic effects which were significant at  $p < 0.05$  from the initial value, with percentage decreases of 38.07% and 51.95% respectively.

## Results and Discussions

The whole plants of *Boerhaavia diffusa* Linn was collected and analyzed the various standardization parameters. Preliminary phytochemical results showed the presence or absence of certain phytochemicals in the drug. The tests performed using n-hexane, chloroform, ethyl acetate, alcoholic and water extracts.

The aqueous leaf extract of *B. diffusa* produced nondose related decreases in blood glucose level in alloxan- induced diabetic rats. The non-dose related hypoglycaemia had its onset of action in less than 2 hours, peaked at 6 hours and lasted for over 8 hours after oral administration of the extract. The mechanism of action of this hypoglycaemic effect of the extract is not elucidated in this study. Some medicinal plants with hypoglycemic properties are known to increase circulating insulin levels in normoglycaemic rats<sup>8</sup>.

A plausible mechanism of action is that the extract might have stimulated the residual pancreatic beta-cell function or produced the hypoglycaemia through an extra-pancreatic mechanism, probably increasing peripheral utilization of glucose as postulated by Farjou and his colleagues<sup>5</sup> and Erah and coworkers<sup>9</sup> to explain the hypoglycemic effects of the extracts of *Artemisia* and *Solenostemon*. Oliver 1980<sup>10</sup> listed glycosides, flavonoids, tannins, organic sulphur compounds, catechol, and alkaloids as active ingredients in hypoglycemic plants. Thus the hypoglycemic effect produced by the extract of *B. diffusa* leaves may be due to the glycosides, flavonoids, tannins and saponins present in the extract. Further investigation is expected to characterise the active hypoglycemic principles and to elucidate the mechanism of action.

## Conclusion

In the present study we can conclude that a plausible mechanism of action is that the extract might have stimulated the residual pancreatic beta-cell function or produced the hypoglycaemia through an extra-pancreatic mechanism, probably increasing peripheral utilization of glucose. The hypoglycemic effect produced by the extract of *B. diffusa* leaves may be due to the

glycosides, flavonoids, tannins and saponins present in the extract. Further investigation is expected to characterize the active hypoglycemic principles and to elucidate the mechanism of action. The plant exhibits a somewhat periodic efficacy, with its maximum activity being noticed in the month of May. These flexible and cost-effective techniques present the advantage of the simultaneous processing of standards and samples with versatile detection possibilities, including a great variety of post-chromatographic reagents. The validation of analytical methods is largely recognized as the best safeguard against the generation of unreliable data and is becoming an absolute requirement in many fields.

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