Pharmacognostical Studies


Family: Perplocaceae

Exomorphic features (Plate 1)

Distribution: Southern Western Ghats of Tamil Nadu.

Status: rare and endemic

Habit: climbing wiry shrub; branchlets pubescent.

Leaves: elliptic-lanceolate, 4-10 x 2-5 cm thick, acute at base, entire or slightly revolute at margin, acute-short acuminate at apex, lateral nerves 7-11 pairs, prominent; petiole ca. 1.2 cm long.

Flowers: terminal and axillary, dichotomous cymes, densely villous; peduncle ca. 5 cm long, densely villous; pedicel ca. 1.2 cm long, densely villous.

Calyx: deeply 5-lobed with ovate glands between the lobes; lobes ovate, ca. 1.5 mm long.

Corolla: rotate, 5-lobed; corona of five, broad membranous scales.

Stamens: 5, inserted on the corolla throat; filaments very short and broad; anthers attached to the style apex and inflexed; pollen masses in pairs in each cell.

Ovary: 2, ovate, ca. 1 mm long; style -flat at apex.

Fruit: 2, divaricate, smooth, follicular mericarps, ca. 10 cm long; seeds ovate-oblong, ridged ventrally, tipped with a long white coma.
Microscopic studies
Anatomy of the stem

The young stem is circular in cross sectional outline, measuring 800 µm in radius. It consists of dark and thick crushed epidermal layer and one or two superficial layers of periderm. The cortical zone is wide measuring 100 µm thick. The cortex is parenchymatous and homogeneous. An endodermoid layer is somewhat clearly visible at the inner boundary of the cortex. The endodermoid cells are spindle shaped and thin walled. Inner to the endodermoid layer occur several discrete masses of fibres arranged in a ring (Plate 2a). The secondary phloem is wide and forms a continuous sheath along the outer boundary of the xylem cylinder. There are small masses of internal phloem or medullary phloem occurring in a ring along the inner boundary of the xylem cylinder (Plate 2b).

The xylem cylinder consists of outer thick portion of secondary xylem elements and inner cylinder of primary xylem elements. The outer secondary xylem consists of compact, radial, long lines of xylem fibres and several small clusters of vessels distributed with wide gaps in between the clusters. The inner part of the xylem cylinder represents primary xylem which includes several long, radial lines of xylem elements which are circular and wide. The outer part of the secondary xylem is somewhat unequal in distribution due to localization of wide vessels (Plate 2a & b).

An old stem is 1.45 mm thick in radius. It is circular in sectional view with smooth outline. The epidermis and subepidermal layer are compressed into thick dark cylinder (Plate 2c). There are two or three layers of superficial periderm which is located inside the dark cylinder (Plate 2d). The cortical zone consists of tangentially
elliptical, thin walled, compact parenchyma cells. Endodermoid layer, sclerenchyma masses are fairly distinct in the old stem.

The vascular cylinder consists of outer continuous zone of secondary phloem and inner thick cylinder of xylem with wavy outer contour (Plate 2d). The phloem cylinder includes small clusters of sieve elements which occur in alternate masses of parenchyma cells (Plate 3a). The vascular cylinder including phloem and xylem is 450 μm thick. The xylem includes outer portion of secondary xylem and inner portion of primary xylem. The secondary xylem consists of inner zone of mostly xylem fibres which occur in compact parallel lines with rectangular cells and thick walls. There are also xylem rays which are thin with single row of cells.

In the outer part of the secondary xylem occur large wide circular thick walled vessel elements which are 60 μm in diameter. The presence of many wide vessels in the outer boundary indicates the initiation of the secondary growth. The inner zone of the xylem cylinder consists of long narrow primary xylem elements (Plate 2d & 3b). The primary xylem consists of wide, circular, thick walled metaxylem cells which are wider in the outer part and becomes gradually narrow, collapsed and crushed protoxylem elements (Plate 3b). The medullary phloem or inner phloem includes large clusters of phloem elements which are wide and angular. These medullary phloem elements are in isolated strands forming an inner circle of medullary phloem.

