Chapter – III

Studies on Borreria hispida
CHAPTER – 3

STUDIES ON BORRERIA *HISPIDA*

3.1 INTRODUCTION

*Borreria hispida* (Linn.) K. Schum\textsuperscript{307 - 309} belongs to the family Rubiaceae. It is known as *Nattaichuri* in Tamil. It is used in the indigenous systems of medicine. The other vernacular names are given below.

- **Bengali**: Madana - banta - Kadu
- **Gujarathi**: Madhuri jadi
- **Hindi**: Guthri, Madanaghanti
- **Kannada**: Madanabudu maegiddah
- **Marathi**: Ghanti-chi-bhaji, gondi
- **Oriya**: Solagathi
- **Sanskirit**: Madanaghanta
- **Telugu**: Madanagrandth

*B. hispida* [BH] (Photograph 2), is a perennial herb, distributed in the tropical region. Seven species are found in India.

It is a procumbent, scarbid, hirsute or hispid herb or sub glabrous, usually with long internodes. Leaves are subsessile, 1.3-5 by 0.8-2 cm. oblong or elliptic, acute, scarbid, pubescent or nearly glabrous with scarbid or ciliate margins; stipules membranous, hispid with few bristles which are usually larger than sheath. About 4-6 flowers in whorl with in the stipular cup, pedicels are short, bracts are lanceolate – subacute and hyaline. Calyx are hispid, 4 mm long tube, narrowly campanulate. Corolla is pale blue or white and about 4-5 metre long.

216
Photograph 2. *Borreria hispida* (whole plant)
3.2 REVIEW OF LITERATURE

3.2.1 Medicinal Properties

In the traditional system of medicine, the juice of plant is used to treat fever, inflammation of eye and gums. A decoction of the herb is used in the treatment of headache, earache, otitis media suppurativa. An extract of the leaves is given as an astringent in haemorrhoids and gall stones and also for the treatment of pimples and sores. The seeds gave demulcent properties and are given diarrhoea and dysentery. Roots are prescribed as a mouth wash to cure toothache.

3.2.2 Phytochemical review on B. hispida

From the petroleum ether extract of the powdered plant material, ß-sitosterol has been isolated and from the methanolic extract ursolic acid was isolated and reported\(^\text{310}\). From the fresh plant material D-mannitol has been identified and reported\(^\text{311}\). Isorhamnetin has been isolated from the seeds of the plant and reported\(^\text{312}\). From the petroleum ether of the air dried, powdered, aerial part of B. hispida, erythrodol was reported. Upon successive extraction of BH with benzene, a triterpene 3-keto 12-ene-29-oic acid was reported. Further successive extraction with chloroform, n-hentriacontanol and a triterpenoid epikatonic acid (Olean 12-ene-3ß-ol-29 oic acid) was reported\(^\text{313}\).

3.2.3 Pharmacological review on B. hispida

Central Drug Research Institute [CDRI], Lucknow, India has done a preliminary pharmacological screening of about 295 medicinal plants in a pilot study and Borreria hispida was one among the plant. 50% of the ethanolic extract of BH reported to have hypothermic and diuretic activity. The study also reported the LD\(_{50}\) of BH as 750 mg / kg.
[i.p. in mice]. Hot water extracts of seeds of BH reported to have a relief of headache in adult human.

3.3 AIM AND OBJECTIVE OF THE STUDY

*Borreria hispida* (Rubiaceae), is used in the Siddha system of medicine. Several medicinal properties have been attributed for the plant and only few phytoconstituents have been reported and ursolic acid being the major triterpenoid. Regarding pharmacological investigations, only hypothermic and diuretic activities have been reported. (vide section 3.2.3).

The aims of the present investigations are as follows.

- To document HPTLC finger printing of the methanolic extract of the whole plant with marker compounds.
- To isolate and identify new phytoconstituents from the methanolic extract of the whole plant.
- To evaluate the following pharmacological and microbiological activities for methanolic extract of the whole plant.
1. Hepatoprotective activity
2. Anti-inflammatory activity
3. *In-vitro* antioxidant activity
4. Analgesic activity
5. Antipyretic activity
6. Local anaesthetic activity
7. Antispasmodic activity
8. Antibacterial activity
3.4 PHYTOCHEMICAL STUDIES ON B. HISPIDA

3.4.1 Materials and Methods

3.4.1.1 Plant Material

The whole plant of *B. hispida* was collected in and around Chennai, during December 2001 and was authenticated by Dr. E. Sasikala, Central Research Institute for Siddha, Arumbakkam, Chennai, India. A voucher specimen was deposited at the herbarium of C.L. Baid Metha College of Pharmacy, Thorapakkam, Chennai, India. The whole plant was shade dried and powdered.

3.4.1.2 Solvents and Chemicals

As given in section 2.4.1.2.

3.4.1.3 Extractive Value

The extractive values for the powdered *B. hispida* was carried out by successive extraction with solvents viz., hexane, chloroform, ethyl acetate and methanol.

3.4.1.4 Preliminary phytochemical screening

All the extracts viz., hexane, chloroform, ethyl acetate and methanol of *B. hispida*, were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in it.

3.4.1.5 HPTLC finger printing

HPTLC instrumentation details and procedures followed are same as given in the section 2.4.1.5. The HPTLC chromatogram for the methanolic extract of the whole plant was recorded with ursolic acid as the marker compound (ursolic acid authentic sample
was supplied by Dr. A. Patra, Department of Chemistry, University College of Science, Culcutta - 9). The extract was hydrolysed and the chromatogram was obtained with isorhamnetin as the marker compound.

### 3.4.1.6 Spectral Analysis

Instruments and methods used for IR, $^1$H NMR and $^{13}$C NMR are same as given in section 2.4.1.6.

#### GC – MS Analysis

GC – MS analysis was carried out on GC – MSD 5973 Agilent instrument. The other details are as follows.

- **Detector**: Mass Selective Detector
- **Column thickness**: 0.25 µ
- **Column length**: 30 metre
- **Internal diameter**: 0.25 mm
- **Carrier gas**: Helium
- **Temperature programme**: Initial temperature 70°C, final temperature 280°C at the value of 10°C/min.
- **Injection temperature**: 250°C
- **Flow rate**: 1ml/min.
- **Library software**: Wiley.

### 3.4.2 Experimental Studies

#### 3.4.2.1 Extraction of the plant material

The shade dried and powdered *B. hispida* (4 kg) was extracted with methanol in the cold (48 h). The extract was filtered and distilled on a water bath and finally dried in
vacuum to get a dark brown mass (500 g). This extract was used for phytochemical investigations.

3.4.2.2 Extractive value

The procedure adopted is same as described in section 2.4.2.2.

3.4.2.3 Preliminary phytochemical screening

The preliminary phytochemical screening for various extracts viz., hexane, chloroform, ethyl acetate and methanol were performed for the presence of various phyto constituents namely steroid, triterpenoid, phenol, flavonoid, tannins, glycoside/sugar, alkaloid, furanoid/indole quinonoid, cumarin and lignan according to the literature and the results are presented in the table 21.

3.4.2.4 HPTLC finger printing

Preparation of the extract and marker compounds

The dried whole plant of *B. hispida* was powdered and passed through 50 mesh. 5 g of the powder was extracted with 50 ml of methanol in soxhlet apparatus. The methanolic extract was concentrated and made up to 50 ml in a standard flask. The marker compound ursolic acid 10 mg was dissolved in 10 ml of methanol. 10 µl of the extract solution and marker compound solutions were spotted for the chromatogram. The scanning wavelength was 200 nm.

HPTLC finger printing method

HPTLC finger printing procedure is same as discussed in section 2.4.2.4. The *R* value and HPTLC spectrum were recorded and the results are presented in the fig 70a.
For the purpose of standardization, another chromatogram was run with acid hydrolysed methanolic extract using the flavonoid isorhamnetin as the marker compound. The hydrolysis was necessary since the structure of isorhamnetin glycoside present in the plant has not been established because it was difficult to isolate the pure glycoside. Purushothaman et al.\textsuperscript{312} has also reported isorhamnetin from the acid hydrolysed methanolic extract of \textit{B. hispida}.

The hydrolysed extract was prepared by refluxing 500 mg of methanolic extract with 10 ml of 5\% ethanolic hydrochloric acid for an hour on water bath. The solution was poured into water and extracted with chloroform. The chloroform was distilled off and the residue was dissolved in methanol and made up to 50 ml.

Isorhamnetin was used as the marker compound in case of acid hydrolysed methanolic extract of \textit{B. hispida}. 10 mg of isorhamnetin was dissolved in 10 ml of methanol. 10 \textmu l of both the hydrolysed extract and the marker compound were taken for spotting. The scanning wavelength was 254 nm. The chromatogram is given in the fig 70b.

\textbf{3.4.2.5 Isolation of phytoconstituents from methanolic extract of \textit{B. hispida}}

The methanolic extract (500 g) obtained from 4 kg (dry weight) of \textit{B. hispida} was chromatographed over silica gel (100 – 200 mesh) in benzene and eluted with solvents of increasing polarity viz., benzene, chloroform and methanol. The results are presented in the scheme 2.
Scheme 2

*Borreria hispida*  
(aerial part 4 kg)

\[ \text{Methanol (6 L)} \]

\[ \text{Methanolic extract (500 g)} \]

\[ \text{Column Chromatography / Silica gel / 100 – 200 mesh} \]

\[ \text{Hexane:Benzene 4:1} \]

\[ \text{Hexane:Benzene 1:1} \]

\[ \text{Benzene:Ethyl acetate 9:1} \]

\[ \text{Chloroform:Methanol 19:1} \]

**Fraction I**

- Menthol  
- Nopinone (by GC-MS)

**Fraction II**

- 6, 10, 14 Trimethyl Pentadecane 2-one  
- Phytol (by GC-MS)

**Fraction III**

- Sterol mixture  
  - Campesterol  
  - Stigmasterol  
  - $\beta$-sitosterol (1:3:2) (by GC-MS)

**Fraction IV**

- Oleanolic acid  
- Ursolic acid
Fraction I

Fraction I was eluted with hexane – benzene (4:1), gave a waxy residue.

