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Evaluation of Reproductive Effect on Indian Traditional Herb Using Female Rats

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Abstract: This study's findings suggest that Premna tomentosa Willd (MEPT) and Premna latifolia Roxb (MEPL) extracts, when taken orally, have potent anti-oxidant, anti-inflammatory, and anti-arthritic activity against the exudative and proliferative phases of inflammation. Based on the results presented here, it appears that taking methanolic extracts of the whole plants of Premna tomentosa Willd (MEPT) and Premna latifolia Roxb (MEPL) can ameliorate various stages of chronic inflammatory states when taken orally. Because of its demonstrated effectiveness across all experimental models, the current investigation lends credence to the traditional medical community's use of the whole plant of Premna tomentosa Willd and Premna latifolia Roxb to treat inflammatory conditions.

1. INTRODUCTION

An extensive literature survey will be made in the first year with regard to Indian herbs The Indian herb will be identified and authenticated by a pharmacognosist or a Botanist. The collected Herbs will be shade dried completely and coarsely powdered. The extracts will be collected by hot percolation process, and they will be subjected to different chromatographic test to identify the presence of basic chemical constituents. The selected extract will be subjected to purification process and complete phyto chemical analysis will be carried out. The solvents required for extraction would be identified and their stability criteria would be established. The extract will be subjected for neurological pharmacological activity using animal models. Animal models will be surveyed to validate implantation.

The extract will be subjected for reproductive activity using animal models by
1. Implantation study
2. Fertility study
3. Uterotrophic assay

The statistical analysis would expresses mean ± SD. Suitable numerical techniques would be evolved to meet the purpose. This study will be explored on importance of Indian herbal plants special emphasis on reproductive system. It gives extensive evidence on free from adverse effects up on usage. I hoped that these selected herbs show more effective, efficient and potent action on reproductive studies. In order to analyses the testing new therapeutic agent, analysis of genetic susceptibility factors and search for bio markers for implementation of have great potential to provide new insights and suggest new lines of research.

Literature Survey
The following are some of the related works:

Research on the effects of Premna tomentosa wild and Premna latifolia roxb extracts on inflammation in rats was done.

Herbal remedies for the male and female reproductive systems were often employed in the past. But there is no study that proves these outcomes scientifically. An example of one of these plants (Premna tomentosa willd) was chosen and gathered from Andhra Pradesh, India for this investigation. S.V. University botanist Dr.K Madhavachetty verified the authenticity of the plant specimens.

All the phytoconstituents in the plant material were extracted through percolation with a methanol and water solvent, after the plant material was dried and ground into a powder. In order to extract the phytoconstituents from plant material, it must first be dried, powdered, and then extracted using a suitable solvent.

In order to determine what phytoconstituents were present in the extract, a series of chemical analyses were performed.

In the month of December 2012, entire plants of the Verbenaceae family members Premna tomentosa Willd and Premna latifolia Roxb were taken from the Tirumala hills in Tirupati (Andhra Pradesh, India). Botany assistant professor Dr. Madhavachetty from Sri Venkateswara University in Tirupati, Andhra Pradesh, India, confirmed the plant's legitimacy.

After 2 weeks of air drying in the shade, we used the dried portions of the Premna tomentosa Willd and Premna latifolia Roxb plants. The dried dugs were ground into a coarse powder and sieved through a No. 40 to prepare them for extraction with solvents of progressively higher polarity. To separate various solvents, a soxhlet apparatus may be used to perform continuous hot percolation at varying temperatures, such as with petroleum ether (60°-80°C), chloroform (59.5-61.5°C), ethyl acetate (55.5-56.5°C), and methanol (79°-81°C). Extracts were filtered and concentrated to dryness under low pressure after they were collected.

Evaluation of Premna latifolia and Premna tomentosa for their pharmacological properties. Macroscopical aspects of Premna tomentosa and Premna latifolia were analysed, including those of the entire plant parts and the powder made by grinding the whole plant parts.

Transverse Section of the Specimen:
Collection of specimens, Sectioning, Staining, Photomicrograph

2. Powder Microscopy
Physico-Chemical Parameters:
Determination of foreign matter, moisture content, ash value, extractive value

Preliminary qualitative phytochemical examination:
Petroleum ether (60°-80°C), Chloroform (59.5-61.5°C), Ethyl acetate(55.5 - 56.5°C), and Methanol (79°-81°C) extracts of whole plants of Premna tomentosa Willd and Premna latifolia Roxb were subjected to preliminary phytochemical screening for Alkaloids, Glycosides, Phytosterols, Volatile oil, Saponins, Phenolic compounds, Tannins, Proteins and free amino acids, Gums and Mucilage, Flavonoids, and Lignins,

Fluorescence analysis:
The fluorescence analysis of the drugs was observed in day light and UV light (254mm) using powder and various extracts of the drugs.

Thin layer chromatography:
All the extracts of Premna tomentosa Willd and Premna latifolia Roxb were subjected to thin layer chromatography using different solvent systems. Different spots developed in each solvent system were identified by means of iodine chamber, UV light and hydrochloric acid.

High Performance Thin layer chromatography
HPTLC profile for the Methanolic extract of Premna tomentosa Willd and Premna latifolia Roxb were performed using CAMAG make HPTLC@ 245nm to 366 nm.

3. Pharmacological study

Acute oral toxicity
The Methanolic extract of whole plants of Premna tomentosa Willd.(MEPT) and Premna latifolia Roxb.(MEPL) was selected for acute oral toxicity study and the dose was selected according to the OECD guidelines 423. The signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma, as well as the onset of toxicity and signs of toxicity were also noted.

In vivo anti oxidant activity:
Experimental groups of rats received, Group I - Received vehicle [Normal control]1% v/v Tween 80, 1ml/100 g, Group II - CCl_4 (1 ml/kg of body weight), i.p [Negative Control], Group III & IV - Received methanol extract of whole plant of Premna tomentosa Willd.(MEPT) (200& 400mg/kg body weight p.o) suspended in 1% v/v Tween 80 + CCl_4 (1 ml/kg of body weight), i.p, Group V& VI - Received methanol extract of whole plant of Premna latifolia Roxb. (MEPL) (200 & 400 mg/kg body weight p.o) suspended in 1% v/v Tween 80 + CCl_4 (1 ml/kg of body weight), i.p On the 15^{th} day all group of animals were kept fasting for 12 h and sacrificed by cervical dislocation. Blood was collected from the jugular vein into tubes containing heparin (anticoagulant), centrifuged at 3000 rpm for 15 min and the resulting buffy coat removed. The packed cells were washed three times with physiological saline (0.9% NaCl), lysed by suspending them in cold distilled water, and then centrifuged at 7000 rpm for 30 min. The resulting pellet contained the erythrocyte membrane and the supernatant represented the haemolysate.

