5. MATERIAL AND METHODS

5.1 SELECTION OF PLANTS

Selection of plants has been based on their ethnomedical & traditional uses. The plant *Hemidesmus indicus* Stem & Leaves and *Lantana camara* Stem & Flowers were chosen for the present investigation.

As discussed with tribal people of Jhabua (M.P.), these plants were widely used by them and other villages of Central India for treatment of pain, inflammation and to cure other illness of CNS disorders, therefore the plants were selected.

5.2 COLLECTION AND AUTHENTICATION OF PLANTS

*H. indicus* stem & leaves and *L. camara* stem & flowers obtained locally from Indore and Jhabua region of Madhya Pradesh. Identification of both plant samples were done by Botanist Dr. S. N. Dwivedi, Professor and Head, Department of Botany, Janta PG College, APS University, Rewa, M.P.

(Voucher No. J/BOT/H-238) for *H. indicus*

(Voucher No. J/BOT/L-251) for *L. camara*.

5.3 PHARMACOGNOSTICAL EVALUATION

5.3.1 MACROSCOPIC EXAMINATION

Color

Untreated samples were examined under diffuse day light. An artificial light source with wavelength similar to those of day light may also be used. The color of sample was observed.

Surface Characteristic, Texture and Fracture Characteristics

Materials was touched to determine if it is soft or hard bend and ruptured it to obtain information on brittleness and the appearance of the fracture plane-whether it is fibrous, smooth, rough, granular etc.
Odor

A small portion of the sample was placed in the palm of the hand and slowly and repeatedly, the air was inhaled over the material.

Taste

A small amount of drug powder was kept over the tongue and the taste was observed.

Loss on Drying

For estimation of loss on drying, it was dried at 105°C for 5 hours in an oven (Memmert), cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air-dried material.

5.3.2 MICROSCOPIC EXAMINATION

Microscopic examinations of medicinal plants are not only essential to the study of the adulterants but also are indispensable in the correct identification.

Classification of Microscopic Particles

The presence of cell contents, such as starch grains, aleurone grains, plastids, fats and oils, may render sections non-translucent and obscure certain microscopic characters. Many reagents that dissolve some of these contents were used in order to make the remaining parts stand out clearly or produce a penetrating effect. This has been rendered the section more transparent revealed details of the structures.

A solution of 10% potassium hydroxide was used as clarifying agent. Phloroglucinol and hydrochloric acid were applied to a sample on a slide by the following method:

The phloroglucinol and hydrochloric acid was placed on one edge of cover slip of a prepared specimen slide. The excessive fluid under the cover slip of the slide was removed by using a strip of tissue paper.

The different cell contents were observed by following methods.
5.3.3 QUANTITATIVE MICROSCOPY

The fresh plant parts leaves of *H. indicus* L. were subjected to standard procedure for the determination of various leaf constants.\(^4\)

**Determination of Stomatal Number**

Leaf fragments of 5x5 mm approx in size were placed in test tube containing 5ml of chloral hydrate solution. This was heated in a boiling water-bath for about 15 minutes or until the fragments become transparent. Fragments were then transferred to a microscopic slide and the mount was prepared with the lower epidermis uppermost, in chloral hydrate solution. Afterward a drop of glycerol-ethanol solution was put on one side of the cover glass to prevent the preparation from drying. It was examined with a 40X objective and with 6X eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (X) for each stomata. Thus for each surface of the leaf, average number of stomata per square millimeter was calculated.