**IR**

\[ \text{KBr} \quad \text{cm}^{-1} \]

(Fig 71) 3338 (hydroxyl), 2917, 2849, 1707 (carbonyl), 1472, 1463, 1377, 729 and 719 (methylene rocking).

GC – MS chromatogram showed two major peaks ‘a’ and ‘b’ at Rt. 8.78 and 13.25 min respectively. (Fig 72). The compounds were identified as **menthol** (peak ‘a’) and **nopinone** (peak ‘b’) respectively by comparison with attached software library data (Wiley).

![Menthol](image1)

**Menthol**

![Nopinone](image2)

**Nopinone**

Peak ‘a’ (menthol) showed fragments at m/z 155 (M⁺-1), 138, 123, 109, 95, 81, 71 (100%), 55, 41. (Fig 73). Peak ‘b’ (nopinone) showed fragments at m/z 138 (M⁺), 123, 109, 95, 83 (100%), 67. (Fig 74).

Fraction II

Fraction II was eluted with hexane – benzene (1:1), gave a waxy residue.
IR $\nu_{\text{KBr}}$ cm$^{-1}$

(Fig 75) 3400(hydroxyl), 2917, 2849, 1705(carbonyl), 1472, 1463, 1301, 729 and 719 (methylene chain).

GC – MS chromatogram showed two major peaks ‘a’ and ‘b’ at Rt. 17.67 and 18.8 min respectively. (Fig 76). The compounds were identified as **6,10,14 trimethyl 2-penta decanone** (peak ‘a’) and **phytol** (peak ‘b’) respectively by comparison with attached software library data (Wiley).

![6, 10, 14 – Trimethyl – 2 – pentadecanone](image)

**Phytol**

Peak ‘a’ (6,10,14 trimethyl 2-penta decanone) showed fragments at m/z 268 ($M^+$), 250, 210, 155, 141, 138, 127, 124, 113, 109, 95, 85, 71, 58(100%) (Fig 77). Peak ‘b’ (phytol) showed fragments at m/z 296 ($M^+$), 278, 263, 249, 211, 207, 183, 123, 113, 109, 95, 85, 81, 71(100%). (Fig 78).

**Fraction III**

Fraction III was eluted with benzene – ethyl acetate (9:1), gave a colourless solid, crystallized from methanol. (mp 145 - 148°C). It answered for Liebermann Butchard test
for sterol. It gave a single spot on TLC over silica gel G with benzene – ethyl acetate (9:1) as the developing solvent ($R_f$ 0.53).

**$\text{IR V}^{\text{KBr}}_{\text{max}} \text{ cm}^{-1}$**

(Fig 79) 3442 & 1062 (hydroxyl), 2936, 2866, 1640, 1463, 1381, 1240, 1023, 969, 959 (trans disubstituted double bond), 838 (trisubstituted double bond), 801, 778.

$^1\text{H NMR (d, CDCl}_3$, 400 MHz)  

(Fig 80a – 80b). 0.68 – 1.001 (sterol methyls), 3.55 (1 H, m, H-3) and 5.00 – 5.35 (olefinic protons).

GC – MS chromatogram showed three major peaks ‘a’, ‘b’ and ‘c’ at Rt. 29.68, 30.77 and 33.13 min respectively (Fig 81). The compound were identified as **campesterol** (peak ‘a’), **stigmasterol** (peak ‘b’) and **ß-sitosterol** (peak ‘c’) respectively by comparison with attached software library data (Wiley).

![Campesterol](image)

227
Peak ‘a’ (campesterol) showed fragments at m/z 400 (M⁺), 385, 367, 354, 340, 328, 301, 289, 273, 255, 231, 213, 199, 185, 173, 159, 145, 133, 119, 105, 93, 81, 67, 55. (Fig 82) Peak ‘b’ (stigmasterol) showed fragments at m/z 412 (M⁺), 394, 379, 369, 351, 327, 314, 300, 285, 273, 255, 242, 231, 213, 199, 186, 173, 159, 146, 133, 119, 105, 83, 69, 55. (Fig 83). Peak ‘c’ (β–sitosterol) m/z 414 (M⁺), 396, 381, 367, 354, 329, 316, 303, 288, 273, 255, 231, 213, 199, 186, 173, 159, 145, 132, 119, 105, 94, 81, 68, 55. (Fig 84).
Fraction IV (Mixture of oleanolic acid and ursolic acid)

Fraction IV was eluted with chloroform – methanol (19:1), gave colourless solid, crystallized from acetone (mp 285 - 289°C). It answered for the triterpenoid. It gave a single spot on TLC over silica gel with chloroform – methanol (19:1) as the developing system. (Rf 0.64).

\[
\text{IR }^\text{KBr}_{\text{max}} \, \text{cm}^{-1}
\]

(Fig 85) 3443 & 1029 (hydroxyl), 2926, 2853, 1690 (carbonyl), 1620 & 826 (trisubstituted double bond), 1462, 1387, 1377 (gem dimethyl), 997, 949, 763.

Acetylation of fraction IV

Fifty mg of the fraction IV was treated with 20 ml of acetic anhydride and 1 ml of pyridine and the mixture was allowed to stand over night at room temperature. It was poured into crushed ice and extracted with chloroform (2 x 100 ml). The combined chloroform extracts was washed with 50 ml of water and dried over anhydrous sodium sulphate. The solution was distilled to dryness and recrystallized from methanol.(mp 262°C).

\[
\text{IR }^\text{KBr}_{\text{max}} \, \text{cm}^{-1}
\]

(Fig 86). 2942, 2867, 1733 (acetate carbonyl), 1696 (acid carbonyl), 1648 (trisubstituted double bond), 1483, 1370 (gem dimethyl), 1245 (acetate carbonyl bending), 827 (trisubstituted double bond).
$^1$H NMR (d, CDCl$_3$, 400 MHz) (Mixture of acetates of oleanolic acid and ursolic acid)

(Fig 87).

0.68 – 1.19 (methyl signals of oleanolic acid and ursolic acid acetates), 0.88 (3H, d, J=6.0 Hz, H-30 of ursolic acid acetate), 0.95 ((3H, d, J=6.0 Hz, H-29 of ursolic acid acetate), 2.22 (1H, d, J=14.0 Hz, H-18 of ursolic acid acetate), 2.82 (1H, dd, J=14.0 & 4.0 Hz, H-18 of oleanolic acid acetate), 4.43 (1H, t, J=8.0 Hz, H-3), 5.29 (1H, t, J=4.0 Hz, H-12), 1.98 (3H, s, OCOCH$_3$).

$^{13}$C NMR (d, CDCl$_3$, 100 MHz) (Mixture of acetates of oleanolic acid and ursolic acid)

(Fig 88) 37.6 (C-1), 22.7 (C-2), 80.3 (C-3), 37.6 (C-4), 55.1 (C-5),
18.0 (C-6), 32.9 (C-7), 39.1(C-8), 47.6 (C-9), 36.7 (C-10),
23.2 (C-11), 122.3, 125.0 (C-12 of oleanolic acid acetate and ursolic acid acetate respectively), 143.4, 137.8 (C-13 of oleanolic acid acetate and ursolic acid acetate respectively), 27.8 (C-15), 22.7 (C-16), 45.6 (C-17), 40.7,
52.2 (C-18 of oleanolic acid acetate and ursolic acid acetate respectively), 46.3, 39.3 (C-19 of oleanolic acid acetate and ursolic acid acetate respectively), 30.5, 38.8 (C-20 of oleanolic acid acetate and ursolic acid acetate respectively), 33.6 (C-21), 32.3 (C-22), 27.9 (C-23), 15.2 (C-24), 16.5 (C-25), 16.9 (C-26), 25.7, 23.8 (C-27 of oleanolic acid acetate and ursolic acid acetate respectively),
183.9 (C-28), 33.6, 21.1 (C-29 of oleanolic acid acetate and ursolic acid acetate respectively), 23.4, 17.9 (C-30 of oleanolic acid acetate and ursolic acid acetate respectively), 170.8 – OCOOCH₃ and 21.1 OCOCH₃.

**GC – MS (Mixture of acetates of oleanolic acid and ursolic acid)**

GC – MS chromatogram showed two major peaks ‘a’ and ‘b’ at Rt. 13.48 and 15.81 min. respectively (Fig 89). The compounds were identified as **oleanolic acid acetate** (peak ‘a’) and **ursolic acid acetate** (peak ‘b’).