Biochemical estimation
Plasma resulting from the initial centrifugation was used for measuring lipid peroxidase following the method of Gutteridge and Wilkins (1982) while the haemolysate was used for the estimation of 1. Superoxide dismutase 2. Catalase 3. Glutathione Reductase and 4. Glutathione peroxidase.

4. Analgesic activity

Analgesic activity by Formalin induced pain (Hunskaar and Hole)
Experimental animals were injected subcutaneously with20µl of formalin into the dorsal hind paw. Group I - Received vehicle control (1% v/v Tween 80, 1ml/100 g) ,Group II& III - Received methanol extract of whole plant of Premna tomentosa Willd. (MEPT) (200&

400mg/kg body weight p.o) suspended in 1% v/v Tween 80,Group IV& V - Received methanol extract of whole plant of Premna latifolia Roxb. (MEPL) (200 & 400 mg/kg body weight p.o) suspended in 1% v/v Tween 80 and Group VI- Received standard drug (Indomethacin, 10mg/kg) p.o, respectively 30 min before formalin injection.

The time the mice spent licking or biting the injected paw or leg was recorded. On the basis of the response pattern described by Tjolsen et al. two distinct periods of intensive licking activity were identified and scored separately. The first period (early phase) was recorded 1-5 min after the injection of formalin and the second period (latephase) was recorded 20–40 min after the injection. The percentage inhibition of licking was calculated by the formula:(C−T)/C×100 where C represents the vehicle treated control group value for each phase and T represents the treated group value for each phase.

Analgesic activity by Eddy's Hot plate method (Turner)
Experimental animals received, Group I - Received vehicle control (1% v/v Tween 80, 1ml/100g) , Group II& III - Received methanol extract of whole plant of Premna tomentosa Willd.(MEPT) (200 & 400mg/kg body weight p.o) suspended in 1% v/v Tween 80, Group IV& V - Received methanol extract of whole plant of Premna latifolia Roxb. (MEPL) (200 400 mg/kg body weight p.o) suspended in 1% v/v Tween 80 and Group VI- Received standard drug morphine sulphate (5 mg/kg s.c) 30 min before the thermal pain stimulus.
Mice were screened by placing them on a hot plate maintained at 55±1 ˚C and recorded the reaction time in seconds for licking of hind paw or jumping.

Anti inflammatory activity
Acute Model: Carrageenan-induced Paw Edema in Rats
Group I - Received vehicle (Negative control) 1% v/v Tween 80, 1ml/100 g, Group II& III - Received methanol extract of whole plant of Premna tomentosa Willd. (MEPT) (200 &400 mg/kg body weight p.o) suspended in 1% v/v Tween 80 ,Group IV& V - Received methanol extract of whole plant of Premna latifolia Roxb. (MEPL) (200 & 400 mg/kg body weight p.o) suspended in 1% v/v Tween 80, Group VI - Received standard drug (Diclofenac sodium, 100mg/kg) p.o. suspended in 1% v/v Tween 80.

One hour after treatment, the rats are challenged by a subcutaneous injection of 0.1ml of 1% freshly prepared solution of carrageenan (1% carrageenan suspension in 0.9% NaCl solution) was injected into the sub-plantar tissue of the right hind paw. The paw volume was measured initially and at intervals of 30, 60, 120, 180 & 240 mins after carrageenan injection by volume displacement method using Plethysmometer by immersing the paw in mercury cell. The inflammation in paw volume is calculated as percentage compared with the basal volume. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically. The percentage inhibition of paw edema was calculated by using the following formula;

Percentage of edema inhibition = (Vc -Vt/Vc) × 100
Vc- Volume of paw edema in control group
Vt- volume of paw edema in treated group

Chronic Model: **Cotton Pellet-Induced Granuloma in Rats**

The same experimental design is used followed by the acute method. One hour after treatment, the animals are anesthetized with Diethyl ether and 20 mg of the sterile cotton pellet was inserted one in each axilla and groin of each rat in all groups by making small subcutaneous incision. The incisions were sutured by sterile catgut. All animals were sacrificed by cervical dislocation on the 8th day and cotton pellets were surgically dissected out. The isolated cotton pellets were separated from extraneous tissue and dried at 60°C until weight become constant. The net dry weight of each cotton pellet was determined (after subtracting the initial weight of the cotton pellet). The mean weights of the cotton pellet of the control and treatment groups were calculated. The percentage of anti-inflammatory activity was calculated by inhibition of increase in the weight of the cotton pellet was estimated.

The percent inhibition increase in the weight of the cotton pellets was calculated by:
% Inhibition = [Wc – Wt / Wc] X 100
Wc = Pellet weight of the control group
Wt = Pellet weight of the drug treated group

Acute non-immunological arthritis

Turpentine oil induced joint oedema in rats

Acute non-immunological inflammatory joint oedema was induced by injecting 0.02 ml of turpentine oil in to the synovial cavity of the knee joint, 30 min after the drug administration. Arthritis was induced to all groups of animal except Group I. Diameter of the joint was monitored at 30 min, 1, 2, 3, 4, 5th and 6th hr, using micrometer screw gauge.

Group I - Received vehicle (Normal control) 1% v/v Tween 80, 1ml/100 g, Group II - Received vehicle (Arthritis control) 1% v/v Tween 80, 1ml/100 g, Group III & IV -Received methanol extract of whole plant of Premna tomentosa Willd. (MEPT) (2000 & 40 mg/kg body weight p.o) suspended in 1% v/v Tween 80, Group V& VI –Received methanol extract of whole plant of Premna latifolia Roxb. (MEPL) (200 & 400 mg/kg body weight p.o) suspended in 1% v/v Tween 80, Group VII - Received standard drug (Diclofenac sodium, 4 mg/kg) p.o, respectively.