**Anatomy of the leaf**

In cross sectional view, the leaf exhibits thick midrib and uniformly thin and even lamina (Plate 4a). The midrib consists of thick adaxial cone and wide semicircular abaxial part. The midrib is 1.2 mm thick; the adaxial cone is 250 μm
thick and the abaxial part is 1.1 mm wide. The epidermal layer of the midrib consists of small squarish thin walled cells with prominent cuticle (Plate 4b). The ground tissue includes circular and angular compact parenchyma cells. The vascular strand is broadly arc shaped and bicollateral. The vascular arc is 800 µm wide and 200 µm thick. It consists of several, radial compact lines of xylem elements. The number of elements in each line is 4 to 6 cells. The cells are angular, wide and thick walled. Phloem elements occur both on the adaxial concavity as well as along the abaxial convex part of the strand. The phloem strands are in discontinuous small units comprising small thick walled angular sieve elements (Plate 4b).

**Lamina**

The lamina is 200 µm thick. The adaxial epidermal layer consists of rectangular or squarish thin walled cells with smooth prominent cuticular layer. The cells are 20 µm thick. The abaxial epidermis is similar to those of the adaxial epidermis. The mesophyll tissue consists of narrow adaxial band of palisade tissue and much thicker abaxial zone of spongy parenchyma. The palisade cells are single layered, short, thin and less compact. The cells are 30 µm in height. The spongy parenchyma cells are many layered. The cells are spherical or lobed. They are loosely arranged forming wide air chambers divided by network of partition filaments formed by spongy parenchyma (Plate 4c). Calcium oxalate druses are sparsely seen in the mesophyll tissue. The druses are isolated and occur in the intercellular spaces of the mesophyll tissue (Plate 4d).
**Epidermal cells and stomata**

The epidermal tissue was studied from surface view of the paradermal sections. The adaxial epidermis consists of small, polyhedral parenchyma cells with thick straight anticlinal walls (Plate 4e). The adaxial epidermis is apostomatic. The abaxial epidermis is stomatiferous. The epidermal cells are rectangular, thin walled and the anticlinal walls are straight. The stomata are aggregated into clusters comprising 10 to 15 stomata in a cluster (Plate 4f). The stomata are tetracytic comprising a pair of lateral subsidiary cells and another pair of polar subsidiary cells. The guard cells are broadly elliptical with narrow stomatal pore. The guard cells are 30 × 20 µm in size.

**Venation pattern**

The venation is densely reticulate. The major secondary veins are thin and less prominent (Plate 5a). The secondary lateral veins are uniformly thick and repeatedly branched and dense. The vein islets are wide and variously shaped. Within the islets occur either simple (unbranched) terminations or branched once or many times forming dendroid outline. Ends of the terminations are thicker due to terminal accumulation of short tracheids (Plate 5b).

**Powder microscopy**

The powder preparation of the sample exhibits the following elements:

**Epidermal cells and stomata**

A small piece of epidermal peeling of the leaf was seen in the powder. The peeling showed epidermal cells and stomatal type. The epidermal cells are polygonal,
small with straight thin anticlinal cells (Plate 6a). Stomata are diffusely distributed in the epidermis. The stomata are tetracytic or cyclocytic type. Each stoma is surrounded by 6 subsidiary cells which are rectangular and thin walled. The guard cells are circular and they are 20 µm in diameter.

**Laticifer**

Long, narrow, cylindrical, thin walled laticifers are seen in the powder. Laticifers are articulated non anastomosing type. Dark granular particles are abundant in the lumen of the laticifers. A segment of laticifer is 480 µm long and 25 µm thick (Plate 6.b).

**Fibres**

Xylem fibres are abundant in the powder (Plate 6c & d). The fibres may be narrow or wide. The narrow fibres are long, thick walled, tapering at the ends and the lumen is narrow. The narrow fibre is 450 µm long and 10 µm thick. The wide fibre has thin wall, wide lumen and the ends are less tapering. The wide fibres are 500 µm long and 15 µm thick. Some of the fibres have elliptical slit like simple pits which occur in crisscross manner. These fibres are called fibre tracheids (Plate 6.e). The pits are multiseriate and they are obliquely oriented. The fibre tracheids are 700 µm long and 25 µm wide.

**Vessel elements**

Vessel elements are less frequent in the powder. They are long, narrow and cylindrical. Some of them have short tails or tailless. On the lateral wall of the vessel element occur circular, multiseriate bordered pits. The end wall perforation is wide,
circular and horizontal or slightly oblique. The vessel elements are up to 450 µm long and 50 µm wide (Plate 6f & g).

**Physicochemical parameters**

The physicochemical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content, ash values and extractive values of stem and leaf of *Bacolepis nervosa* were determined. The results are depicted in Table 2.