![Diagram of oleanolic acid acetate (R1 = R2 = CH₃, R3 = H) and ursolic acid acetate (R1 = CH₃, R2 = H, R3 = CH₃)]

Peak ‘a’ (oleanolic acid acetate) showed peaks at m/z 498 (M⁺), 456, 438, 392, 377, 323, 300, 281, 249, 248, 233, 219, 203, 189, 175, 163, 147, 133, 119, 95, 81, 69, 57, 43. (Fig 90). Peak ‘b’ (ursolic acid acetate) showed peaks at m/z 498 (M⁺), 456, 377, 300, 281, 269, 248, 233, 219, 203, 190, 189, 175, 163, 147, 133, 119, 95, 81, 69, 57, 43, 28, 15. (Fig 91).
3.4.3 Results and Discussion

3.4.3.1 Introduction

As mentioned in the review of literature, the following phytoconstituents viz., ursolic acid, methyl ursolate, 3 keto 12-ene 28-oic acid, isorhamnetin, \( \beta \)-sitosterol and D-mannitol have been reported from \textit{B. hispida}. In this section, we report the identification of menthol, nopinone, 6, 10, 14 tri methyl pentadecane-2-one, phytol, campesterol, stigmasterol and \( \beta \)-sitosterol through GC – MS analysis. We also isolated and characterized the mixture of oleanolic acid and ursolic acid by the application of spectroscopic data as IR, \(^1\)H NMR and \(^{13}\)C NMR. Preliminary phytochemical screening and HPTLC finger printing with marker compounds are also reported.

3.4.3.2 Extractive value (Successive extraction)

The results of the successive extraction of the whole plant powder of \textit{B. hispida} with hexane, chloroform, ethyl acetate and methanol were found to be 3.3, 6.4, 9.3 and 12.5 w/w respectively.

3.4.3.3 Preliminary qualitative analysis

The preliminary qualitative analysis of hexane, chloroform, ethyl acetate and methanol extracts (successive extraction) of \textit{B. hispida} was carried out and their results are presented in the table 21. The following phytoconstituents viz., triterpenoids, glycosides, flavonoids, steroids, alkaloids and phenolic compounds were answered for the qualitative tests.
3.4.3.4 HPTLC finger printing

The HPTLC finger printing analysis is shown in the fig 70a. The methanolic extract of *B. hispida* was developed with chloroform – methanol (19:1) as the developing system. The scanning wavelength was 200 nm. The chromatogram showed 6 peaks (track ‘a’). Track ‘b’ shows the marker ursolic acid at \( R_f \) value 0.74. From the chromatogram, the peak 6 of track ‘a’ coincides with that of ursolic acid (track ‘b’). Thus the methanolic extract of *B. hispida* can be standardized by using ursolic acid as the marker compound.

Purushothaman et al\textsuperscript{312} have reported the presence of isorhamnetin glycoside in the methanolic extract of *B. hispida*. But they could not isolate the pure flavonoid glycoside. Hence the extract was hydrolysed with hydrochloric acid and they have reported isorhamnetin as the aglycone. Hence the methanolic extract was hydrolysed with 5% ethanolic hydrochloride and the hydrolyzate was subjected to HPTLC with isorhamnetin (Sigma chemicals, USA) as the marker compound (Fig 70b). the scanning wavelength was 254 nm and developing system was toluene – ethyl acetate (9:1).

The HPTLC chromatogram of the hydrolysed extract (track ‘a’), showed 7 peaks. The marker compound isorhamnetin is shown in track ‘b’. The marker compound peak is corresponded to peak 3 of track ‘a’. (\( R_f \) 0.47).

3.4.3.5 Characterization of phytoconstituents from methanolic extract of *B. hispida*

**Fraction I**

Fraction I was eluted with hexane – benzene (4:1). The IR spectrum (Fig 71) showed the presence of hydroxyl (3338 cm\(^{-1}\)), carbonyl (1707 cm\(^{-1}\)) and methylene bending (729 & 719 cm\(^{-1}\)). GC – MS of fraction I (Fig 72) showed two major peaks (a and b), which are identified as menthol (Fig 73) and nopinone (Fig 74) respectively.
Fragmentation patterns for menthol and nopinone are given in Fig 92 and 93 respectively. The data corresponds to the reported values for menthol\textsuperscript{316} and nopinone\textsuperscript{317, 318}. The identity of the compound menthol was also confirmed by the comparison with authentic sample.

**Fraction II**

Fraction II was eluted with hexane – benzene (1:1). The IR spectrum (Fig 75) showed the presence of hydroxyl (3400 cm\(^{-1}\)), carbonyl (1705 cm\(^{-1}\)) and methylene bending (729 & 719 cm\(^{-1}\)). GC – MS of fraction I (Fig 76) showed two major peaks (a and b), which are identified as 6, 10, 14 trimethyl 2-pentadecan-2-one (Fig 77) and phytol (Fig 78) respectively. Fragmentation patterns for 6, 10, 14 trimethyl 2-pentadecan-2-one and phytol are given in Fig 94 and Fig 95 respectively. The data corresponds to the reported values for 6, 10, 14 trimethyl 2-pentadecan-2-one\textsuperscript{319} and phytol\textsuperscript{320, 321}. The identity of the compound phytol was also confirmed by the comparison with authentic sample.

**Fraction III**

Fraction III was eluted with benzene – ethyl acetate (9:1), gave a sterol mixture campesterol, stigmasterol and \(\beta\)-sitosterol in the ratio 1:3:2. (mp 145 - 148°C). The IR spectrum (Fig 79) showed the presence of hydroxyl (in all the three sterols) (3442 cm\(^{-1}\)), tris substitututed double bonds \(\gamma,\delta,\epsilon\) in all the three sterols (1640, 838 cm\(^{-1}\)) and trans olefinic linkage in the side chain of stigmasterol (959 cm\(^{-1}\)). \(^1\)H NMR spectrum of the sterol mixture (Fig 80a – 80b) showed the sterol methyls in the region of \(\delta\) 0.68 – 1.001 and H – 3 at \(\delta\) 3.55 as a multiplet. The olefinic protons appeared in the region \(\delta\) 5.00 – 5.35. The upfield peaks due to side chain double bond of stigmasterol. The \(^1\)H NMR values corresponds to those reported in the literature\textsuperscript{322}. GC – MS chromatogram of the sterol
(Fig 81) also showed the presence of campesterol (Fig 82), stigmasterol (Fig 83) and β-sitosterol (Fig 84) at Rt 29.68, 30.77 and 33.13 min. respectively (1:3:2). The mass spectral fragmentation pattern of all the three sterols are given in fig 96, 97 and 98 respectively. The fragmentation pathways coincide with those reported in the literature. The occurrence of sterol mixture corresponding to campesterol, stigmasterol, β-sitosterol and α-spinasterol has been reported in the literature. To avoid ambiguity, the identity of the sterols was confirmed by comparison with authentic samples supplied by Dr. J.D. Connolly, Department of chemistry, University of Glasgow, Scotland, UK.

**Fraction IV**

Fraction IV was eluted with chloroform – methanol (19:1) (mp 285 - 289°C). It answered for the triterpenoid. It gave a single spot on TLC with chloroform – methanol (19:1) (Rf 0.64) corresponded to oleanolic acid. The IR spectrum (Fig 85) showed the presence of hydroxyl (3433 & 1029 cm⁻¹), acid carbonyl (1690 cm⁻¹), trisubstituted double bond (1620 & 826 cm⁻¹) and gem dimethyl (1377 cm⁻¹).

The compound was acetylated with acetic anhydride and pyridine to increase the solubility and for the purpose of taking GC – MS (mp 262°C). The IR spectrum of the acetate (Fig 89) showed the peaks for acid carbonyl (1696 cm⁻¹) and acetate carbonyl (1733 cm⁻¹). It also showed peaks for trisubstituted double bond (1648 & 827 cm⁻¹) and gem dimethyl (1377 cm⁻¹).

The ¹H NMR as well as ¹³C NMR of the acetate clearly showed it to be a mixture of oleanolic acid acetate and ursolic acid acetate. Both the ¹H NMR as well as ¹³C NMR spectra showed signals for olean-12-ene and urs-12-ene systems and thus clearly establishing the presence of both oleanolic acid and ursolic acid in the fraction IV (details
are discussed under experimental section). The presence of the mixture was finally confirmed by GC – MS (Fig 90) which showed ursolic acid acetate being the major compound. The characteristic fragments are shown in Fig 99. The fragments are shown for ursolic acid acetate. The corresponding fragments have been obtained for oleanolic acid acetate also, in which both methyl groups in ring E are at C-21. The mass spectral fragments correspond to those reported in the literature.

3.5 PHARMACOLOGICAL STUDIES

3.5.1 Materials and Methods

3.5.1.1 Plant material

Arial part of *Borreria hispida* [BH] was collected in and around Chennai. The plant material is shade dried and powdered. The powdered material of *B. hispida* (4 kg) was extracted with methanol in cold for 48 h. The extract was filtered, distilled and dried in vacuum (500 g).

3.5.1.2 Preparation of the drug for the pharmacological study

The methanolic extract of *B. hispida* was prepared in the form of emulsion with 1% v/v Tween 80 and the same was administered orally to animals. Standard drugs viz., silymarin, diclofenac sodium and paracetamol were prepared in the form of suspension with 1% w/v SCMC.

3.5.1.3 Animals

Wistar albino mice (25-30 g), wistar albino rats of either sex (140-200 g) were obtained from the inbred colony of department of pharmacology, C.L. Baid Metha College of Pharmacy, Thorapakkam, Chennai-96. The animals were kept in polypropylene cages at 25 ± 2° C with relative humidity 45-55% under 12 h light and
12 h dark cycle. They were fed with standard laboratory animal feed (Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India) and tap water *ad libitum*.