**Determination of the Stomatal Index**

Fragments of leaves, approx 5x5 mm in size were placed in test-tube containing about 5 ml of chloral hydrate TS. Heat it on water-bath for about 15 minutes or until the fragments are transparent. Then transferred fragment to a slide and prepare it as described above. The lower epidermis uppermost, in chloral hydrate TS, placed a small drop of glycerol-ethanol TS at one side of the cover-glass to prevent the material from drying. This was examined under a microscope with a 40X objective and with 6X eyepiece, equipped with a drawing apparatus. Mark on the drawing paper a cross (χ) for each epidermal cell and a circle (O) for each stoma. The stomatal index was calculated as follows:

\[
\text{Stomatal index} = \frac{S \times 100}{E+S}
\]

Where, \(S\) = the number of stomata in a given area of leaf,
\(E\) = the number of epidermal cells (including trichomes) in the same area of leaf.
Determination of Palisade Ratio

The leaf fragments of size about 5×5 mm placed in test-tube, which contain 5 ml of chloral hydrate solution. It was then heated on boiling water-bath for about 15 minutes or until the fragments become transparent. Fragment to a microscopical slide was transferred and the mount of the upper epidermis in chloral hydrate solution was prepared and a small drop of glycerol solution was put on one side of the cover-glass to prevent from drying. It was examined with 40X objective and 6X eye piece. Drawing apparatus was attached. The four adjacent epidermal cells on paper traced, focused gently downward to bring the palisade into view. Then palisade cells under four epidermal cells were counted. Then a cell is intersected (include it in the count only when more than half of it, is within the area of the epidermal cells). The average number of palisade cells beneath one epidermal cell; divide the count by 4 was calculated.

Determination of Vein-Islet Number

Piece of leaf lamina (area not less than 4² mm) from the central portion of lamina has been taken. Margin and Midrib of leaf has been excluded. The pieces of lamina have been cleared by heating in test tube which contains chloral hydrate solution on a boiling water-bath for 30 to 60 minutes or until become clear. Prepared a mount in glycerol-solution or stain it with safranin solution (if desired). Examinations were done by 4X objective and 6X eye piece. By means of microscopical drawing apparatus, a line has been drawn, which represents 2 mm on a sheet of paper. Then construct a square on line representing an area of 4² mm. The paper has been moved, so that square has been seen in the center of field of eyepiece. Then placed a slide with clear leaf piece on microscope stage and veins and vein lets have been drawn, included within the square. It completes the outlines of those vein-islets which were overlapped two adjacent sides of the square. The numbers of vein-islets with in the square including those overlapping on two adjacent sides has been counted and exclude those intersected by other two sides. The obtained results were the number of vein-islets in 4² mm and the average number of vein-islets per square millimeter was calculated.
5.3.4 Fluorescence Analysis of Powdered drug

Powdered plant parts were screened for fluorescence characteristics with and without chemical treatment. The observations pertaining to the color, in day light and under ultraviolet (short and long) were noticed.\textsuperscript{5,6}

5.4 MICROMERITIC EVALUATION \textsuperscript{7,9}

Angle of Repose

Angle of repose is the maximum angle possible between the surface of a pile of powder and the horizontal plane.

\textbf{Procedure}: A glass funnel has been held in place with a clamp on a ring support over a glass plate. Approximately 50 gm of powder has been transferred into the funnel by keeping the orifice of the funnel blocked by the thumb. As the thumb removed, the lab-jack was adjusted, so as to lower the plate and maintain about 6.4 mm gap between the bottom of the funnel stem and the top of the powder pile. When the powder was emptied from the funnel, the angle of heap to the horizontal plane was measured with the protractor. Angle of repose was calculated by the formula.

\[
\tan \theta = \frac{h}{r}
\]

\[
\theta = \tan^{-1} \frac{h}{r}
\]

where,

- \( h \) = height of pile,
- \( \theta \) = angle of repose
- \( r \) = radius of the base of the pile
Bulk Density

Bulk density is the mass of powder divided by its bulk volume.