**Moisture content**

Percentage of loss on drying or moisture content of the stem and leaf was found to be 70% and 78% respectively which signifies that the drug is properly dried and stored (Table 2).

**Ash values**

The analytical results for total ash of stem and leaf were found to be 7.0% and 7.7% respectively. The amount of acid insoluble ash presents in stem and leaf were 1.3% and 1.7% respectively. The water soluble ash of stem and leaf were found to be 4.2% and 4.0% respectively. The amount of sulphated ash presents in stem and leaf were 6.2% and 4.8% respectively.

**Extractive values**

Percentage of the extractive values of various extracts is given in Table 2. The results showed that various extracts of leaf contain greater proportion by mass of the extractive values than the various extracts of stem. Petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and aqueous soluble extractive values of
stem were 4.6%, 7.6%, 8.6%, 7.4%, 9.2%, 11.7% and 15.3% respectively and leaf were 6.2%, 6.8%, 9.2%, 8.2%, 15.2%, 16.2% and 16.1% respectively (Table 2). In stem and leaf extracts, water soluble extractive values were higher followed by alcohol and chloroform soluble extractive values while the least amount of extractive value was observed in petroleum ether extract.

Fluorescence analysis

Fluorescence analysis of stem and leaf powder were studied at day light and UV light (245 nm and 365 nm) and the observations are presented in Tables 3 and 4. Fluorescence studies of stem powder revealed the presence of fluorescent green with 1N aqueous NaOH, 1N alcoholic NaOH, 1N HCl, Conc. HCl, Conc. H₂SO₄, 50% H₂SO₄, Conc. HNO₃ and benzene under UV light of shorter wavelength. The leaf powder treated with 1N aqueous NaOH, 1N alcoholic NaOH, Conc. HCl, Conc. HNO₃ and ethanol revealed the presence of fluorescent green under UV light of shorter wavelength.

Preliminary phytochemical screening

The distribution of different phytochemical constituents in petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of stem and leaf powder of B. nervosa were evaluated qualitatively and the results are presented in Table 5. The presence of alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein have been confirmed in the methanol and ethanol extracts of stem and leaf of B. nervosa.
Quantitative estimation

The total phenol content, flavonoid content and tannin content of ethanol and methanol extracts of stem and leaf of *B. nervosa* are presented in Fig. 1. In the present study, total phenol, flavonoid and tannin contents were higher in methanol extract than ethanol extract. The present study revealed that, the total phenol content, flavonoid content and tannin content are more in leaf than stem of *B. nervosa*.

HPTLC analysis

The HPTLC chromatogram profiles of alkaloid, flavonoid, glycoside, saponin, steroid and terpenoid in visible light and UV lights at 254 nm and 366 nm wave lengths are given in Plates 7 - 12. The Rf values, peak area and the assigned substances are given in Tables 6 - 11. The HPTLC densitograms of alkaloid, flavonoid, glycoside, saponin, steroid and terpenoid are given in Fig. 2 - 7. The methanol extracts of stem and leaf showed the presence of alkaloids, flavonoids, glycosides, saponins, steroids and terpenoids. The results also confirmed the presence of 12 types of alkaloids, 10 types of flavonoids, 13 types of glycosides, 10 types of saponins, 17 types of steroids and 9 types of terpenoids in leaf of *B. nervosa*. The HPTLC profile of *B. nervosa* stem revealed the presence of 8 types of alkaloids, 9 types of flavonoids, 11 types of glycosides, 9 types of saponins, 12 types of steroids and 8 types of terpenoids.

GC-MS analysis

The chemical composition of stem and leaf of *B. nervosa* were analysed by using GC-MS. The chromatogram of ethanol extracts of stem and leaf of *B. nervosa* is given in Fig. 8a, 8b and 8c. The identification of the phytochemical compounds was
confirmed based on the retention time (RT), molecular formula, molecular weight (MW) and peak area in percentage and are presented in Tables 12 and 13. The GC-MS analysis of B. nervosa stem revealed the presence of thirty compounds. The first compound identified with less retention time (2.33 min) was octane, 3, 5-dimethyl whereas, lupeol was the last compound which took longest retention time (33.32 min) to identify. The results revealed that, thymol (30.47%) was found as major component followed by undecane (10.02%), eugenol (9.53%), vellerdiol (6.13%), piperine (5.66%) and 9, 17 octadecadienal, (z)-(5.45%).