All the pharmacological and toxicological experimental protocols were approved by Institutional Animals Ethics Committee [IAEC] of Committee for the Purpose of Control and Supervision on Experimentation on Animals [CPCSEA]. Vide sanction letter no.

1. IAEC/VIII/1/CLBMCP/2003-2004
2. IAEC/XII/13/CLBMCP/2004-2005

3.5.1.4 Chemicals

All fine chemicals were purchased from Sigma chemicals (USA) and other chemicals from SRL (Mumbai), Aldrich (USA) and CDH (New Delhi).

3.5.1.5 Acute toxicity studies

Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development-423 [OECD-423] guidelines (acute toxic class method). Wistar rats (n=6) of either sex was selected by random sampling technique. The animals were kept fasting for overnight, had access only to water. The methanolic extract of *B. hispida* was administered orally at the initial dose 5 mg/kg body weight by intra gastric tube and observed for 14 days. The animals are observed individually after dosing once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h, and daily there after, for a total of 14 days. Since there was no mortality with 5 mg/kg for 14 days, the procedure was repeated for next higher doses such as 50, 500 and 2000 mg/kg.
3.4.1.6 Dose fixation

In order to fix the dose for methanolic extract of *B. hispida*, a pilot study was performed in which analgesic activity by acetic acid induced writhing reflex was chosen as the model. Albino mice of either sex were divided into six groups (n=6). The methanolic extract of *B. hispida* were administered orally in geometric progression starting from 25 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg, 800 mg/kg and 1600 mg/kg respectively. Half an hour before the drug treatment, acetic acid (3% v/v), 1ml/100 g, i.p.) was administered to all the animals. The number of writhings is calculated for 20 min and the % protection of analgesia is calculated.

3.5.2 Experimental Studies

3.5.2.1 Hepatoprotective activity

Method

Carbon tetrachloride toxic model\(^{194}\).

Animals

Rats of six per group were used.

Materials

Methanolic extract of BH in the form of emulsion with 1% v/v Tween 80 and silymarin (SM) in 1% w/v SCMC suspension was employed as standard drug. 1% v/v Tween 80 solution was used as control vehicle. For inducing acute hepatic damage, carbon tetra chloride in olive oil (1:1) was employed.

Serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum alkaline phosphatase (ALP), serum bilirubin (BILN), total proteins (TPN) and total albumin (TAL), were estimated by using standard kits from M/S Ranbaxy Laboratories.
Ltd., New Delhi India. All the reagents used were of analytical grade. Silymarin (Silybon, M/S Microlabs, Bangalore) was used as standard drug.

**Experimental Protocol**

Rats (n=6) are randomly divided into five groups. I group served as vehicle control and received only 1% v/v Tween 80 solution for 5 days [10ml/kg, p.o.]. II group served as toxic control and received carbon tetrachloride olive oil (1:1) (2 ml/kg, s.c.). III group animals served as standard drug control and received silymarin 25 mg/kg, p.o., in 1% w/v SCMC for 5 days. IV and IV group animals received methanolic extract of BH 200 and 400 mg/kg, p.o., in 1% v/v Tween 80 for 5 days. All groups except I group animals received carbon tetrachloride - olive oil (1:1) (2mg/kg, s.c.) on 2\(^{nd}\) and 3\(^{rd}\) days, 30 min after the administration of the test compounds. On the fifth day, after 4 h of last dose, animals were sacrificed by decapitation. Blood was collected by excising the jugular vein. It is allowed to clot and then centrifuged at 3000 rpm for 15 min to separate the serum for various biochemical estimations like AST, ALT, ALP, total albumin (TAL), total protein (TPN) and bilirubin (BILN).

**Estimation serum aspartate transaminase (AST)**

Experimental details are similar as described in the section 2.5.2.2.

**Estimation of serum alanine transaminase (ALT)**

Experimental details are similar as described in the section 2.5.2.2.

**Assay of serum alkaline phosphatase (ALP)**

Experimental details are similar as described in the section 2.5.2.2.
Estimation of serum bilirubin (BILN)\textsuperscript{197}

Experimental details are similar as described in the section 2.5.2.2.

Estimation of total protein (TPN)\textsuperscript{198}

Experimental details are similar as described in the section 2.5.2.2.

Estimation on of total albumin (TAL)\textsuperscript{198}

Experimental details are similar as described in the section 2.5.2.2.

The results of the hepatoprotective activity data are presented in the table 22 and fig 100.

Histopathological examination\textsuperscript{199}

Small fragments of liver is washed in ice-cold saline, fixed in 10\% w/v formalin solution, dehydrated in the ethanol (50\% v/v) embedded in paraffin and cut into 5 µm thick sections using a microtome. The sections were stained with eosin-haemotoxylin dye for photo microscopical observations.

Statistics

The results are expressed as mean ± SEM. The statistical difference between control and treated groups were tested by Student’s ‘t’ test. In all cases, a difference was considered significance when p<0.05.
3.5.2.2 ANTI-INFLAMMATORY ACTIVITY

Method

Carrageenan induced oedema method\textsuperscript{200}.

Animals

Wistar albino rats.

Materials

Methanolic extract of BH in the form of emulsion with 1% v/v Tween 80 and Diclofenac sodium (DS) in 1% w/v SCMC suspension was employed as standard drug. 1% v/v Tween 80 (10 ml/kg, p.o.) solution was used as control vehicle.

Plethysmograph

Indigenously prepared graduated plethysmograph was used for the study. The mercury displacement due to dipping of the paw was directly read from the scale attached to the mercury column.

Experimental protocol

Rats (six per group) were randomly divided into four groups. I Group animals received 1% v/v Tween 80 (10 ml/kg, p.o.). Group II animals received DS 5 mg/kg, p.o., in 1% w/v SCMC. III and IV Group animals received methanolic extract of BH 200 and 400 mg/kg p.o., in 1% v/v Tween 80 emulsion respectively. Diclofenac sodium was employed as the standard drug.
A mark was made on both the hind paw (right and left) just beyond tibio tarsal joint, so that every time the paw is dipped in the mercury column upto the fixed mark to ensure constant paw volume. The initial paw volume (both right & left) of each rat was measured by mercury displacement.

After 30 min of drug administration, 0.1 ml of 1% w/v carrageenan is injected in the right hind paw sub-plantar region of each rat. The left paw served as reference (non-inflammatory paw) for comparison. The paw volumes of both legs of control and test compound treated rats were measured at 1 h, 2 h, 3 h, 4 h and 5 h after carrageenan administration.

The results of the anti-inflammatory effects of the test compounds are presented in the table 23 and fig 101. The percentage inhibition for each rat and each group was obtained by using the formula C-T/C x 100, where C is the oedema rate of control group and T as that of treated group.

Statistics

The results are expressed as mean ± SEM. The statistical difference between control and treated groups were tested by Student’s ‘t’ test. In all cases, a difference was considered significance when p<0.05.
3. 5.2.3 IN-VITRO ANTI OXIDANT ACTIVITY

**DPPH Assay** \(^{209, 210}\)

**Chemicals and Instrumentation**

DPPH (Aldrich, USA), Naphthylene diamine dichloride (Loba Chemie, Mumbai). All other reagents used were of analytical grade. UV spectra used is Shimadzu UV-1601 model.

**Experimental details**

The free radical scavenging activity of the methanolic extract of BH at different concentrations was examined using DPPH radical. The reaction mixture consisted of 1 ml of 0.1 mM DPPH in ethanol, 0.95 ml of Tris-HCl buffer (pH 7.4), 1 ml of ethanol and 0.05 ml different concentrations of BH.

The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding the extract. The experiment was performed in triplicate and % scavenging was calculated using the formula 100-[100/blank absorbance x sample absorbance]. The antioxidant activity was compared with Vitamin C which was used as the standard antioxidant.

The results are presented in the table 24 and fig 102.

**Nitric Oxide radical Inhibition Assay** \(^{217}\)

**Chemicals**

Sodium nitroprusside, naphthyl ethylene diamine (Acros organics, Belgium) and sulphanilamide (Suvchem Chemicals, Mumbai).
Experimental details

Sodium nitroprusside (5 µM) prepared in standard phosphate buffer solution was incubated with different concentrations of the methanolic extract of BH, dissolved in standard phosphate buffer (0.025 M, pH4) and the tubes were incubated at 25°C for 5 h. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% w/v sulphanilamide in 2% v/v phosphoric acid and 0.1% w/v naphthyl ethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner. The experiment was performed in triplicate and % scavenging was calculated using the formula 100-[100/blank absorbance x sample]. The activity was compared with ascorbic acid, which was used as a standard anti-oxidant.

The results are presented in the table 25 and fig 103.

3.5.2.4 ANALGESIC ACTIVITY

Acetic acid induced writhing reflex method\textsuperscript{219}.

Materials

Methanolic extract of \textit{Borreria hispida} (BH) in 1% v/v Tween 80 emulsion and Paracetamol (PA) (100 mg/kg, p.o.) in 1% w/v SCMC suspension were used for the study. 1% v/v Tween 80 (10 ml/kg, p.o.) solution was used as vehicle control.

Animals

Wistar albino mice (25 – 30 g) of either sex.
Experimental protocol

Mice of either sex were randomly divided into four groups, each group containing six animals. Group I animals received only 1% v/v Tween 80 solution (10 ml/kg, p.o.). Group II animals received PA (100 mg/kg, p.o. in 1% w/v SCMC), Group III & IV animals received methanolic extract of BH 200 and 400 mg/kg, p.o., in 1% v/v Tween 80 emulsion respectively.