**Procedure:** A Powder (60 gm) has been passed through a standard sieve number 20. A weighed amount (50 gm) has been introduced into the bulk density apparatus and the timer knob was set for 100 tapings. The volume occupied by the powder was noted. Further, another 50 taps continued and the process of tapping continued until concurrent volume has been achieved. This final volume was the bulk volume. Bulk density was calculated by using the equation.

\[
\text{Bulk density (ρ)} = \frac{\text{Mass of powder}}{\text{Bulk volume}}
\]

Tapped Density

Tapped density is an increased bulk density attained after mechanically tapping of container.

**Procedure:** A powder sample has been filled in the graduated measuring cylinder. After observing the initial volume, the cylinder was mechanically tapped and a volume reading was taken until little further volume change was observed.

\[
\text{Tapped density (ρ)} = \frac{\text{Mass of powder}}{\text{Tapped volume}}
\]

5.5 PHYSICOCHEMICAL EVALUATION 10-12

**Determination of Foreign Matter**

About 10 gm of sample has been weighed and spread on a white tile uniformly, without overlapping. Then the sample was inspected by means of 5X lens and the foreign organic matter has been separated. After complete separation, the matter was weighed and percentage w/w was determined.
DETERMINATION OF SOLVENT EXTRACTION VALUE

Determinations of Water-Soluble Extractive Value

Five gm of powdered drug was macerated with 100ml of water closed flask for 2hr and was occasionally shakes with 6hr time period and was allowed to stand for 18hr. After filtration the 25ml of the filtrate evaporated to dryness in a tarred flat-bottomed shallow dish. Dried at 105°C and weighed. Percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

Determinations of Ethanol-Soluble Extractive Value

Ethanol is an ideal solvent for extraction of various chemicals like tannins, alkaloids, resins etc. Ethanol (95% V/V) was used for determination of ethanol soluble extractive. Five gm of powdered drug was macerated with 100ml of ethanol closed flask for 24 hours and was occasionally shakes with 6 hours time period and was allowed to stand for 18 hours. After filtration the 25 ml of the filtrate evaporated to dryness in a tarred flat-bottomed shallow dish. Dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

Determinations of Moisture Content

The percentage of active constituents in crude drug is mentioned on air dried bases. Hence, the moisture content of the crude drugs should be determined and should also be controlled. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

Procedure: The powdered sample of stem of *H. indicus* weighed 5gm accurately and kept in IR moisture balance. The loss in weight was recorded as percentage (%) moisture with respect to air-dried sample of crude drug.

Determinations of Ash Value

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drugs or adhering to it or deliberately added to it as a form of adulteration. Many a time the crude drugs are admixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with
different inorganic content. Ash value is a creation to judge the purity of crude drugs. Generally, either ash value or acid-insoluble ash value or both is determined. Total ash usually consists of phosphates, silicates and silica. On the other hand, acid-insoluble ash, which is a part of total ash insoluble in dilute hydrochloric acid, contains adhering dirt and sand.

**Determination of Total Ash**

Total ash was determined by weighing 2 gm of the air-dried crude drug in the tarred platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then was cooled and weighed.

**Determination of Acid Insoluble Ash**

The ash obtained from the previous process was boiled with 25ml of 2M HCl for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a desiccator and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.

**Determination of Water-Soluble Ash**

The ash was boiled with 25ml of water for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug.

**Determination of Foreign Matter**

Weigh 100-500 gm of the drug sample to be examined or the maximum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and calculate the percentage present.
5.6 Extraction of Plant Material

The crude dried powdered drugs 50 gm were kept for maceration in 200 ml ethanol for 7 days. These drugs were re-macerated and obtained extracts were further used for chemical evaluation. Same process has been repeated with water as a solvent.\textsuperscript{11,12}

5.7 PRELIMINARY PHYTOCHEMICAL EVALUATION OF EXTRACT\textsuperscript{11,12}

The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals.

Alkaloids

Small portion of alcoholic extract stirred separately with a few drops of dilute hydrochloric acid and then filtered. The filtrate is then tested carefully with various alkaloid reagents such as:

**Mayer’s Reagents**

Alkaloids give precipitate with Mayer’s reagents. One ml of Mayer’s reagent (Potassium mercuric iodide solution) was added to 1 ml extract, whitish yellow or cream-colored precipitate indicated the presence of alkaloids.