Thirty one compounds were identified in the ethanol extract of leaf of B. nervosa. The major constituents recorded from leaf of B. nervosa were thymol (28.51%), eugenol (10.74%), 12 - oleanen - 3 - yl acetate, (3a) - (7.97%), piperine (5.54%), 13, octadecenal, (Z) - (5.21%) and oleic acid (5.02%). Fig. 9 - 12 show the mass spectrum and structure of different compounds of stem and leaf of B. nervosa.

FTIR analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum of B. nervosa stem and leaf powder was illustrated in Fig. 13a & b. The outcome of FTIR functional groups of stem and leaf of B. nervosa are represented in Table 14a & b.

Antioxidant activity

In the present study, antioxidant capacity of various solvents of B. nervosa stem and leaf extracts was examined using five different assays.
DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of stem and leaf of *B. nervosa* is shown in Fig. 14a and 15a. The DPPH activity of stem was in the order of ethanol, petroleum ether, methanol, benzene and ethyl acetate respectively (Fig. 14a). The IC$_{50}$ values of stem extracts were 18.40 µg/mL, 23.84 µg/mL, 25.18 µg/mL, 26.13 µg/mL, 26.16 µg/mL and ascorbic acid with IC$_{50}$ value of 21.10 µg/mL (Fig. 14f). The present study revealed that the ethanol extract of leaf had the highest scavenging activity with IC$_{50}$ value of 17.24 µg/mL and benzene leaf extract had the lowest scavenging activity with IC$_{50}$ value 26.16 µg/mL. (Fig. 15f). The different concentrations of various extract of *B. nervosa* showed antioxidant activities in a dose dependent manner.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of stem and leaf extracts of *B. nervosa* is shown in Fig. 14b and 15b. The activity of stem was in the order of petroleum ether, methanol, ethyl acetate, ethanol and benzene respectively (Fig. 14b). The IC$_{50}$ values of stem extracts were 16.28 µg/mL, 18.34 µg/mL, 18.94 µg/mL, 19.22 µg/mL, 20.16 µg/mL and ascorbic acid with IC$_{50}$ value of 18.16 µg/mL (Fig. 14f). The hydroxyl radical scavenging activity of leaf extracts of *B. nervosa* was in the order of ethyl acetate, ethanol, methanol, petroleum ether and benzene respectively (Fig. 15b). The IC$_{50}$ values of various solvent leaf extracts were 18.27 µg/mL, 20.16 µg/mL, 22.67 µg/mL, 23.41 µg/mL 24.22 µg/mL and ascorbic acid with IC$_{50}$ value of 21.34 µg/mL (Fig. 15f). The present study revealed that ethyl acetate leaf extract has prominent hydroxyl radical scavenging activity than the other solvent extracts.
Superoxide radical scavenging activity

In the present study, the superoxide radical scavenging effect of different extracts was compared with the same dose of ascorbic acid ranging from 50 to 800 µg/mL. (Fig. 14c and 15c). The superoxide radical scavenging activity of stem extracts was in the order of ethanol, methanol, ethyl acetate, petroleum ether and benzene (Fig. 14c). The superoxide radical scavenging activities of leaf extract were in the order of methanol, ethanol, benzene, petroleum ether and ethyl acetate (Fig. 15c). When compared to ascorbic acid, the superoxide radical scavenging activity of the alcoholic extracts (both methanol and ethanol) were found to be high (Fig. 15f).

ABTS radical assay

The different extracts of stem and leaf of *B. nervosa* were subjected to ABTS radical cation scavenging activity and the results are shown in Fig. 14d and 15d. Among various solvent extracts of *B. nervosa* stem, ethanol extract possessed the highest ABTS radical cation scavenging activity with IC<sub>50</sub> value 17.91 µg/mL, while benzene showed the lowest ABTS radical scavenging activity with IC<sub>50</sub> value 27.98 µg/mL (Fig. 14d). Differences for the ABTS radical cation scavenging capacity of various solvents and reference compound (Trolox) was recorded in this study. Among various solvent extracts of *B. nervosa* leaf, ethyl acetate possessed the highest ABTS radical cation scavenging activity (IC<sub>50</sub> value = 24.11 µg/mL), while benzene showed the lowest ABTS radical cation scavenging activity (IC<sub>50</sub> value = 27.98 µg/mL). When compared to Trolox, all the extracts possessed less ABTS scavenging activity (Fig. 15f).
Reducing power assay