All the animals received intra peritoneal (i.p.) injection of 3% v/v of acetic acid (1 ml/100g) 30 min after the administration of drugs BH (200 & 400 mg/kg) in 1% v/v Tween 80 emulsion and PA (100 mg/kg). Paracetamol was used as the standard drug.

The number of writhings produced by each animal was observed individually under a glass jar for a period of 20 min and the same was counted. The % protection of analgesic activity was calculated by using the formula C-T/C x 100, where C is the number of writhings in the control group and T is the number of writhings in the treated group. The results of the analgesic activity data are presented in table 26 and fig 104.

Tail immersion model

Animals

Wistar albino female rats.

Materials

Methanolic extract of BH in 1% v/v Tween 80 emulsion and Morphine (MO) injection.
Experimental protocol

Rats (six per group) were randomly divided into four groups. Group I animals received only 1% v/v Tween 80 solution (10 ml/kg, p.o.). Group II animals received MO (5 mg/kg, s.c.), Group III & IV animals received methanolic extract of BH 200 and 400 mg/kg, p.o., in 1% v/v Tween 80 emulsion respectively.

The animals were screened for the sensitivity test by immersing 3 cm of the tail of the rat gently in hot water maintained at 55 ± 0.5°C. Within a few sec, the rats reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0 and 10 min interval. The average of the two values was taken as the initial reaction time. After 30 min, tail withdrawal time of each group animals was noted and the % protection of analgesia was calculated by using the formula C-T/C x 100 where ‘C’ represents the tail withdrawal (in sec) of control and ‘T’ to that of treated groups.

The results of the analgesic activity data are presented in table 27 and fig 105.

Statistics

The results are expressed as mean ± SEM. The statistical difference between control and treated groups were tested by Student’s ‘t’ test. In all cases, a difference was considered significance when p<0.05.

3.4.2.5 ANTIPYRETIC ACTIVITY

Introduction

Regulation of body temperature requires a delicate balance between the production and loss of heat; the hypothalamus regulates the set point at which body
temperature is maintained. In fever, this set point is elevated and non steroidal anti-inflammatory drugs promote its return to normal. These drugs do not influence body temperature when it is elevated by such factors as exercise or increase in the ambient temperature.

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, malignancy or other disease status. A common feature of these conditions is the enhanced formation of cytokines such as interleukin-1β, interleukin-6, α-Interferon, β-Interferon and Tissue Necrosis Factor (TNF). The cytokines increase the synthesis of PGE$_2$ in circumventricular organs in and near the preoptic hypothalamic area and PGE$_2$ in turn increases in cyclic AMP, triggers the hypothalamus to elevate body temperature by promoting increase in heat generation and decrease in heat loss. Antipyretic agents suppress this response by inhibiting the synthesis of PG E$_2$.

**Method**

Yeast induced pyrexia model in rats$^{329}$.

**Principle**

Pyrexia can be induced in rats by injecting yeast and since it is a large molecular weight protein, which acts as a pyrogen. The pyrexia so caused can be reduced by antipyretic agents.

**Animals**

Wistar albino rats.
**Materials**

Methanolic extract of BH in 1% v/v Tween 80 emulsion solution, 20% w/v brewers yeast suspension in normal saline and Paracetamol (PA) in 1% w/v SCMC suspension. 1% v/v Tween 80 (10 ml/kg, p.o.) was used as the vehicle.

**Experimental protocol**

The rats of either sex are grouped into four containing ten in each group. The body temperature of each rat is measured rectally using telethermometer. The rats were made febrile by subcutaneous injection into the dorsal region (1 ml/100 g of a 20% w/v brewers yeast suspension in normal saline). Then the animals were fasted, but allowed access to water, 19 h later, those rats which develop a satisfactory pyrexia, indicated by increase in body temperature of at least 1°C were randomly divided into four groups, each containing six rats. First group received 0.9% w/v sodium chloride solution (hypothermic control group), the second group received PA 200 mg/kg, p.o., third and fourth groups received methanolic extract of *B. hispida* 200 mg/kg, p.o., & 400 mg/kg p.o. (both as emulsion with 1% v/v Tween 80) The rectal temperature was recorded on a telethermometer inserted 4 cm in the rectum for 45 sec before and after 20, 21, 22 and 23 h after yeast injection.

The results of antipyretic activity data are presented in the table 28 and fig 106.

**Statistical analysis**

All data were represented as mean ± standard error mean (SEM) and are subjected to student’s ‘t’ test. In all cases, a difference was considered significance when p<0.05.
3.5.2.6 LOCAL ANAESTHETIC ACTIVITY

Introduction

Local anaesthetics are drugs, which, when applied locally to nerve tissue, cause reversible blockade of nerve impulse conduction. At effective concentrations, local anaesthetics block transmission of autonomic, somatic sensory and somatic motor impulses. Local anaesthetics inhibit the generation and propagation of nerve impulses by blockade of voltage gated sodium channels in the nerve membrane.

Method

Nerve plexus anaesthesia in frogs

Principle

The local anaesthetic activity can be easily studied by using three methods.

i) Nerve block anaesthesia - where the drug is added close to the nerve trunk.

ii) Surface anaesthesia - where the drug is administered into the conjunctiva of the eye and corneal reflex was studied.

iii) In-filtration anaesthesia - where the drug is administered intradermally and the injection site is tested for reaction to pin pricks.

Nerve block anaesthetic agents block generation and conduction of nerve impulse all parts of the neurone where they come in contact, without causing any structural damage. In nerve block anaesthesia model, the local anaesthetic drug added to the abdominal pouch in which the spinal nerves are exposed. Irritant chemicals such as O.1N HCl, when introduced to the foot of the frogs, due to the action of local anaesthetic, the foot withdrawal reflex will be delayed.
Animals

Frogs (100-150 g)

Materials

O.1N HCl, Lignocaine 1% w/v solution, methanolic extract of B. hispida (1%, 5%, 10%, 20% and 40% w/v emulsion in 1% v/v Tween 80).

Experimental protocol

Frogs are grouped into six (n=6). First group meant for standard drug (1% w/v Lignocaine) control. Group second to six for studying methanolic extract of B. hispida (1%, 5%, 10%, 20% and 40% w/v respectively)

Procedure

The frogs were decerebrated and the upper part of the spinal cord was destroyed. Abdomen cut opened and all the abdominal organs were removed, so that a pouch was made of abdominal walls. Spinal naves in the cavity were exposed. The frogs were fixed on the frog board with two of its hind legs hanging free from the board. A beaker containing 0.1 N HCl was introduced into the right hind leg. The brisk reflex withdrawal of the leg was noted. Then washed the right leg by dipping the leg in the beaker containing normal saline. The above procedure is repeated for left hind leg and reflex withdrawal was noted and the left leg was washed by dipping with normal saline. 10 ml of 1% w/v lignocaine was added to the abdominal pouch. The sciatic nerve flex is exposed to the local anaesthetic actions of the drug. Allowed the drug to act for 5 min. Then, immersed right and left hind legs in succession in the beaker containing 0.1N HCl as before. The delay in the reflex withdrawal of legs was noted. The above study
performed for various concentrations of methanolic extract of \textit{B. hispida} such as 1\%, 5\%, 10\%, 20\% and 40\% w/v solutions. The reflex withdrawals of legs in the various concentrations of \textit{B. hispida} treated frogs were noted.

The results of local anaesthetic activity was presented in the table 29.

3.4.2.7 ANTI SPASMODIC ACTIVITY

Introduction

Antispasmodics, often called spasmylytics and are used to ease griping or colic pain, expel wind (flatus) and relieve dyspepsia or indigestion. The plant kingdom is rich in such compounds. In fact most remedies used in conventional medicine include at least one antispasmodic of plant origin.

Antispasmodics are used to treat colic and indigestion. Examples of plant derived antispasmodics include: some tropane alkaloids (atropine, hyoscine); opium alkaloids (papaverine, codeine); essential oils (peppermint, dill, caraway, anise, thyme, garlic, and chamomile) and flavonoids (apigenin, kaempherol, quercetin).

Method

Antispasmotic activity in rat duodenum\textsuperscript{331}.

Principle

The contraction and hence spasm of the smooth muscle is mediated by acetylcholine, which is released from the post ganglionic parasympathetic nerve ending and acts as muscarinic receptor (M\textsubscript{3}) on smooth muscles. Anti cholinergic drugs act by blocking the action of acetylcholine, mediated through muscarinic receptors.
Antispasmodic drugs acts by blocking M₃ receptor and there by reduce the motility as a result of which the spasm may be relieved.

**Animals**

Male waster rats (150-175 g).

**Materials**

Acetylcholine (Ach) (10 µg/ml), Atropine (10 µg/ml), methanolic extract of *B. hispida* (50, 100, 200, 400 and 800 µg/ml) solutions.

**Physiological solution**

Tyrode solution.

**Instrument**

Student organ bath.

**Procedure**

The rats (n=6) were subjected to 24 h fasting and had access to water *ad libitum* prior to experimentation. The animals were killed by cervical dislocation. Duodenum of approximately 1.5 cm length were prepared and mounted in 20 ml inner organ tube containing tyrode solution. The bath solution was maintained at 33 ± 1°C and constantly oxygenated with 95% O₂+5% CO₂. The preparation was allowed to equilibrate for at least 1h under 1g resting tension. After the stabilization period (1 h), cumulative dose response curves (DRC) of acetylcholine was obtained. Atropine (10 µg/ml) and different
concentration of methanolic extract of BH (50, 100, 200, 400 and 800 µg/ml) were added to the organ bath after 5 min and again DRC of acetylcholine was taken.