Formaldehyde induced arthritis in rats

Similar experimental design was followed in continuation On day 1, 30 min after the drug administration chronic non immunological arthritis was induced by sub-plantar injection of 0.1ml of 2% formaldehyde solution and repeated on day 3. Arthritis was induced to all groups of animal except Group I. Arthritis was assessed by measuring the mean increase in paw diameter over a period of 10 days using micrometer screw gauge.

Histological processing and assessment of arthritis damage

On day 10, animals were sacrificed; knee joints were removed and kept in 5% formaldehyde. After decalcification in 5% formic acid, processed for paraffin embedding tissue sections (7 μm thick) were stained with haematoxylin and eosin.

Chronic Immunological Arthritis

Chronic immunological CFA-induced arthritis in rats (New bould)

Similar experimental design was followed as non- immunological 1 day before the complete freund's adjuvant injection and daily treatment continued for 21 days. The oedema of the left and right hind paws was evaluated at 4, 8, 14 and 21days post injection of complete freund's adjuvant using micrometer screw gauge. After the 21st day, animals were sacrificed by cervical dislocation and their legs were amputated at knee joints.

Histological processing and assessment of arthritis damage
Histopathological changes of arthritis damage were scored as: (none, mild, moderate, severe).

Estimation of haematological parameters
On the 21stday after arthritis induction, rats were sacrificed by cervical dislocation and blood samples were collected into ethylene diamine tetraacetic acid coated tubes by cardiac puncture. Estimation of RBC count, WBC count, neutrophils, easinophils, lymphocytes and haemoglobinby manual techniques established in the laboratory. The estimation of rheumatoid factor (RF) by turbidimetric method (BTR-810, Ranbaxy) was determined.

Table 1: Pharmacognostical studies of Premna latifolia and Premna tomentosa
Macroscopical Features of whole plant of Premna tomentosa Willd

Characteristics	Observations			
	Bark	**Leaves**	**Flowers**	**Powdered plant material**
Colour	Light greyish brown	Green	Pinkish white	Pale green
Odour	Odourless	Pleasant odour	Characteristic	Characteristic
Taste	Characteristic	Slightly bitter	Characteristic	Slightly bitter
Texture	Slightly rough	Slightly rough	Smooth	---
Shape	Branches and branchlets obtusely quadrangular	Simple, decussate-opposite, lanceolate-elliptic, ovate-cordate	bisexual, numerous, pedicels	---
Size	Up to 25ft	5-22 × 3-14 cm	2-4 mm long, 1-2mm wide	---

Table 2: Macroscopical Features of whole plant of Premna latifoliaRoxb

Characteristics	Observations			
	Bark	**Leaves**	**Flowers**	**Powdered plant material**
Colour	Dark greyish	Green	Creamish white, yellowish green,	Pale green
Odour	Odourless	Unpleasant	Unpleasant	Characteristic
Taste	Characteristic	Slightly bitter	Characteristic	Slightly bitter
Texture	Slightly rough	Slightly	Smooth	---

		rough		
Shape	Obtusely quadrangular, lenticellate, nodes annulate	Lanceolate-ovate, elliptic-ovate to rhomboid	bisexual, numerous	---
Size	Up to 25ft	3-9 x 1.5-6 cm	Pedicels 1-3 mm long	---

Anatomy of the leaf

In sectional view, the lamina shows a median thick midrib and smaller lateral views. The midrib and lateral views are prominent and they hang on the abaxial side of the lamina (Figure 4.3.1). The midrib is elliptical in section view and it projects above the adaxial side into wide, short, hump. On the abaxial part it forms large and thick major part of the midrib. The midrib is 670 µm thick. The adaxial hump is 150 µm wide and the abaxial part of the midrib is 570 µm wide.

The midrib consists of distinct epidermal layer, parenchymatous ground tissue and a circular vascular cylinder (Figure 4.3.2). The epidermal cells are small, circular and thick walled. Glandular trichomes are occasionally seen on the epidermal layer. The ground tissue includes 3 or 4 outer layers of thick walled cells and circular, less compact, thin walled inner parenchyma cells. The ground tissue in the adaxial hump includes small, circular collenchymas cells.

The vascular cylinder consists of a wide and deep cup shaped major vascular strand and a large circular vascular strand located in the adaxial part of the adaxial hump. The main vascular system consists of numerous, long, radial and compact, angular, thick walled xylem elements and several, independent masses of phloem tissue located on the outer periphery of the xylem strand. There are thick, discontinuous layer of sclerenchyma elements on the outer end of the phloem. The adaxial circular vascular strand consists of 2 opposite segments of xylem; phloem occurs in between the 2 xylem segments. The 2 vascular strands have phloem just opposed.

Lateral Vein

The lateral vein is planoconvex with flat adaxial side and thick abaxial part. The structure is more or less similar to midrib. The vascular strand consists of a bowl shaped collateral main structure and a small, circular adaxial strand. The main strand includes several radial compact lines of xylem elements and thick layer of phloem elements on the lower part of the xylem. A thin layer of sclerenchyma cells is located along the outer border of the vascular structure. The adaxial strand is circular with thin outer ring of xylem elements enclosing a central mass of phloem elements

Lamina

It is dorsiventral measuring about 100 µm in thickness. The adaxial epidermis consists of thick epidermal cells which are wide and squarish in outline and possess thick cuticle. The adaxial epidermis is apostomatic. Thenabaxial epidermis is thin and includes small, rectangular or circular thick walled cells. The mesophyll tissue consists of 2 horizontal layers

of columnar palisade cells and 2 or 3 layers of lobed, loosely arranged spongy parenchyma cells.

Epidermal trichomes are commonly seen on the lower epidermis of the lamina. The trichomes are either glandular or non glandular type. The glandular trichomes are capitates type. They have a single, short, thin walled stalk cell and a spherical head of 4 compact secretory cells.

The glandular trichomes are 25 µm in height. The glandular head is 20 µm in diameter. The non glandulartrichomes are 2 or 3 celled, uniseriate and unbranched. They have thick lignified walls and narrow lumen

Epidermal cells and stomata
In surface of epidermal cells in paradermal sections appear small cells with thick wavy anticlinal walls. The adaxial epidermis has no stomata. The cell walls are smooth.