**Dragendorff’s Reagents**

Alkaloids give orange brown precipitate with Dragendorff’s reagents. One ml of Dragendorff’s reagent (Potassium bismuth iodide solution) was added to 1 ml extract, an orange-red precipitate indicated the presence of alkaloids.

**Hager’s Reagents**

Alkaloids give yellow colored precipitate with Hager’s reagents. In to the 1 ml extract, 3 ml of Hager’s reagent (saturated aqueous solution of picric acid) was added, a yellow colored precipitate indicated the presence of alkaloids.

**Wagner’s Reagents**

Alkaloids give reddish brown precipitate with Wagner’s reagents. In to 1 ml extract, 2 ml of Wagner’s reagent (iodine in potassium iodide) was added and the formation of reddish-brown precipitate indicated the presence of alkaloids.
Pharmacological Screening of Some Indigenous Plants for Various CNS Activities

Carbohydrates and Glycosides

A small quantity of each extract dissolved separately in distilled water and was filtered. The filtrate is subjected to the following test for Carbohydrates.

Molisch’s Test

One ml of α-napthol solution and concentrated sulphuric acid was added in 2 ml of the extract, through the side of the test tube. The formation of purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

Fehling’s Solution

Equal volume of Fehling’s A (copper sulphate in distilled water) and Fehling’s B (potassium tartrate and sodium hydroxide in distilled water) reagent was mixed along with few drops of extract solution and boiled, a brick red precipitate of cuprous oxide forms.

Benedict’s test

Extract solution was treated with few drops of Benedict reagent (alkaline Solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms, if reducing sugar is present.

Proteins and Free Amino Acids

Small quantity of alcoholic extract was dissolved in to the few ml of water and subjected to Millon’s, Biuret and Ninhydrin tests.

Gums and Mucilage

About 10 ml of extract were slowly added to the 25 ml of absolute alcohol with constant stirring, filtered, dried in air and examine for its swelling properties.

Terpenoids / Triterpenoids/ Steroids

(a) Salkowski Test: Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids.
(b) **Liebermann-Burchard Test:** Chloroform solution of the extract with few drops of acetic anhydride and one ml of concentrated sulphuric acid from the sides gives reddish ring at the junction of 2 layers.

**Volatile oil**

**By Hydro distillation method**

**Tannins**

Small quantities of alcoholic extracts were taken separately in water and tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%), 1% solution of gelatin containing 10% sodium chloride, 10% lead acetate and aqueous bromine solutions.

**5.8 ACUTE TOXICITY STUDY OF EXTRACT (LD\textsubscript{50})\textsuperscript{13,14}**

Acute oral toxicity studies have been conducted separately followed by using OECD guideline 423. The method used defined doses of 5, 50, 300, 2000 mg/kg \textit{p.o.} body weight. Results were allowed substance rank and classify according to the Globally Harmonized System (GHS) for classification of chemicals which causes acute toxicity. From LD\textsubscript{50} determination, 1/10\textsuperscript{th} of the dose was focused as the medial for pharmacological screening. Since all the animals were alive; no mortality, no toxicity and no significant changes in the body weight between the control and treated group were observed at a dose of 2000 mg for duration of 72 hours. This finding probably suggests that the ethanol and aqueous extract are relatively safe or non-toxic in rats at the doses used for this study.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>LD\textsubscript{50}</th>
<th>ED\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{H. indicus} L.</td>
<td>2000 mg/kg</td>
<td>200 mg/kg</td>
</tr>
<tr>
<td>\textit{L. camara} L.</td>
<td>2000 mg/kg</td>
<td>200 mg/kg</td>
</tr>
</tbody>
</table>

*Table 5.1: Acute Toxicity Study for \textit{H. indicus} & \textit{L. camara}.*

The result indicates 200 mg/kg dose has been considered as effective dose (ED\textsubscript{50}), for \textit{H. indicus} & \textit{L. camara}. *(Table 5.1)*
The present study has been carried out to evaluate the LD$_{50}$ and all Pharmacological activities of ethanolic extract & aqueous extract of *H. indicus* L. stem & leaves and also ethanolic extract & aqueous extract of *L. camara* L. stem & flowers.