3.5.3 Results and discussion

3.5.3.1 Acute toxicity study

The methanolic extract of *Borreria hispida* did not cause any mortality upto 2000 mg/kg and was considered as safe (OECD – 423 guideline unclassified).

3.5.3.2 Dose fixation

In order to fix the dose for methanolic extract of *B. hispida*, a pilot study was performed in which analgesic activity by acetic acid induced writhing reflex was chosen as the model. The % protection of analgesia was statistically insignificant in the lower doses such as 25, 50 and 100 mg/kg. Statistically significant analgesic activity was obtained at 200 mg/kg, p.o., and also in 400 mg/kg. A graded dose response was observed between 200 and 400 mg/kg, p.o. At higher doses such as 800 and 1600 mg/kg, p.o., the increment of % protection of analgesia was minimal and there was no graded dose response observed at these two higher dose levels. Probably, a ceiling dose response would have reached at 800 and 1600mg/kg p.o dose levels. Hence 200 and 400 mg/kg, p.o., was fixed as the dose for all the pharmacological studies.

3.5.3.3 HEPATOPROTECTIVE ACTIVITY

3.5.3.3.1 Results

BH at both dose levels (200 and 400 mg/kg) exhibited significant (P<0.001) hepatoprotective activity. The serum AST levels in BH (200 & 400 mg/kg) treatment group showed 190 ± 2.80 and 122.60 ± 3.11 IU/L respectively, serum ALT values as 128.5 ± 2.2 and 93.33 ± 3.16 respectively, serum ALP values as 231.67 ± 4.43 and
167.33 respectively. Total albumin levels as 3.45 ± 0.18 and 4.44 ± 0.17 respectively, total protein levels as 5.56 ± 0.15 and 6.44 ± 0.12 respectively and serum bilirubin levels as 2.81 ± 0.11 and 1.71 ± 0.09 respectively. (Table 22 and fig 100).

Normal lobular architect of the liver with central vein and portal tracts and cords of hepatocytes with sinusoids in between was observed in normal rats (Fig 107). Rats treated with carbon tetrachloride shown fatty degeneration of hepatocytes (Fig 108). In BH 200 mg/kg treated group (Fig 109), the patchy areas of fatty changes interspersed with normal hepatocytes. Central vein appears congested. In BH 400 mg/kg treated group (Fig 110), the fatty changes confined to perivenular region but the peripheral hepatocytes appears normal.

3.5.3.3.2 Discussion

Treatment of rats with CCl₄ is known to elevate the serum enzymes such as AST, ALP and bilirubin level. In the present study also, it was seen that the administration of CCl₄ (Group II) elevated the levels of serum marker enzymes such as ALT, AST, ALP and bilirubin. The levels of total protein and total albumin were lowered. Treatment with both silymarin and BH (200 & 400 mg/kg) decreased the elevated levels of AST, ALT, ALP and bilirubin compared to CCl₄ treated groups. The stabilization of serum bilirubin, AST, ALT and ALP by the drug treatment is a clear indication of the improvement of the functional status of the liver cells. This finding is further correlated with the histopathological studies.

In the present study, methanolic extract of B. hispida was found to contain the triterpenoids oleanolic and ursolic acids. Both oleanolic acid and ursolic acid are reported to have hepatoprotective property. Hence, the observed hepatoprotective
activity of methanolic extract of *B. hispia* may be, at least in part, be attributed to the presence of these triterpenoids.

### 3.5.3.3 Conclusion

This present study exhibited the hepatoprotective property of methanolic extract of *B. hispida* against CCl₄ induced hepatotoxicity in rats. These findings validate the ethnic claims of *B. hispida* and may be due to the active principles such as oleanolic acid and ursolic acid.

### 3.5.3.4 ANTI-INFLAMMATORY ACTIVITY

#### 3.5.3.4.1 Results

In carrageen an induced paw oedema (acute model), the standard anti-inflammatory drug (Diclofenac sodium 5 mg/kg, p.o.) as well as the test drug BH (200 & 400 mg/kg) exhibited a significant reduction (P<0.001) in the volume of paw oedema in rats as compared to the control rats. All the drugs showed maximum inhibition of the carrageenan induced rat paw oedema at the end of 3h. Their % inhibition of oedema are written within parenthesis. Diclofenac sodium showed maximum of 76.92% inhibition of oedema. BH 200 & 400 mg/kg produced 44.23 & 61.53% inhibition respectively. (Table 23 and fig 101).

#### 3.5.3.4.2 Discussion

Carrageenan induced paw edema is commonly employed technique for the evaluation of anti-inflammatory studies. Nitric oxide free radical is one of the mediators of inflammation. Drugs which scavenge the NO free radical reported to have anti-inflammatory activity. Since BH is having NO free radical scavenging property...
(discussed in section 3.5.3.5), the same, may be, in part, responsible for the anti-inflammatory activity of BH.

Oleanolic acid and ursolic acid are reported to have anti-inflammatory activity\textsuperscript{337 - 339}. The mechanism of anti-inflammatory effects of oleanolic acid and/or ursolic acid have been attributed to the following aspects

i) Inhibition of histamine release from mast cells induced by the compound 48/80 and concanavalin A\textsuperscript{340}.

ii) Inhibition by lipoxygenase and cyclo oxygenase activity\textsuperscript{341 & 342}.

iii) Inhibition of elastase\textsuperscript{343}.

iv) Inhibition of complement activity, possibly through the inhibition on C\textsubscript{3}-convertase of the classical complement pathway\textsuperscript{344}.

Both oleanolic acid and ursolic acid present in the methanolic extract of \textit{B. hispida} may be responsible for the observed anti-inflammatory activity.

3.5.3.4.3 Conclusion

Methanolic extract of \textit{B. hispida} showed significant anti-inflammatory activity and may be due to

i) Scavenging of NO free radical activity

ii) Presence of active principles such as oleanolic acid and ursolic acid.
3.5.3.5 IN-VITRO ANTI OXIDANT ACTIVITY

3.5.3.5.1 Results

DPPH Assay

The methanolic extract of BH studied for its in-vitro anti-oxidant activity by DPPH assay. Vitamin C was used as the reference standard.

The IC$_{50}$ values of methanolic extract of B. hispida and Vitamin C in DPPH method were found to be 470 µg/ml and 0.64 µg/ml respectively. (Table 24 and fig102 and Table 14 and Fig 36).

Nitric Oxide radical inhibition assay

The IC$_{50}$ values of methanolic extract of B. hispida and Vitamin C by NO radical inhibition assay were found to be 480 µg/ml and 0.72 µg/ml respectively. (Table 25 and fig 103 and Table 18 and Fig 40).

3.5.3.5.2 Discussion

The methanolic extract of B. hispida exhibited significant in-vitro anti-oxidant activity against DPPH and Nitric oxide radicals. NO radical scavenging activity is a desirable property since the involvement of NO is implicated in many diseases such as cancer, diabetes and in inflammation.

3.5.3.5.3 Conclusion

Mediation of free radicals in the pathophysiology of hepatitis and inflammation is reported. Methanolic extract of B. hispida showed significant hepatoprotective and anti-inflammatory activity. The above activities by BH, may be correlated with its antioxidant activity.
3.5.3.6 ANALGESIC ACTIVITY

3.5.3.6.1 Results

Acetic acid induced writhing response in mice

In the acetic acid induced writhing method methanolic extract of *B. hispida* and PA showed a significant reduction in the number of writhings mice. This reduction was dose related. BH (200 & 400 mg/kg) produced 44 and 69% protection (P<0.001) respectively. The standard drug Paracetamol (PA) produced a maximum of 80% protection (P<0.001) of analgesia. (Table 26 and fig 104).

**Tail immersion model**

In tail immersion model, the standard drug morphine (5 mg/kg) showed 205% protection (P<0.001) of analgesia while the BH (200 & 400 mg/kg) produced 66.66 & 128.90% protection of analgesia respectively. (Table 27 and fig 105).

3.5.3.6.2 Discussion

Acetic acid induced writhing reflex can be inhibited by both opioid as well non-opioid types of drugs. In the present study, BH exhibited significant analgesic activity against both acetic acid induced writhing reflex model and tail immersion model. Analgesic activity by tail immersion method is used for evaluation of opioid type analgesics.

3.5.3.6.3 Conclusion

BH, exhibited significant analgesic activity in both the models and its mechanisms may be due to both peripheral and central mediation. When compared with the % protection among two models, BH seems to preferably exert its analgesic activity
by central than peripheral mechanism. This present findings support the analgesic claims of *B. hispida*.

3.5.3.7 ANTIPYRETIC ACTIVITY

3.5.3.7.1 Results

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 19 h of administration. Treatment with methanolic extract of *B. hispida* at doses of 200 and 400 mg/kg decreased the rectal temperature of the rats in dose dependent manner. The antipyretic effect was maintained for 5 h, after yeast administration. The standard drug paracetamol at the dose of 200 mg/kg significantly reduced the yeast provoked elevation of body temperature. The results are presented in the table 28 and fig 106.

3.5.3.7.2 Discussion

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection or other disease states. The observed antipyretic effect of BH seems to support the view that the plant has some influence on prostaglandin biosynthesis, because prostaglandin is believed to be a regulator of body temperature.