Figures 14e and 15e show the dose response curves for the reducing power of various solvent extracts (50 - 800 µg/mL) from B. nervosa. It was found that the reducing power of plant extracts increases with increasing concentration of each solvent. The ranking order for reducing power of stem extracts was methanol, ethanol, petroleum ether, ethyl acetate and benzene respectively (Fig. 14e). The ranking order for reducing power of leaf extracts was methanol, ethanol, petroleum ether, benzene and ethyl acetate respectively. Higher reducing power was (0.588 ± 0.027 µg/mL in stem; 0.436 ± 0.61 at 800 µg/mL in leaf) evident in methanol extract (Fig. 15e).

Anticancer activity

Antitumor activity of ethanol extracts of B. nervosa stem (BNS) and leaf (BNL) against EAC (Ehrlich Ascites Carcinoma) tumor bearing mice was assessed by the parameters such as relative organ weight, tumor volume, viable and non viable cell count, mean survival time and percentage increase in life span. The results are shown in Tables 15 - 17.

Effect on organ weight

There was a significant decrease in the body weight of B. nervosa stem and leaf treated groups compared to the tumor control. A highly significant ($p<0.01$) decrease was observed in groups IV and VI treated with 300 mg/kg of stem and leaf extracts. The administration of the extracts indicated a dose dependent decrease in the weight of different organs. The relative organ weight of all the vital organs were restored to normal on treatment with 300 mg/kg body weight of the extracts
Similar reduction was noticed on treatment with the standard drug vincristine (80 mg/kg body weight).

**Effect on solid tumor volume**

Solid tumor volume was observed on 15th, 20th, 25th and 30th day in the tumor control and drug treated mice. Treatment with *B. nervosa* stem and leaf extracts at the dose of 300 mg/kg body weight for a period of 14 consecutive days in EAC induced solid tumor bearing mice showed a significant (p<0.001) reduction in tumor volume (2.63 ± 0.026 and 3.02 ± 0.054) respectively when compared to the tumor alone group (13.4 ± 0.68) on 30th day. The administrations of *B. nervosa* stem and leaf extracts showed a dose dependent decrease in tumor volume. The animals treated with the standard drug (vincristine 80 mg/kg body weight) were found to be also efficient (2.64 ± 0.013) in preventing the development of solid tumor on the same day as shown in Fig. 16.

**Effect on mean survival time, life span, packed cell volume, viable and non viable cell count**

The effect of *B. nervosa* stem and leaf extracts at the doses of 150 and 300 mg/kg on the mean survival time and increase in life span of EAC bearing mice is shown in Table 16. In the EAC control group, the mean survival time was 16.54 ± 0.12 (days) while it increased to 22.34 ± 0.65 (150 mg/kg), 28.92 ± 0.72 (300 mg/kg) in stem extract treated groups and 20.43 ± 0.18 (150 mg/kg), 26.53 ± 0.84 (300 mg/kg) in leaf extract treated groups. The group treated with the standard drug vincristine (80 mg/kg) showed 27.22 ± 0.64 days of mean survival time. The increase in the life span of tumor bearing mice treated with stem and leaf extracts at different doses (150 and 300 mg/kg body weight) and the standard vincristine was found to be
35.06%, 74.84%, 23.51%, 60.39%, and 64.57% respectively. In the present study, animals administered with different doses of *B. nervosa* extracts showed a dose dependent increase in mean survival time and the life span with respect to control. Administration of stem and leaf extracts at the dose of 300 mg/kg body weight significantly (*p* < 0.001 and *p* < 0.01) decreased the packed cell volume and viable cell count. Furthermore, non viable cell counts at the dose of 300 mg/kg body weight of *B. nervosa* leaf extract were significantly (*p* < 0.01) increased in a dose dependent manner.

**Effect on Haematological parameters**

The effects of *B. nervosa* stem and leaf extracts treated animals are shown in Table 17. Haematological parameters of tumor bearing mice were found to be altered when compared to normal group. The haemoglobin content, RBC and WBC counts in the EAC control group were decreased as compared to the normal control. Treatment with *B. nervosa* stem and leaf extracts at the dose of 300 mg/kg significantly (*p* < 0.05) increased the haemoglobin content, RBC and WBC counts to more or less normal levels. In differential count of WBC, the percentage of neutrophils and eosinophils increased while the lymphocyte count decreased in the EAC control group. Groups treated with *B. nervosa* stem and leaf extracts showed restoration of these haematological parameters in dose dependent manner.