**Drugs**

All drugs have been obtained from Pallav Chemicals Pvt. Ltd., Bombay. All extracts were suspended with the help of gum acacia in distilled water at the time of oral administration.

**Experimental Protocols**

All experimental protocols were reviewed and accepted by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of allied experiments.

*Protocol approval reference number (PBRI/IAEC/PN-17047a).*

**Experimental Animals**

Adult rats of Westar strains of 150-250 grams and Swiss albino mice of 18-25 grams of either sex have been obtained from local market of Indore. The animals have been placed in a controlled room, with normal room temperature 25 ± 3°C and humidity 35 - 50 %. Normal rat feeds and water *ad libitum* have been provided at regular interval of time. Animals have been housed in polypropylene cages. The animals have been allowed to acclimatize to laboratory conditions prior to experimental procedures.

**Administration of Dose**

The test extract has been administered in a single dose by gavages using a stomach tube or suitable feeding canula. If single dose is not possible in unfavourable conditions, then dose has been given in smaller fractions over a period not exceeding to 24 hours. Only water *ad libitum* has been provided to animals, rest they were fasted overnight, before dosing. Following the period of fasting, the animals were weighed and the test substances were administered. The dose level used as the starting dose has been selected from one of four fixed levels 5, 50, 300 and 2000 mg/kg body weight.

Acute toxicity studies have been performed with three animals and observations found after studies are:

i) Maximum 2000 mg/kg body weight of dose has been given.
ii) Depends on LD$_{50}$ determination, $1/10^{th}$ of the dose has been fixed as the medial dose for further pharmacological screenings.

iii) At maximum level of dose, no mortality rate has been found.

5.9 PHARMACOLOGICAL SCREENING FOR CNS $^{15-31}$

EXPERIMENTAL DESIGN

60 healthy & adult Swiss albino mice & 60 Westar rats were assigned to randomly to 10 groups as follows: (Table 5.2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>Drug administered</th>
<th>Drug administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal Control</td>
<td>Normal saline or Normal water</td>
<td>Normal saline or Normal water</td>
</tr>
<tr>
<td>2.</td>
<td>Positive control</td>
<td>Standard drug as per activity</td>
<td>Standard drug as per activity</td>
</tr>
<tr>
<td>3.</td>
<td>Test 1 (200 mg/kg)</td>
<td>5% W/V $H. indicus$ stem extract (ethanol)</td>
<td>5% W/V $L. camara$ stem extract (ethanol)</td>
</tr>
<tr>
<td>4.</td>
<td>Test 2 (400 mg/kg)</td>
<td>10% W/V $H. indicus$ stem extract (ethanol)</td>
<td>10% W/V $L. camara$ stem extract (ethanol)</td>
</tr>
<tr>
<td>5.</td>
<td>Test 3 (200 mg/kg)</td>
<td>5% W/V $H. indicus$ stem extract (aqueous)</td>
<td>5% W/V $L. camara$ stem extract (aqueous)</td>
</tr>
<tr>
<td>6.</td>
<td>Test 4 (400 mg/kg)</td>
<td>10% W/V $H. indicus$ stem extract (aqueous)</td>
<td>10% W/V $L. camara$ stem extract (aqueous)</td>
</tr>
<tr>
<td>7.</td>
<td>Test 5 (200 mg/kg)</td>
<td>5% W/V $H. indicus$ leaves extract (ethanol)</td>
<td>5% W/V $L. camara$ flower extract (ethanol)</td>
</tr>
<tr>
<td>8.</td>
<td>Test 6 (400 mg/kg)</td>
<td>10% W/V $H. indicus$ leaves extract (ethanol)</td>
<td>10% W/V $L. camara$ flower extract (ethanol)</td>
</tr>
<tr>
<td>9.</td>
<td>Test 7 (200 mg/kg)</td>
<td>5% W/V $H. indicus$ leaves extract (aqueous)</td>
<td>5% W/V $L. camara$ flower extract (aqueous)</td>
</tr>
<tr>
<td>10.</td>
<td>Test 8 (400 mg/kg)</td>
<td>10% W/V $H. indicus$ leaves extract (aqueous)</td>
<td>10% W/V $L. camara$ flower extract (aqueous)</td>
</tr>
</tbody>
</table>
5.9.1 ANALGESIC ACTIVITY