3.5.3.7.3 Conclusion

The present findings showed that the methanolic extract of *B. hispida* possesses a significant antipyretic effect in yeast provoked elevation of body temperature in rats. Hence the results support the claim of traditional uses in fever.
3.5.3.8 LOCAL ANAESTHETIC ACTIVITY

**Results**

The standard drug lignocaine (1% w/v) exhibited local anaesthetic activity after 10 sec of its administration. The methanolic extract of *B. hispida*, did not produce local anaesthetic activity at all the concentrations tested. The results are presented in the table 29.

3.5.3.9 ANTI SPASMODIC ACTIVITY

**Results**

The methanolic extract of *B. hispida* did not exhibit anti spasmodic activity upto 800 µg/ml in the rat duodenum smooth muscle.

3.6 ANTI MICROBIAL ACTIVITY

3.6.1 Introduction

Infectious disease account for 19% mortality globally\(^\text{174}\). Despite the progress made in the understanding of microbiological diseases and their control, incidents of epidemics due to drug resistant micro-organisms and the emergence of hither to unknown disease due to microbes, pose enormous public health concerns. Historically, plants have provided a good source of anti-infective agents. Plant based antimicrobials represent a vast untapped source for medicines.

3.6.2 ANTI-BACTERIAL ACTIVITY\(^\text{332}\)

**Method**

Agar well diffusion
Medium

Mueller Hinton agar

Organism

i) Gram positive bacteria - *Staphylococcus aureus (ATCC 25923)*

ii) Gram negative bacteria - *Escherichia coli (ATCC 25922)*

The standard bacterial strains were procured from American type culture collection (ATCC # 21301, Park Lawn Drive Rockville, MD 20852, U.S.A) and were obtained from the department of microbiology, Madras Medical College, Chennai, India.

Preparation of the test drug (stock solution)

Ten mg of methanolic extract of *B. hispida* is dissolved in 10 ml of dimethyl sulfoxide (DMSO).

Preparation of the standard drug (stock solution)

Ten mg of ciprofloxacin dissolved in 10 ml of dimethyl sulfoxide.

- Test drug concentration : 100 to 1000 µg/ml
- Standard drug used : Ciprofloxacin
- Standard drug concentration : 10 µg/ml
- Petri dish employed : Borosilicate glass petridish (148 x 20mm)

Procedure

The petri dish employed was cleaned and sterilized (150°C for 2 h in oven). The sterilized (autoclaved at 120°C for 30 min) molten Muller Hinton Agar (between 40-50°C) was inoculated with the suspension of the micro organism (1ml/100ml of the
medium) and poured into the petri dish to give a depth of 3 to 4 mm, precautions were taken to produce uniform layer of medium on the plate. After the inoculum has completely solidified, a clean, sharp, sterilized cork borer was chosen. With single movement exactly perpendicular to the plate, a well made with the cork borer to the base of the plate and each plug is removed with the tip of small scalpel. Drawn up enough of a different concentrations of samples (in triplicate) and concentration of standard drug (in triplicate) by squeezing the pipette, so as the lower meniscus was at its tip and there was no air below the fluid. All the plates were refrigerated for 2 h at 4°C as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of the different solution. The plates were then incubated at 35-37°C for 24 h and the zone of inhibition were observed. All the operations were carried out under vertical laminar air flow bench.

Results

The standard drug ciprofloxacin exhibited significant anti bacterial activity, while the methanolic extract of *B. hispida*, did not show significant antibacterial activity at all the tested concentrations.

3.6.3 ANTI-FUNGAL ACTIVITY

Method

Agar well diffusion

Medium

Sabouraud dextrose agar
**Organism**

*Aspergillus fumigatus*

*Candida albicans*

*Candida Kru*

The above fungal strains were obtained from the department of microbiology, Madras, Madras Medical College, Chennai.

**Preparation of the test drug**

Ten mg of methanolic extract of *B. hispida* is dissolved in 10 ml of dimethyl sulfoxide (DMSO).

**Preparation of the standard drug**

Ten mg of ketokonazole is dissolved in 10 ml of dimethyl sulfoxide.

| Test drug concentration : | 100 to 1000 µg/ml |
|--------------------------:|------------------:
| Standard drug used       : | Ketokonazole     |
| Standard drug concentration used : | 10 µg/ml |
| Petri dish employed      : | Boro silicate glass petri dish (148 x 20mm) |

**Procedure**

The petri dish employed was cleaned and sterilized (150°C for 2 h in oven). The sterilized (autoclaved at 120°C for 30 min) molten Muller Hinton Agar (between 40-50°C) was inoculated with the suspension of the micro organism (1ml/100ml of the medium) and poured into the petri dish to give a depth of 3 to 4 mm, precautions were taken to produce uniform layer of medium on the plate. After the inoculum has
completely solidified, a clean, sharp, sterilized cork borer was chosen. With single movement exactly perpendicular to the plate, a well made with the cork borer to the base of the plate and each plug is removed with the tip of small scalpel. Drawn up enough of a different concentrations of samples (in triplicate) and concentration of standard drug (in triplicate) by squeezing the pipette, so as the lower meniscus was at its tip and there was no air below the fluid. All the plates were refrigerated for 2 h at 4°C as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of the different solution. The plates were then incubated at 35-37°C for 48 h and the zone of inhibition were observed. All the operations were carried out under vertical laminar air flow bench.

Results

The standard drug ketoconazole exhibited significant anti bacterial activity, while the methanolic extract of *B. hispida*, did not show significant antifungal activity at all the tested concentrations.
Table – 21. Preliminary qualitative tests on various extracts of *B. hispida*

<table>
<thead>
<tr>
<th>Tests</th>
<th>Hexane extract</th>
<th>Chloroform extract</th>
<th>Ethyl acetate extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside / Sugar</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Furan/ Indole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinonoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cumarin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lignan</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates positive test  - indicates negative test
HPTLC Finger Print of the methanolic extract of *B. hispida* with marker compounds

**(b)** Ursolic acid

**(a)** BH methanolic Ext.

Developing systems: CHCl₃: CH₃OH (19:1)

Scanning Wave Length: 200 nm.

Instrument: CAMAG - SCANNER 3

Software: CATS V 4.05 Version
Fig. 70b.

HPTLC Finger Print of the hydrolysed methanolic extract of B. hispida with marker compounds

(b) Isohamnetin

(a) Hydrolysed BH methanolic Ext.

Developing systems: Tolune : EtoAc (9:1)
Scanning Wave Length: 254 nm.
Instrument: CAMAG - SCANNER 3
Software: CATS V 4.05 Version
Fig. 71. IR Spectrum of Fraction 1

Transmittance / Wavenumber (cm$^{-1}$)

- 3338
- 2917
- 2849
- 1707
- 1472
- 1463
- 1377
- 729
- 719
Fig. 72. Gas Chromatography Spectrum of Fraction I

Abundance

Time ->

9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00

a 8.78

b 13.25

19.37 20.38 21.35 22.28 23.17 24.11 24.47
Fig. 73. GC-MS Matching Spectrum of peak 'a' of Fraction I with software database.

Mass Spectrum Corresponding to R: 8.78 minutes

#55992: Menthol (Database - wiley)
Fig. 74. GC-MS Matching Spectrum of peak b’ of Fraction I
with software data base

Mass Spectrum Corresponding to Rt 13.25 minutes

#33374: Nopinone (Database: Wiley)
Fig. 78. IR Spectrum of Fraction II

Transmittance / Wavenumber (cm⁻¹)
Fig. 77. GC-MS Matching Spectrum of peak 'a' of Fraction II

with software data base

Mass Spectrum Corresponding to Rt. 17.57 minutes

6,10,14−Trimethyl−2−pentadecanone

#211397: 6,10,14−trimethyl−2−pentadecanone (Database - wiley)

6,10,14−Trimethyl−2−pentadecanone
Fig. 78. GC-MS Matching Spectrum of peak 'b' of Fraction II with software data base.

Mass Spectrum Corresponding to Rt. 18.6 minutes:

#245668: Phytol (Data Base Wiley)
Fig. 81. Gas Chromatography Spectrum of Fraction III
Fig. 83. GC-MS Matching Spectrum of peak 'b' of Fraction III
with software data base

Mass Spectrum Corresponding to Rt. 30.71 minutes

#336889; Stigmasterol (Database - wiley)
Fig. 84. GC-MS Matching Spectrum of peak 'c' of Fraction II with software data base

Mass Spectrum Corresponding to Rt. 33.12 minutes

#340163: beta-Sitosterol

#340163: \(\beta\)-Sitosterol (Database - wiley)
Fig. 85. IR Spectrum of Fraction IV

Transmittance / Wavenumber (cm⁻¹)
Fig. 86. IR Spectrum of acetate of Fraction IV
Fig. 87. $^1$H NMR Spectrum of Acetate of Fraction IV ( δ, CDCl$_3$, 400 MHz)
Fig. 88. $^{13}$C NMR Spectrum of Acetate of Fraction IV (δ, CDCl$_3$, 100 MHz)