The abaxial epidermis consists of epidermal cells with wavy thick anticlinal walls. Stomata are densely distributed in the abaxial epidermis. The stomata are actinocytic, each stoma is surrounded by 4 or 5 radially stretched subsidiary cells. The guard cells are broadly elliptical and measure 15 x 25 µm in size. In surface view of the epidermis there are also seen cross sectional view of the glandular trichomes The secretory head of the gland includes a circle of 4 cells. Calcium oxalate crystals are seen densely clotting the veins. The crystals are short, spindle shaped. They occur within the epidermal cells which form bundle sheath cells of the view.

Venation pattern
The lamina consists of densely reticulate venation system. The major lateral veins give rise to smaller vein lets which form polyhedral vein islets. The vein lets have thick and distinct vein boundaries and within the islet there are mostly simple, unbranched vein terminations. The terminations are thin and long. They are mostly straight.

Petiole
In cross section view the petiole is triangular with adaxial flat side. The epidermal layer of the petiole is very thin and less prominent. The cells are circular, thin walled and darkly stained. The ground tissue is homogeneous with compact, polygonal parenchyma cells. The ground tissue in the central part of the petiole is larger with thin compact cell. The vascular system consists of a triangular outline which is wavy with shallow, ridges and furrows. The ridged layer of vascular strands consists of xylem inside the ridge and phloem outside. Each vascular segment has thick and prominent sclerenchyma cap. The xylem elements are in short, parallel radial lines each line having 2 to 5 elements. The phloem is thick and massive and includes sieve elements and phloem parenchyma cells. The petiole is 2.1 mm in vertical plane and 1.6 mm in horizontal plane.

Stem
The stem is circular in cross sectional view. It consists of 2 or 3 successive thin cylinders of periderm. The epidermal layer is thin with small, squarish, thick walled cells. The epidermis is broken at several places. Inner to the epidermis is a layer of first formed periderm which includes 4 layers of radially aligned suberized phellem cells.

Inner to the first periderm occurs a circle of highly thick masses of fibres. Inner to the fibre masses occurs a second layer of periderm. In this region the periderm cells are rectangular and radially elongated

The second layer of periderm is followed by a thick cylinder of wide, thin walled sclerenchyma elements. This is followed by a third layer of periderm which consists of radially arranged tabular or rectangular phellem cells. The inner boundary of the third layer of periderm consists of a thin continuous cylinder of sclerenchyma.

Secondary phloem occurs in the form of a thin continuous cylinder in between the fibre cylinder and secondary xylem. The phloem consists of sieve elements with companion cells and phloem parenchyma.

Secondary xylem cylinder is in the form of hollow, dense structure and includes vessels, fibres and rays. The vessel elements of the secondary xylem are solitary or in radial multiples of 2 or 3. The vessels are circular or elliptical, fairly thick walled and are 40µm in diameter. A growth ring is seen in the middle part of the xylem cylinder

Xylem fibres are polyhedral in sectional view thick walled and lignified. The lumen of the fibre is fairly wide. The xylem rays are 1 or 2 cells thick, they are straight and the cells are fairly thick and lignified

Root

The roots of thin and thick types were studied. The thin root is 1.5mm in diameter. The thin root consists of comparatively thin periderm. However the xylem cylinder is thick and dense. The thin root has fairly distinct superficial periderm which consists of 7 layers of cells which are tabular in shape and suberized.

Inner to the periderm is a wide cortical zone where the cells have dark dense inclusions. In the middle part of the cortex occurs a thin continuous cylinder of sclerenchyma cells.

Secondary phloem occurs in thin continuous layer around the xylem cylinder. Secondary xylem includes several successive rings of vessels. Each ring represents a growth ring within a growth ring are seen only 2 or 3 layers of vessels. The vessels are all mostly uniform in diameter and a few vessels may be narrow. The vessels are 40µm in diameter.

Thick root

The thick root has heterogeneous thick periderm which is 200 µm in thickness the periderm consists of regular, radial rows of tabular cells. The periderm shows 2 thin rings of cells. Each ring being one cell in thickness. These uniseriate rings have lignified walls and they are called phelloid cells. The remaining cells of the periderm have suberized cell walls and they are called phellem cells. Inner to the periderm occurs the cortex where the cortical cells have dense dark inclusions. The secondary xylem exhibits several concentric rings of vessels and each ring appears to be a growth ring boundary. The vessels of the xylem are mostly solitary or in short radial multipliers. They are circular or elliptical. Both narrow and wide vessels are seen. The wide vessels are 40µm in diameter and the narrow vessels are 20 µm in diameter. Xylem fibres are highly thick walled and lignified. Xylem rays are narrow and straight. The ray cells are radially elongated and thin walled. Calcium oxalate crystals are seen in the

xylem fibres. The crystals are druses. The crystals are not abundant and occur diffusely in the secondary xylem.

POWDER MICROSCOPY
The powder preparation exhibits the following elements:

Trichomes:
A unique type of trichome called dendroid trichome is abundant in the powder These trichomes are densely distributed on the lower surface of the lamina. Some of the dendroid trichomes have a long stalk which consists of 4 or 5 rectangular cells. The stalk cells are thin walled and they may be 130 to 310µm in length

At the tip of the stalk occur a cluster of horizontally oriented branches of trichome. These horizontal trichomes appear stellate in surface view

Some of the dendroid trichomes have a large number of lateral trichome which appear like a bunch. Lateral trichomes are mostly vertical in position. Some of the dendroid trichomes are subsessile having a short, less prominent stalk with terminal cluster of lateral trichomes. The lateral branches are up to 360µm long and 5µm thick. The cell walls of the terminal trichomes are thick and lignified.

A second type of nonglandulartrichome is less common. This trichome consists of 3 or 4 cells and the trichome is uniseriate and unbranched. They have comparatively thin walls and wide lumen. These uniseriatetrichomes are more than 300µm in length and 15µm thick

Glandular trichome
Detached from the lamina are seen the secretory body of the glandular trichomes. The trichome is circular with 4 cells arranged in cruciate form. The cell walls are thin and have dense content

Xylem elements:e powder of the xylem tissue includes 3 major components namely vessel elements, fibres and parenchyma cells. Tracheids are also occasionally seen in the powder

Tracheids: The tracheids are narrow, much elongated cylindrical cells with dense bordered pits on the lateral walls. No perforation plate is evident. The tracheid is 320µm long and 20µm thick

Fibres: The fibres are mostly narrow and libriform type. They have thick lignified walls and narrow lumen. The ends of the fibre are gradually tapering into pointed tip ,Some fibre appear to be short and wide. The fibres are upto 550µm long and 10µm thick.
Vessel elements: The vessel elements are mostly cylindrical, narrow and long. The vessel elements have dense, multiseriate lateral wall pits The end wall perforation is wide circular and horizontal Some of the vessel elements have short tails and oblique elliptical perforations

Microscopical Studies of Premna latifolia Roxb
Anatomy of the leaf
The leaf consists of prominent and thick midrib and thin lamina with fairly dense trichome. The midrib is wide and thick in the abaxial region and thin wide hump in the adaxial region.