**Antidiabetic activity**

**Effect of stem and leaf extracts of *B. nervosa* on body weight**

In the antidiabetic activity, the effect of *B. nervosa* stem and leaf extracts on body weight is measured on 14th day of post induction and was compared with normal
and diabetic control groups. The values are shown in Table 18. Alloxan induced diabetic rats showed decrease in body weight compared to normal rats. Oral administration of stem extract at the dose of 300 mg/kg showed an increase in body weight on 14th day of post induction when compared to other drug treated diabetic rats.

**Effect of stem and leaf extracts of B. nervosa on fasting glucose level**

A marked rise in fasting blood glucose level was observed in diabetic control group (Group II) as compared with normal control rats (Group I). Stem and leaf extracts of B. nervosa (150 and 300 mg/kg) exhibited a dose dependent significant (p<0.05) antihyperglycemic activity on 0, 7th and 14th day posttreatment. The antihyperglycemic effect of stem extract (Group III & IV) was found to be more effective than leaf extract (Group V & VI). The reference standard, glibenclamide showed a significant (p<0.001) reduction in blood glucose compared to diabetic control (Table 18).

**Effect of stem and leaf extracts of B. nervosa on serum profile**

The effect of stem and leaf extracts of B. nervosa on the serum insulin, glucose, urea, creatinine and glycolyted Hb of normal and diabetic treated rats are shown in Table 19. The results revealed that the insulin level was significantly (p<0.001) reduced in diabetic rats (Group II) compared to normal rats (Group I) but the other biochemical parameters like glucose, urea, creatinine and glycolyted Hb were significantly (p<0.01; p<0.001) increased in diabetic rats than control rats. A significant (p<0.05; p<0.01) increase in insulin level and decrease in other
biochemical parameters mentioned above were observed in stem and leaf extracts of
*B. nervosa* treated diabetic rats (Groups III - VI).

**Effect of stem and leaf extracts of *B. nervosa* on protein and liver marker enzyme**

The levels of total protein, albumin, globulin and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats are presented in Table 20. When compared with normal control rats (Group I), the diabetic control rats (Group II) had decreased levels of serum total protein, albumin, globulin and elevated levels of liver marker enzymes such as SGPT, SGOT and ALP. After treatment with the stem and leaf ethanol extracts of *B. nervosa* at 150 and 300 mg/kg body weight doses (Groups III, IV, V and VI) and glibenclamide (Group VII), the total protein, albumin, globulin and liver marker enzymes were brought back to near normal levels. It was further evident that stem extract (300 mg/kg body weight) treated rats showed significant (*P*<0.01) reduction of liver marker enzyme when compared to diabetic control and other treated groups.

**Effect of stem and leaf extracts of *B. nervosa* on serum lipid profile**

Table 21 illustrates the effect of stem and leaf extracts of *B. nervosa* on the levels of total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C and VLDL-C in the serum of experimentally induced diabetic rats. It is evident from the results that the serum levels of total cholesterol (TC), triglycerides (TG), very low density lipoproteins (VLDL-C) and low density lipoproteins (LDL-C) were significantly increased (*p*<0.01) whereas, serum high density lipoproteins (HDL-C) level was significantly reduced (*p*<0.05) in diabetic rats compared to normal control group. It was further evident that stem and leaf extracts treated groups significantly (*p*<0.05;
$p<0.01$) reduced the levels of TC, TG, VLDL-C, and LDL-C whereas significantly ($p<0.05$) increased HDL-C respectively in a dose dependent manner (Table 21).

**Effect of stem and leaf extracts of B. nervosa on antioxidant enzymes**

Alloxan induced diabetic rats were found to have decreased SOD, GSH, GPX and CAT enzyme in serum, liver and kidney as compared with control. Administration of stem and leaf extracts to the diabetic rats resulted in significant ($p<0.05; p<0.01$) increase in the activities of SOD, GSH, GPX and CAT (Tables 22-24). Diabetic rats were found to exhibit significant ($p<0.01; p<0.001$) increase of lipid peroxidase in serum, liver and kidney compared to control rats. Treatment with stem and leaf extracts produced significant ($p<0.05$) decrease in LPO.