- Oleanolic acid acetate: $R_1 = R_2 = CH_3$, $R_3 = H$
- Ursolic acid acetate: $R_1 = R_3 = CH_3$, $R_2 = H$
Fig. 91. GC-MS Matching Spectrum of peak 'b' of acetate of Fraction IV

with software data base

Mass Spectrum Corresponding to Rt: 15.81 minutes

@3:20:44: Ursolic acid acetate (Data base - Wiley)
Fig 92. Mass fragmentations pattern of Menthol (C\textsubscript{10}H\textsubscript{20}O) (M\textsuperscript{+} = 156)

\[ \begin{align*}
\text{CH}_3 & \quad \text{OH} \\
\text{H}_3\text{C} & \quad \text{CH}_3
\end{align*} \]

\[ \begin{align*}
\text{M}^+ - 1 & = 155 \\
\text{M}^+ - \text{H}_2\text{O} & = 138 \\
\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3 & = 123 \\
\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3 - \text{CH}_2 & = 109 \\
\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3 - \text{CH}_2 - \text{CH}_2 & = 95 \\
\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 & = 81 \\
\text{Other Peaks m/z} & 71, 55, 41
\end{align*} \]
Fig 93. Mass fragmentations pattern of Nopinone (C$_9$H$_{14}$O) ($M^+ = 138$)

$$M^+ = 138$$
$$M^+ - CH_3 = 123$$
$$M^+ - CH_3 - CH_2 = 109$$
$$M^+ - CH_3 - CH_2 - CH_2 = 95$$
$$M^+ - CH_3 - CH_2 - CH_2 - CO = 67$$
$$m/z 83 (100\% )$$
Fig 94. Mass fragmentation pattern of 6, 10, 14 – Trimethyl - 2 - Pentadecanone
\((C_{18}H_{36}O) (M^+ = 268)\)

\[
\begin{align*}
M^+ &= 268 \\
M^+ - H_2O &= 250 \\
M^+ - CH_3(CO)CH_2 - H &= 210 \\
CH_3 - C = CH_2 \text{ (McLafferty fragment)} &= 58 \text{ (100%)}
\end{align*}
\]
Fig 95. Mass fragmentation pattern of Phytol (C₂₀H₄₀O) (M⁺ = 296)

\[ M^+ = 296 \]
\[ M^+ - H₂O = 278 \]
\[ M^+ - H₂O - CH₃ = 263 \]
\[ M^+ - H₂O - CH₃ - CH₂ = 249 \]
\[ 278 - \text{CH}_2 - \text{CH}_2 = 207 \]
Fig 96. Mass fragmentation pattern of Campesterol ($C_{28}H_{48}O$) ($M^+ = 400$)

$M^+ = 400$
$M^+ - \text{CH}_3 = 385$
$M^+ - \text{H}_2\text{O} = 382$
$M^+ - \text{H}_2\text{O} - \text{CH}_3 = 367$
$M^+ - \text{Cleavage C 22} - \text{C 23 bond} = 315$
$M^+ - \text{Cleavage C 20} - \text{C 22 bond} = 301$
$M^+ - \text{Side Chain} = 273$
$M^+ - \text{Side Chain} - \text{H}_2\text{O} = 255$
$M^+ - \text{Side Chain} - \text{Ring D fragment 42} = 231$
$231 - \text{H}_2\text{O} = 213$
Fig 97. Mass fragmentation pattern of Stigmasterol (C_{29}H_{48}O) (M^+ = 412)

M^+ = 412  
M^+ - H_2O = 394  
M^+ - H_2O – CH_3 – 2H = 379  
M^+ - Isopropyl = 369  
M^+ - Cleavage C 23 – C 24 bond = 327  
M^+ - Side Chain = 273  
M^+ - Side Chain – H_2O = 255  
M^+ - Side Chain – Ring D fragment 42 = 231
Fig 98. Mass fragmentation pattern of β-Sitosterol (C_{29}H_{50}O) (M^+ = 414)

M^+ = 414
M^+ - H_2O = 396
M^+ - H_2O - CH_3 = 381
M^+ - Side Chain = 273
M^+ - Side Chain - H_2O = 255
M^+ - Side Chain - Ring D fragment 42 = 231
231 - H_2O = 213
m/z 303 is more intense than m/z 300 characteristic of β - Sitosterol
Fig 99. Mass spectral fragmentation of Ursolic acid acetate
Table - 23 Anti-inflammatory activity of methanolic extract of *B. hispida* [BH] and Diclofenac sodium [DS] on rat hind paw oedema induced by carrageenan model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Increase in paw volume ± SEM (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>1% v/v Tween 80</td>
<td>10 ml/kg</td>
<td>0.21±0.013</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>5 mg/kg</td>
<td>0.12±0.009</td>
</tr>
<tr>
<td></td>
<td>[42.85] *</td>
<td>[55.55] *</td>
</tr>
<tr>
<td>Methanolic extract of BH</td>
<td>200 mg/kg</td>
<td>0.20±0.016</td>
</tr>
<tr>
<td></td>
<td>[4.76] *</td>
<td>[22.22] *</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.19±0.020</td>
</tr>
<tr>
<td></td>
<td>[09.52] *</td>
<td>[38.88] *</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SEM and are in seconds. Percentage of protection against carrageenan induced paw oedema are in parenthesis. The statistical difference between control and treated groups were tested by Student’s ‘t’ test. * P<0.0001, **P<0.01, ***P<0.05.
Fig. 10.1. Anti-inflammatory activity of BH after 3rd hour of carrageenan administration
Table – 24. *In-vitro* free radical scavenging effect of methanolic extract of *B. hispida* by DPPH method.

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Concentration of the test substance (µg / ml)</th>
<th>% Scavenging (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract of BH</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>33.85 ± 0.002*</td>
<td>33.94 ± 0.003 *</td>
</tr>
</tbody>
</table>

IC₅₀ of Methanolic extract of *B. hispida* is 470 µg/ml (r=0.99) P<0.001 compared to reagent blank. (Student’s ‘t’ test).

Table – 25. *In-vitro* free radical scavenging effect of methanolic extract of *B. hispida* by NO method.

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Concentration of the test substance (µg / ml)</th>
<th>% Scavenging (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract of BH</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>12.90 ± 0.002*</td>
<td>13.01 ± 0.004 *</td>
</tr>
</tbody>
</table>

IC₅₀ of Methanolic extract of *B. hispida* is 480 µg/ml (r=0.99) P<0.001 compared to reagent blank. (Student’s ‘t’ test).
Fig 102. *In-vitro* free radical scavenging effect of BH by DPPH method

Fig 103. *In-vitro* free radical scavenging effect of BH by NO method
Table – 26. Analgesic activity of methanolic extract of *B. hispida* [BH] and Paracetamol [PA] by acetic acid induced writing reflex method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Mean writhings</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% v/v Tween 80</td>
<td>10ml/kg</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100 mg/kg</td>
<td>09</td>
<td>80 ± 0.83*</td>
</tr>
<tr>
<td>Methanolic extract of BH</td>
<td>200 mg/kg</td>
<td>24</td>
<td>44 ± 0.64*</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>14</td>
<td>69 ± 0.76*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SEM and are in seconds. The statistical difference between control and treated groups were tested by Student’s ‘t’ test.
* P<0.0001, **P<0.01, ***P<0.05.
Table – 27. Analgesic activity of *B. hispida* [BH] and Morphine [MO] by tail immersion model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Reaction time in seconds</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial (Mean ± SEM)</td>
<td>60 Min (Mean ± SEM)</td>
</tr>
<tr>
<td>1% v/v Tween 80</td>
<td>10 ml/kg</td>
<td>1.77±0.049</td>
<td>1.90±0.040</td>
</tr>
<tr>
<td>Morphine</td>
<td>5 mg/kg</td>
<td>1.61±0.042</td>
<td>4.92±0.031</td>
</tr>
<tr>
<td>Methanolic extract of BH</td>
<td>200 mg/kg</td>
<td>1.68±0.035</td>
<td>2.80±0.013</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>1.66±0.044</td>
<td>3.80±0.185</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SEM and are in seconds. Percentage of protection against thermally induced pain by warm water are in parenthesis. The statistical difference between control and treated groups were tested by Student’s ‘t’ test.

* P<0.0001, **P<0.01, ***P<0.05.
Fig. 104. Analgesic activity of BH by acetic acid induced writhing reflex method

Fig. 105. Analgesic activity of BH by tail immersion model
Table – 28. Effect of methanolic extract of *B. hispida* [BH], Paracetamol [PA] on yeast induced pyrexia in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rectal temperature (C°) after yeast injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O h</td>
</tr>
<tr>
<td>1% v/v Tween 80</td>
<td>37.2 ± 0.09</td>
</tr>
<tr>
<td>Paracetamol (200mg/kg)</td>
<td>37.1 ± 0.06</td>
</tr>
<tr>
<td>B.H (200mg/kg)</td>
<td>37.4 ± 0.09*</td>
</tr>
<tr>
<td>B. H (400mg/kg)</td>
<td>37.0 ± 0.14*</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM of six rats. *p<0.0001, ? p<0.005. Student’s ‘t’ test.
Fig 106. Effect of BH on yeast induced pyrexia in rats.
Table 29. Local anaesthetic activity of *B. hispida* [BH], Lignocaine on nerve plexus anaesthesia in frogs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug/Dose</th>
<th>Reaction in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline 0.9% w/v</td>
<td>0 5 10 15 20</td>
</tr>
<tr>
<td>II</td>
<td>Lignocaine 1% w/v</td>
<td>+ + - - -</td>
</tr>
<tr>
<td>III</td>
<td>BH 1% w/v</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>IV</td>
<td>BH 5% w/v</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>V</td>
<td>BH 10% w/v</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>VI</td>
<td>BH 20% w/v</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>VII</td>
<td>BH 40% w/v</td>
<td>+ + + + +</td>
</tr>
</tbody>
</table>

+ Positive response  - Negative response