It is more than 1mm thick, the adaxial hump is 450 µm wide and the abaxial part is 1mm wide.

The epidermal layer of the midrib consists of small, circular, slightly echrinate thick walled cells. The ground tissue in the abaxial part consists of angular, compact, thin walled parenchyma cells. In the adaxial hump, the ground tissue is collenchymatous. The vascular system is multi-stranded and includes adaxial and abaxial bundles. In the adaxial part, there are two vertically cylindrical vascular bundles which are collateral. They consist of a small group of narrow, thick walled, angular, xylem elements and a large mass of phloem located on the upper part of the bundle.

The abaxial part consists of a wide and deep arc of radial and parallel xylem elements, with gaps in between the xylem rows. The xylem elements are narrow, angular, and thick walled with narrow lumen. Phloem occurs in circular, wide masses beneath the xylem strands.

Calcium oxalate crystals are sparsely distributed in the group parenchyma cells outside the phloem strands of the midrib. The crystals are without definite shape and size. They are diffusely distributed in the parenchyma cells.

Lamina

The lamina is distinctly dorsiventral and posses dense glandular and non-glandular trichomes. The lamina consists of thick adaxial epidermis and wide rectangular or squarish epidermal cells with thick cuticle. The abaxial epidermis is thin and the cells are narrowly rectangular with prominent cuticle. The mesophyll tissue consists of two horizontal rows of cylindrical palisade cells.

The spongy mesophyll tissue includes about four layers of small, spherical or lobed and loosely arranged parenchyma cells. There is shallow and wide concavity in the abaxial epidermis. The glandular trichomes are located within the abaxial concavity of the epidermis.

The lamina is 160 µm thick. The glands are peltate type. The gland consists of short and thick two celled stalk. At the apex of the stock occurs a circular plate of typically four cells which radiate from the centre. The terminal plate is the secretory body of the gland.

The second type of trichome is non-glandular, three or four celled uniseriate and unbranched. They occur on the veins and intercoastal area of the lamina. The trichome has very thick and lignified walls and narrow lumen. The trichome may be either straight or curved. The trichome arises from a wide circular epidermal cell which is surrounded by about eight radially rectangular epidermal cells which are called rosette ells.

Epidermal cells and Stomata

Adaxial epidermal cells

In paradermal section the epidermis is viewed in surface view. The cells are wide and possess highly wavy thick walled, smooth anticlinal walls. The adaxial epidermis is apostomatic.

Abaxial epidermis

The abaxial epidermis is seen in the surface view. The epidermal cells are comparatively smaller with thicker, slightly wavy anticlinal walls. The stomata are seen I random distribution. The stomata are actinocytic type. Each stoma is surrounded by radially stretched,

wide thick walled subsidiary cells. The guard cells are broadly elliptical and measure 15x20 µm in size. The stomatal pore is narrow and slit-like.

Calcium oxalate crystals are densely distributed along the veins. Surrounding the veins are seen druses type of crystals. The crystals are not seen in the mesophyll tissue.

Venation Pattern

Both the major and minor veins are thin. The vein islets are well defined with distinct darkly stained vein boundaries. The vein islets are either circular or rectangular or polyhedral. Vein terminations are seen in some of the vein islets. The terminations may be unbranched, long, straight or curved. There are also branched vein terminations which posses two dichotomous. The branched veins are also long and straight.

Petiole

Both proximal and distal parts of the petiole were studied. The proximal part, of the petiole is planoconvex with flat adaxial side and semicircular abaxial part. The proximal petiole is 1.4mm thick and 1.65mm wide. The epidermal layer of the petiole is thin but includes distinct, small, circular and thick walled epidermal cells. The ground tissue is homogenous and parenchymatous with angular, thin walled parenchyma cells.

In the adaxial part of the petiole, the ground tissue consists of collenchymas cells. The vascular system consists of deeply curved and wide multistranded vascular cylinder with wide gap in the adaxial part of the cylinder. The cylinder includes about 12 small and large collateral vascular bundles. The bundles are closely arranged with narrow gaps in between. The cylinder consists of short and long radial lines of angular, thick walled xylem elements. On the outer part of the xylem strands occur several independent masses of phloem elements. Small sclerenchymatous cap occurs for each phloem strand.

The distal part of the petiole is semicircular with wide, shallow, adaxial concavity. It is 950µm thick and 1.3mm wide. The epidermal layer is thin with small, circular and thick walled cells. The ground tissue is parenchymatous. The vascular system consists of wide, somewhat shallow main vascular strand and one or two vascular strands situated within the lateral veins. The main arc of the vascular strand consists of several short, parallel lines of xylem elements and sclerenchyma cells. The xylem elements are circular, thick walled with fairly wide lumen. Phloem occurs in small groups all along the outer periphery of the xylem arc. Fairly thick masses of fibres occur adjacent to phloem strands.
The wing bundles are circular with small central core of phloem surrounded by xylem elements and fibres. A small segment of sclerenchyma occurs as a cap on the outer part of the phloem.

Stem

The stem is circular in sectional view with small and wide two ridges situated opposite to each other. The vascular system is thin and hollow with wide parenchymatous pith. The stem is 2.2mm in diameter. The stem consists of an intact epidermal layer, wide cortical zone and collateral vascular cylinder and wide pith. The epidermis consists of wide and thick walled, elliptical cells. Inner to the epidermis occur about four to eight layers of rectangular, wide, collenchyma cells. The vascular cylinder consists of numerous, fairly long radial lines of xylem elements which include outer secondary xylem and inner primary xylem. The xylem

cylinder also consists of wide and thick walled xylem fibres and prominently thin, straight xylem rays.