**Hepatoprotective activity**

**Body weight**

The administration of CCl$_4$ caused a significant ($p<0.05$) decrease in the body weight of rats as compared with the control rats. The animals treated with stem and leaf extracts of B. nervosa (150 mg/kg and 300 mg/kg) also gained weight during the experimental period (Table 25).

**Biochemical parameters**

The results of serum biochemical parameters are presented in Tables 26 and 27. In the CCl$_4$ control group, significant ($p<0.05; p<0.01$) decreased levels of total protein, albumin and globulin were observed. But the group which received the drug of stem and leaf extracts at the dose of 300 mg/kg body weight showed a significant ($p<0.05$) increase in the reduced levels of protein. The elevation of SGOT, SGPT and ALP in CCl$_4$ intoxication was significantly ($p<0.01$) high when compared to the
normal. The elevated levels of SGOT, SGPT and ALP in groups IV and VI animals (posttreated with stem and leaf extracts, 300 mg/kg) were significantly ($p<0.05$; $p<0.01$) reduced as depicted in Table 26.

Table 27 shows the levels of total bilirubin, conjugated and unconjugated bilirubins and GGTP levels. When compared to normal control rats (Group I), the serum of CCl$_4$ treated rats (Group II) showed a significant elevation in the total bilirubin, conjugated and unconjugated bilirubins. In all the other groups treated with the stem and leaf ethanol extracts of B. nervosa (Group III to VI), the above said biochemical parameters were found to decrease when compared to CCl$_4$ treated diabetic control rats (Group II). However, the decrease in the concentration of total bilirubin, conjugated and unconjugated bilirubins levels were found to be greater in the liver damaged rat group IV, followed by group VI, treated with stem and leaf extracts of B. nervosa respectively.

The effect of B. nervosa stem and leaf extracts on LPO, GP$_X$, GRD, SOD, GSH and CAT activities are shown in Table 28. It showed that GP$_X$, GRD, SOD, GSH and CAT activities were significantly ($p<0.001$; $p<0.01$) decreased in liver damage control group. On the other hand, the groups received with both stem and leaf extracts (300 mg/kg), the values of above enzymatic parameters were near normal control (Group I). The results are well compared with silymarin standard drug treated group (Group VII).

**Histopathology**

Liver section of the control group showed normal liver parenchyma with the central vein clearly seen along with the bile ducts and hepatic arteries. The
hepatocytes were neatly arranged in anastomosing plates with the sinusoids radiating from the central vein (Plate 13a). Venous congestion, necrosis and mononuclear infiltration were the histopathological changes observed in the CCl₄ treated animals (Plate 13b). Histoarchitecture of both low and high dose of stem extract groups showed normal cellular architecture with well brought out central vein, well presented cytoplasm and prominent nucleus (Plate 13c & d). Mild necrosis with mononuclear cell infiltration and edema were the changes observed in rats administered with low dose leaf extract (Plate 13e). Histopathological examination of the liver tissue of rats pretreated with high dose leaf extract showed a high degree of protection when compared with liver damaged control (Plate 13f). The sections of liver taken from the animals treated with standard drug silymarin showed the normal hepatic architecture, which was similar to that of control (Plate 13g).

Antifertility activity

Body and reproductive organ weight

The treatment of rats with stem and leaf extracts of B. nervosa showed decrease in body weight compared to control. The treatment with stem and leaf extracts treated rats caused a highly significant ($p<0.05; p<0.01$) decrease in the accessory sex organ weights namely testis, epididymis and seminal vesicle in all treated groups. In Group III and V (300 mg/kg body weight), the sex organ weights were significantly ($p<0.01$) reduced when compared to that of Group II and IV (150 mg/kg body weight) (Table 29).
Sperm count and sperm motility

Table 30 shows that the sperm motility and sperm density in epididymis were decreased to a significant ($p<0.01; p<0.001$) level in treated animals with the ethanol extracts of stem and leaf of *B. nervosa* compared to control group. The reduction was very severe in rats treated with stem and leaf extracts of *B. nervosa* at the dose of 300 mg/kg body weight (Group III & V) followed by Group II and Group IV rats treated with plant extracts at the dose of 150 mg/kg body weight respectively. The same trend was seen in the caput epididymal sperm density when compared to control rats (Group I).

Sperm abnormality

Sperm nature, in caput and caudal regions, was affected by the ethanol extracts of *B. nervosa* (Table - 30). The results obtained from all the treated groups showed that the tail region of the sperms was much affected than the head.