5.9.1 A. Chemical method

By acetic acid - induced writhing response  

Swiss albino mice were divided into 10 groups of 6 mice each (20-22 gm). Pentazocine (3mg/kg) i.p. was used as standard drug. The first group was given 10 ml/kg of normal saline orally, served as normal control and rest groups received drugs orally as per given experimental design. After 30 minutes of drug administration, mice of all groups were treated with Acetic acid (0.06% 1 ml acetic acid per 100 gm i.p). Then after five minutes of acetic acid injection mice were placed in individual cage and the number of abdominal contractions was counted for each mouse for a time period of 10 minutes.

Percentage inhibition of writhing was calculated using the expression:

\[
\text{Inhibition (\%)} = \frac{\text{Mean number of writhing (control)} - \text{mean no of writhing (test)}}{\text{Mean number of writhing (control)}} \times 100
\]

5.9.1 B. Thermal method

By Eddy’s hot-plate  

This test was carried out on a group of 6 Swiss mice of either sex (18-22 gm, n=6) using Eddy’s hot-plate apparatus. Only mice which showed initial nociceptive responses within 20 seconds were selected for the experiment. Animals were kept fasting of 16-18 hours. The extracts were dissolved in 2% gum acacia and administered orally. The reaction time (hind paw licking / jump response) of animals were delayed on hot plate maintained at 55 ± 1°C temperature was recorded & tabulated, after 30 minutes of drug administration. A cut off time was fixed of 10 seconds to avoid the injury to the paws. Diclofenac sodium (5 mg/kg), p.o. has been used as standard analgesic.
5.9.2 ANTI-INFLAMMATORY ACTIVITY

By Carrageenin induced paw edema

The adult Westar Albino rats (150-200 gm) were divided into 10 groups and each group has 06 rats. Inflammation induced by injecting 0.1ml of 1% Carrageenin into the sub planter tissue of the hind paw of either one side. All group administered drug as per schedule design and phenylbutazone (100 mg/kg) i.p was used as standard drug. It should be given 30 minutes prior to the Carrageenin injection. The paw volume has been measured before and 03 hours after Carrageenin administration by the volume displacement of water-mercury column using a plethysmometer.

5.9.3 ANTI-PYRETIC ACTIVITY

By Brewer’s yeast induced pyrexia

The adult Westar albino rats (180-200 gm) were divided into 10 groups of 06 rats each. Each group received drugs as per treatment design. Paracetamol (100mg/kg) i.p. was used as standard drug. Then after 30 minutes all the groups received 12% W/V suspension of Brewer’s yeast in the dose level of 1ml/100gm body weight s.c. into the loose connective tissue between the shoulder blades. Twelve hours after the injection, the rectal temperature of each rat has been measured using a digital thermometer, only rats that showed an increase in temperature of at least 0.7°C were used for the experiments. The rectal temperature was measured at 1, 2, 3 and 5 hours after drug administration.