The xylem elements are mostly vessels which are circular and thick walled. The vessels are 35µm wide. Along the outer border of the xylem cylinder occur small groups of phloem elements. Along with the phloem also occur phloem parenchyma cells. On the outer border of the phloem are seen several, thick, semicircular masses of highly thick walled lignified fibres with narrow lumen.

The epidermis of the stem bears several prominent glandular trichomes. The trichomes are peltate type. The gland has a single short and wide stalk cell and a thick circular plate of four secretory cells.

In the cortical zone, the cells of the glandular tissue include calcium oxalate crystals. Some of the crystals appear to be to druses while some other crystals are minute spindle shaped crystals.

Root

Both thin and thick roots were studied. The thin root is circular measuring 1.2mm in thickness. The root has wide superficial periderm, thick cortex and circular, dense vascular cylinder.

The epidermis of the root is broken at different places. Where the cells are intact the epidermal cells are very thin and rectangular. The periderm varies in thickness at different places of the root. Where the periderm is prominent, it consists of about seven layers of tubular, thin walled phellem cells. The cortical zone includes fairly wide rectangular air chambers divided radially by thin filaments.

The inner cortex includes four or five layers of compact, thin walled parenchyma cells. The vascular cylinder is circular with central solid core of xylem elements surrounded by secondary phloem. The secondary phloem cylinder is thick and well developed. It includes small clusters of sieve elements mixed with large phloem parenchyma cells and phloem rays.

The xylem cylinders consist of diffusely distributed wide and narrow, thick walled vessels and thick walled lignified fibres with fairly wide lumen.

Thick root

The thick root has well developed two successive cylinders of periderm with parenchymatous tissue located in between the two periderm cylinders. The periderm cells are homogeneous, rectangular and tabular in shape. They have many radial walls. The inner cylinder of periderm is thin comprising four or five layers of thin walled, tabular, suberized cells

Secondary phloem is thick measuring about 200µm in radial plane. The phloem cells occur in regular, compact, radial lines. The sieve elements are located in small groups along the radial lines of cells. The phloem tissue also includes fairly wide thin walled parenchyma cells.

Secondary xylem is in the form of a wide cylinder and includes vessels and xylem fibres. The vessels in the inner circle are narrow and those in the outer circle are wide. The vessels are

circular and thin walled. The outer wider circles are 100µm in diameter. The narrow vessels are 30µm in diameter.

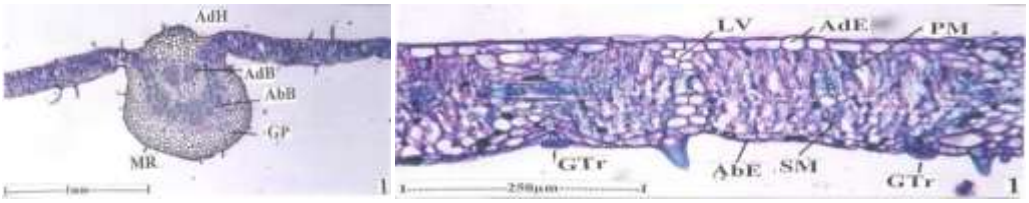


Figure 1: T.S. of leaf through midrib T.S. of lamina with peltate type of glandular trichome

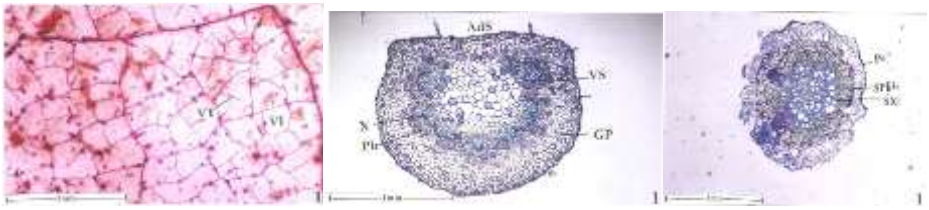


Figure 2: Vein islets and vein terminations enlarged T.S. of petiole enlarged view T.S. of thin root entire view

POWDER MICROSCOPY

The powder preparation of the sample includes the following elements:

Epidermal trichomes:

Two types of epidermal trichomes are common in the powder. Some of the trichomes are nonglandular, they are three or four celled, uniseriate and unbranched. They are broad at the base and narrowly conical at the tip. These nonglandulartrichomes are 70 to 100µm long and 20µm thick at the base. These trichomes occur along the veins as well as on the lamina.

Glandular trichomes are fairly abundant on the epidermal layer of the lamina. The trichomes are of two types. Some are small and the others are large. They have short, central stalk and terminal circular plate of four triangular cells forming a circle. These smaller trichomes are 50µm in diameter and wide trichomes are 80µm in diameter). The trichomes are peltate type. They have horizontally oriented secretory head.

Xylem elements:

The xylem elements include fibres and vessel elements. There are also xylem parenchyma cells.

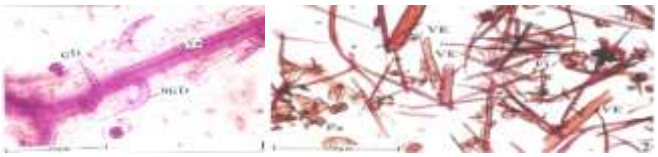
The fibres are all narrow type. They are very long or short with thick walls and narrow lumen with tapering ends. The walls are lignified. No pits are evident in the fibre. The fibres are upto 600µm long and 15µm thick.

Vessel elements:

The vessel elements are mostly long, narrow and cylindrical. The vessel elements are 350µm long and 80µm wide. They have dense, circular or elliptical, multiseriate bordered pits. The end wall perforation is wide and circular. It is either horizontal or oblique. Some vessel elements have short conical tails at one end. There are also vessel elements without tails.

Parenchyma cells:
There is abundance of parenchyma cells in the powder. They are spherical thin walled and do not posses any prominent cell inclusions.