Serum antioxidants

The activities of CAT, SOD, GPx, GST and GRD in the serum of control and plant extracts treated rats were presented in Table - 31. In the present study, plant extracts treated rats showed decreased activities of all the studied antioxidants when compared to control rat.

Serum biochemical profile

Serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) of the control and plant extracts treated rats are depicted in Table 32. The results showed no significant changes in the serum
protein, albumin and globulin. The level of creatinine slightly increased in rats treated with the plant extracts at 150 mg/kg body weight and 300 mg/kg body weight dose. The level of urea and liver marker enzymes like SGOT, SGPT and ALP increased in a dose dependent manner, in both the stem and leaf treated groups, when compared to the control group.

**Reproductive hormone profile**

**Serum testosterone level**

The ethanol extracts of stem and leaf of *B. nervosa* (150 and 300 mg/kg body weight) repeated treatment for 14 days caused a significant (*p* < 0.05; *p* < 0.01) decrease in serum level of testosterone in male rats. The level of testosterone decrease was dose related (Table - 33).

**Serum Luteinizing Hormone (LH) level**

Repeated treatment of male rats with the ethanol extracts of *B. nervosa* for 14 days caused a dose related decrease in the serum level of LH (Table 33).

**Serum estrogen level**

The ethanol extracts stem and leaf of *B. nervosa* (150 and 350 mg/kg) caused an increase in the level of serum estrogen in male rats. Doses of 150 and 250 mg/kg body weight, administered daily for 14 days, caused rise in the serum level of estrogen.

**Serum Follicle Stimulating Hormone (FSH) level**

Pretreatment with ethanol extracts of stem and leaf of *B. nervosa* caused a decrease in the serum level of FSH in male rats compared to control (Table 33).
**Fertility test**

The results presented in Table 34 showed that intragastric administration of stem and leaf extracts of *B. nervosa* (150 mg/kg and 300 mg/kg body weight), for 14 days to male rats, caused a significant (*p*<0.01) decrease in the number of females impregnated by plant extracts treated male rats. When compared to females impregnated with untreated male rats, the number of viable foetuses formed decreased significantly (*p*<0.05; *p*<0.01) in female rats impregnated by treated males. Similarly, the number of resorption sites was found to be reduced in female rats impregnated by treated male rats when compared to control.

**Antiinflammatory activity**

Carrageenan rat paw edema is the suitable method for evaluating the antiinflammatory activity. The percentage of inhibition of edema values of carrageenan induced rat paw edema is given in the Table 35. The stem and leaf of *B. nervosa* extracts at the doses of 150 and 300 mg/kg body weight showed 77.08%, 82.28%, 76% and 79.64% inhibition of edema and at 300 mg/kg dose, the inhibition was higher in both the extracts. Indomethacin (10 mg/kg) was used as standard antiinflammatory drug. It exhibited a protective effect and the percentage of inhibition of edema was 81.82%. The ethanol extract of stem and leaf of *B. nervosa* at the dose level of 150 and 300 mg/kg decreases the edema significantly (*p*<0.01; *p*<0.001) at 3rd and 4th hour administration of the extracts when compared to the control group.

**Antibacterial activity**

In the present study, the antibacterial activity of stem and leaf extracts of *B. nervosa* was carried out by disc diffusion method. The antibacterial property of
petroleum ether, chloroform, ethyl acetate, methanol and ethanol extracts of
*B. nervosa* was tested against 16 pathogenic bacteria and the results are presented in
**Fig. 17 and Plates 14-16.** The methanol extract of *B. nervosa* exhibited higher activity
against *Mycobacterium smegmatis* and *Staphylococcus aureus* (14 mm). The same
extract showed least activity (6 mm) against *Bacillus thuringiensis* and *Escherichia
coli*. The ethyl acetate extract showed zones of inhibition of 13 mm against
*Enterococcus foecalis* and 6 mm against *Staphylococcus aureus* (methicilin sensitive).

The ethanol extract of *B. nervosa* exhibited considerable amount of inhibition
against the tested organism, the highest activity against *Pseudomonas aeruginosa,*
*Bacillus subtilis, Bacillus thuringiensis* (12 mm). *Proteus mirabilis* was resistant to
chloroform, ethyl acetate and methanol extracts of *B. nervosa*. Similarly, *E. coli*
strain was highly resistant to various solvent extracts of *B. nervosa.*