5.9.4 CENTRAL MUSCLE RELAXANT PROPERTY

By Rota rod apparatus

Rota rod is a horizontal metal rod coated with rubber. Its diameter is 3cm, rotates with 25 rpm. The metal rod is about 50 cm above the surface to prevent the animal from jumping off from the roller. The mice have been placed on the revolving rod. Mice remains on Rota-Rod for 2 minutes or more in low successive trials after the administration of test drugs & control vehicle has been selected for the test. The test and standard drug (diazepam, 4mg/kg, i.p.) has been administered 1 hour before placing the rat on the Rota rod. The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and treated rats was taken as an index of muscle relaxation.
5.9.5 ANTI-CONVULSANT ACTIVITY

By Maximal Electro Shock Model\textsuperscript{35,36}

Adult albino male rats were randomly grouped in to respective designed groups (n=6). All groups received dose per orally (p.o). Standard group received Phenobarbitone (30mg/kg, i.p). Current was given after 2 hours of drug administrations. Seizures were induced by electro convulsiometer delivering electrical shock of 150mA of AC current for 0.2 ms through ear electrodes attached to the pinna. The phases of convulsion namely latent phase, tonic flexion of fore limbs, tonic extension of hind limbs & post ictal depression were observed in all groups. Decrease in the duration of hind limb extension & post ictal depression was considered as protective response & considered for statistical analysis. Percentage inhibition of tonic extension was calculated by considering the duration of tonic extension in control group as 100%.

5.9.6 CNS STIMULANT / DEPRESSANT ACTIVITY

By Actophotometer\textsuperscript{37,38}

In digital actophotometer, continuous beam of light falls on photoelectric cells. When the reading is considered as zero, any cut off in the continuity of light by the animal has been recorded on a digital counter in the form of counts. Depending on CNS action of the drug, the animals show locomotor activity either increased or decreased. Each animal has been placed in the actophotometer for 10 minutes and the initial locomotor reading has been taken. The mice have been divided into groups as per experimental design. The animals have been placed in the actophotometer for 10 minutes, after 30 minutes & 01 hour of drug administration. Hence locomotor activity has been measured. Diazepam, 4mg/kg, i.p. used as a standard drug.

5.9.7 ANXIOLYTIC ACTIVITY

By Elevated Plus Maze Apparatus\textsuperscript{39,40}

The test apparatus was validated by Lister.\textsuperscript{41}

An elevated plus maze consisting of two open arms (35×6 cm) and two enclosed arms (35 × 6 × 15 cm) has been used. The maze has been elevated to the height of 40 cm. Mice has been placed individually in center of apparatus, facing towards enclosed arm. The time spent by the
mouse during the next 05 minutes in open & enclosed arm has been recorded on second and seventh day of dosing schedule. The animals received vehicle (1ml/kg) or plant extracts 60 minutes before and diazepam (1mg/kg, *i.p.*), 30 min. before placement on the apparatus. The increased exploratory activity in the open arm has been taken as an indicator of anxiolytic activity.

### 5.9.8 SEDATIVE-HYPNOTICS ACTIVITY

**By Thiopental Sodium induced sleep**

Thiopental sodium (60 mg/kg *i.p.*, sub-hypnotic dose) has been injected, 30 minutes after administration of plant extracts. After these 30 minutes and after 15 minutes of diazepam treatment, thiopental sodium was administered to each animal. The control group (*n* = 6) has been given distilled water 10 ml/kg p.o & positive control group (*n* = 6) has been treated with diazepam (3 mg/kg *i.p.*). Then the animals were observed for the time to lose their righting reflex. The effects have been recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time has been considered to be the time interval between disappearance and reappearance of the righting reflex.

### 5.9.9 STATISTICAL ANALYSIS

Results were tabulated and the data was expressed as mean ± SEM.

The difference between experimental group were determined using one-way analysis of variance (ANOVA) followed by Dunnet test.

P ≤ 0.05 was considered significant.