Vein of the leaf bearing glandular and non-glandular trichomes Two fibres



Physico-chemical parameters:

Table 3: Ash values

Ash values	Whole plants of Premna tomentosaWilldpowder (%w/w)	Whole plants of Premna latifoliaRoxb powder (%w/w)
Total Ash value	7.50	7.00
Acid insoluble ash value	0.80	1.00
Water soluble ash value	1.20	2.00
Sulphated ash value	8.50	8.00

Table 4: Extractive values

Extractive values	Whole plants of Premna tomentosa Willd powder (%w/w)	Whole plants of Premna latifolia Roxb powder (%w/w)
Alcohol soluble extractive	2.40	3.20
Ether soluble extractive	0.50	0.80
Water soluble extractive	3.50	4.00

Preliminary phytochemical screening
Preliminary phytochemical screening showed the presence of Premna tomentosa –Fixed oils, Steroids, Cardiac glycosides, Flavonoids, Phenols and carbohydrates.

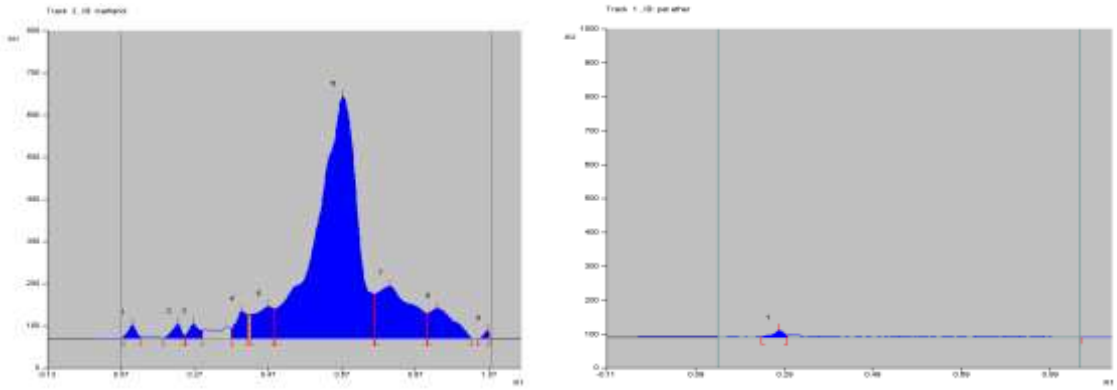
- Premna latifolia - Fixed oil, Steroids, Cardiac glycosides, Alkaloids, Phenols, Tannins, Flavonoids and Saponins
- Fluorescence were observed in dark light and UV light for all the extracts
- **Thin layer chromatography**- of all the solvents methanolic extract of Premna tomentosa and Premna latifolia showed number of characteristic spots in all the solvent systems

Data Showing the Thin Layer Chromatography of methanol extract of Premna tomentosa Willd.

Table 5: Thin Layer Chromatography of methanol extract of Premna latifolia Roxb

Parameters	Stationary phase	Mobile phase	No of spot	R_f value

Alkaloids	Silica gel G	Benzene:Ethanol (9:1)	4	0.34, 0.62, 0.65, 0.71, 0.75
Glycosides		Ethyl acetate:nbutanol :Water (4:4:3)	5	0.22, 0.44, 0.62, 0.14, 0.74
Flavonoids		Petroleum ether:ethyl acetate (2:1)	3	0.26, 0.32, 0.46, 0.54
Steroids		Chloroform:ethanol (24:1)	6	0.2, 0.24, 0.52, 0.44, 0.5, 0.62
Essential oils		Pure chloroform	3	0.74, 0.52,0.38



PHARMACOLOGICAL RESULTS

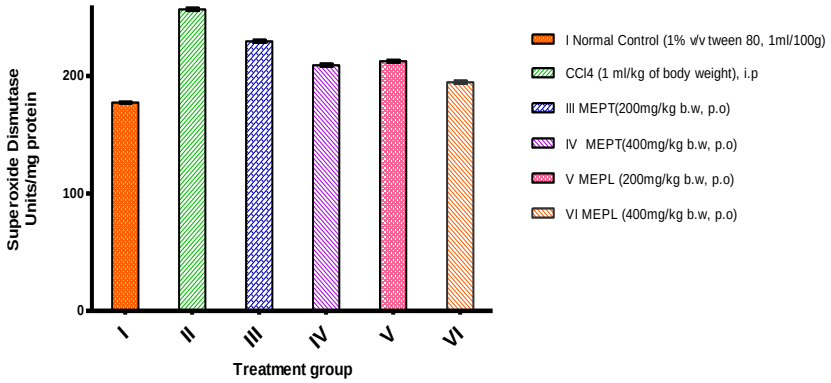
Acute oral toxicity studies

No lethality or toxic reactions such as tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma found ,hence1/10th (200mg/kg) &1/5th (400mg/kg) of this minimum and maximum oral doses were selected for further study.

In vivo Antioxidant activity

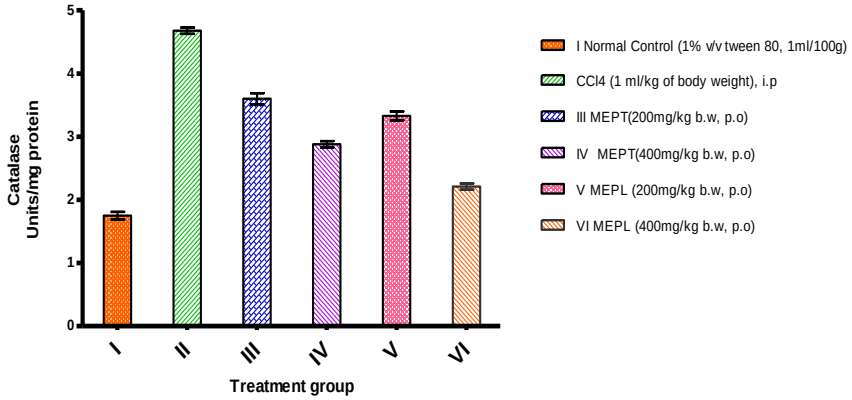
The results of invivo antioxidant activity of MEPT and MEPL clearly indicated that the rigidity of the membranes after administration of test drugs. MEPT and MEPL prevented changes in membrane phospholipids as well as those in membrane fluidity

Effect of MEPT and MEPLon Superoxide Dismutase of the erythrocytes of carbon tetrachloride -intoxicated rat



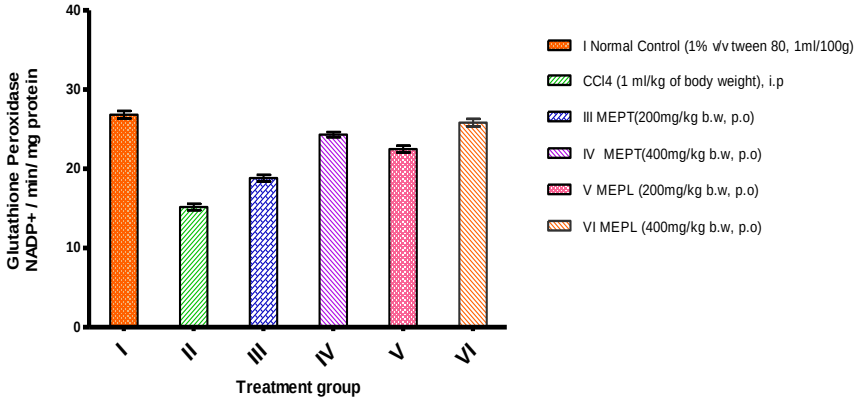
Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. a - Comparison between Group I Vs Group II. b - Comparison between Group II Vs Group III, IV, V & VI. *p<0.05;** p<0.01

Effect of MEPT and MEPLon Catalase of the erythrocytes of carbon tetrachloride -intoxicated rat



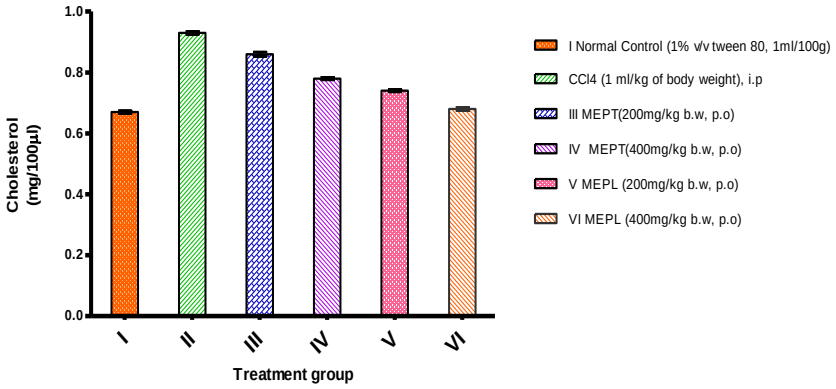
Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. a - Comparison between Group I Vs Group II. b - Comparison between Group II Vs Group III, IV, V & VI. *p<0.05;** p<0.01

Effect of MEPT and MEPLon Glutathione Peroxidase of the erythrocytes of carbon tetrachloride -intoxicated rat



Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. a - Comparison between Group I Vs Group II. b - Comparison between Group II Vs Group III, IV, V & VI. *p<0.05;** p<0.01

Effect of MEPT and MEPL on erythrocyte membrane Cholesterol of carbon tetrachloride -intoxicated rats

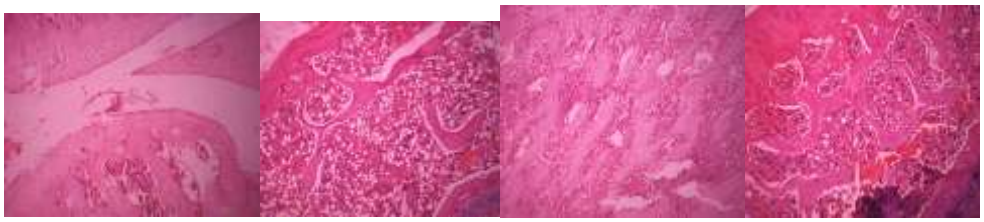


Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. a - Comparison between Group I Vs Group II. b - Comparison between Group II Vs Group III, IV, V & VI. *p<0.05;** p<0.01

Effect of MEPT and MEPL on histopathological changes in Formaldehyde induced arthritic rats

In normal control animals shows no lesions in articular cartilage and vascularity formation into the joint space Arthritis control showed edematous synovium, destructive lesions in articular cartilage and vascularity formation into the joint space in adjuvant-treated animals. Arthritis rats treated with MEPT and MEPL observed well protected synovium, articular cartilage into the joint space with normal cellular characteristics like standard drug Diclofenac sodium treated group.

Effect of MEPT and MEPLinFormaldehyde induced arthritis in rats



Normal Control, Arthritis Control, MEPT (200mg/kg b.w, p.o), MEPT (400mg/kg b.w, p.o)

MEPL (200mg/kg b.w, p.o), MEPL (400mg/kg b.w, p.o), Diclofenac sodium (4mg/kg b.w, p.o)

Immunological Arthritis

Effect of oral administration of MEPT & MEPL on Complete Freund's Adjuvant (CFA)-induced arthritis in rats

The assessment made on the 21st day showed that the MEPT & MEPL (200 & 400mg/kg, body wt.) treatments significantly reduced. Oral dosage of MEPT & MEPL significantly inhibited joint inflammation on CFA-induced arthritis in rats (62.15, 64.51&63.35, 69.72%) respectively.

Innovations:-

Research work which remains to be done under the project (for on-going project):

It was observed that the traditional herbs Premna tomentosa and Premna latifolia both possess good antirheumatic activity. This was confirmed by its antioxidant analgesic and anti-inflammatory actions by both acute and chronic tests. Premna latifolia was more effective when compared to that of Premna tomentosa.

a. **Immediate:** The individual chemical constituents can be isolated and tested for the proved activities.
b. **Long Term**: Based on the innovations, the chemical constituents can be isolated and can be formulated as an ointment or oral dosage for the treatment of rheumatism.

5. CONCLUSION

From the results obtained in the present study, it may be concluded that the oral dosage of methanolic extract of whole plant of Premna tomentosa Willd (MEPT) and Premna latifolia Roxb (MEPL) possesses potent anti-oxidant, anti-inflammatory and anti-arthritic activity against both the exudative and proliferative phases of inflammation. The present observations suggested that oral dosage of methanolic extract of whole plant of Premna tomentosa Willd (MEPT) and Premna latifolia Roxb (MEPL) has a positive influence on different phases of chronic inflammatory states. On the basis of the present study, whole plant of Premna tomentosa Willd and Premna latifolia Roxb seems to be a promising source of anti-inflammatory and anti-arthritic agent as it exhibited its efficacy against all experimental models, thus justifying its use in various inflammatory conditions by the followers of traditional system of medicine.

6. Acknowledgement

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