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PHARMACOLOGICAL EVALUATION OF DIFFERENT EXTRACTS OF LEAVES OF *SALACIA FRUTICOSA WALL*

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ABSTRACT

The herbal powder that was supplied included alkaloids, carbs, phenols, flavonoids, glycosides, saponins, and terpens in its alcoholic and water-based extracts. Oral administration of an alcoholic and water-based extract of the specified herbal powder at doses of 200 mg/kg and 400 mg/kg body weight for a duration of five days. Carragenan-induced edema is a biphasic response, the first phase is mediated through the release of histamine, serotonin and kinins where as the second phase is related to the release of prostaglandins and mediated by bradykinin, leucotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages. Subcutaneous injection of yeast suspension markedly increased the rectal temperature 19 hrs after injection. This type of fever, which is induced by yeast, is called pathogenic fever. Since almost all anti pyretic agents act by inhibiting PG synthesis it is logical to presume that METO and AETO also exerts their effects through the same. Some of the mutant strains of gram –ve rods are used in vaccine preparations (salmonella). The cell wall of this type of bacteria consists of endotoxins (lipoplysaccharides), which activates the macrophages thereby releasing interleukin-1, which causes pyrexia. The results obtained in the present study shown that the Methanolic extract exert concentration or dosed-dependent antinociceptive effects, the rank order of relative potencies in the Eddy's Hot plate test was :ME>AE. The reaction time was found to be 11.4, and 10.4sec respectively, while the standard drug Tramadol was 11.6sec. The methanol extract shows most significant increased reaction time for mice than Tramadol.

KEYWORD: Bradykinin, leucotrienes, polymorphonuclear, lipopolysaccharides, antinociceptive.

1.0. INTRODUCTION

From olden days, traditional systems of medicine are of global importance. Even today in many developing countries, majority of population relies greatly on traditional practioners and medicinal plants to meet primary health care needs,

despite of availability modern medicines. These herbal phytomedicines have become popular for historical and cultural reasons. Currently many people in developed countries have began to turn to alternative herbal medicines. Scientific evaluation of medicinal herbs for their medicinal value and application is on rise. Safety and efficacy of data of many herbs, their extracts and active ingredients, and the preparations containing them was well established**.** However the market of herbal drugs is poorly regulated and assurance of safety efficacy and quality of such herbal products has now become a key issue in industrialized and developing countries.[1] Significant basic and clinical research has been carried out on the medicinal plants and their formulations, with the state-of-the-art methods in a number of Institutes/Universities. There are some good examples. Indian medicinal plants also provide a rich source for antioxidants that are known to prevent/delay different diseased states. The antioxidant protection is observed at different levels. The medicinal plants also contain other beneficial compounds like ingredients for functional foods. In the traditional system of medicine, most of the remedies were taken from plants and they were proved to be useful through the rational behind their use is not well established through systemic pharmacological and clinical studies except for some composite herbal drugs and plants. The plant kingdom undoubtedly still holds many species of plants containing substances of medicinal value which have yet to be discovered large number of plants are constantly being screened for their possible pharmacological effects.[2]

2.0. INTRODUCTION TO SALACIA FRUTICOSA

TAXONOMIC CLASSIFICATION[3]

KINGDOM: Plantae

SUBKINGDOM: tracheobionta

DIVISION: Magnoliophyta

CLASS: Magnoliatae

SUBCLASS: Rosidae

ORDER: Celastrales

FAMILY: Hippocrateaceae

GENUS: Salacia

SPECIES: Fruticosa.

Vernacular names[4]

English: Common willow

Hindi: Dudhi, Baran

Sanskrit: Lootari, Payaswini

Telugu: Patri

Malayalam: Ponkarandi, Ekanayakam

Kannada: Kaddushavanthi

Urdu: Bathur

Unani: Bedmushk, Bedsaada

Ayrvedic: Saptachakra, Swarnmula

3.0. OBJECTIVE OF THE STUDY

The over all aim of the study is to explore the application of traditional medicinal plants of India. The Aim of the present study is

- To explore the possibilities of traditional uses of the drug with proper chemical and pharmacological profile.
- To conduct systematic chemical investigation of Salacia *fruticosa*.
- To evaluate the anti-inflammatory and anti-pyretic activity of Salacia *fruticosa* leaf extract in rat model. However, for analyzing and correlating the data obtained, correctly and more precisely, the present study is designed and carried out in different steps, which are schematically represented as follows.

Pharmacognostic &Phytochemical investigations

- Collection and authentication
- Extraction
- Preliminary qualitative chemical analysis

Pharmacological screening

- Acute toxicity studies
- Evaluation of analgesic activity
- Evaluation of anti inflammatory activity
- Evaluation of anti pyretic activity

4.0. METHODOLOGY

1. Procurement of plant material

For the present investigation *Salacia fruticosa* Wall was collected in and around Thirupathi, Chitoor.

2. Drying and size reduction

The leaves were carefully shade dried for 15 days. To ensure complete dryness plant leaves were kept in hot air oven at 45Cfor 5 minutes. Then leaves are subjected to size reduction to make power by using mechanical grinder. The crushed mass of leaves was then carried out for the process of extraction.

3. Extraction

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures.

General Methods of Extraction of Medicinal Plants

- Maceration
- Infusion
- Digestion
- Decoction
- Percolation
- Hot continuous extraction (Soxhlation)

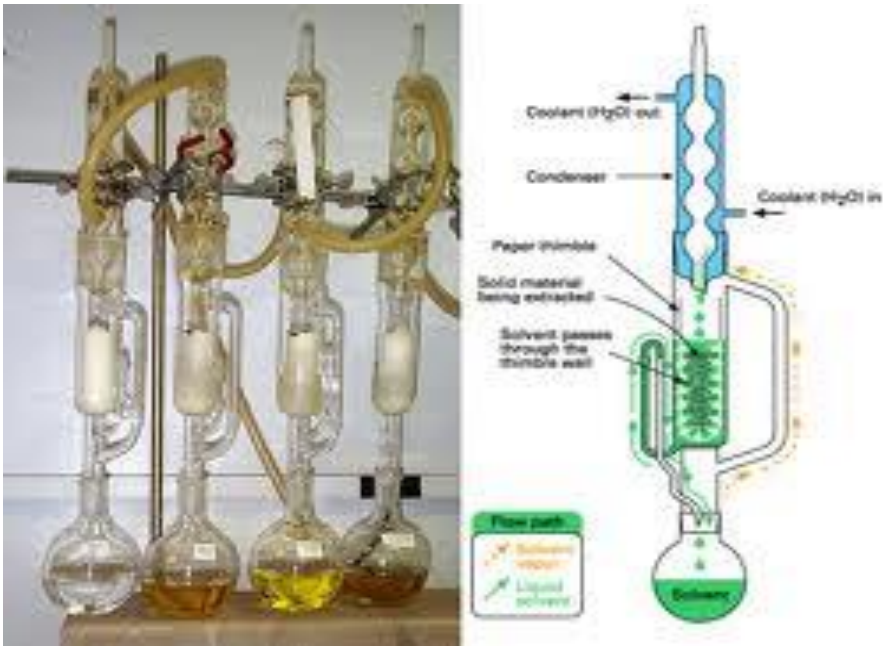


Figure 2: Photograph of Soxhlet apparatus.

Principle and Mechanism

Classic techniques for the solvent extraction of active constituents from medicinal plant matrices are based on the choice of solvent coupled with the use of heat or agitation. Existing classic techniques used to obtain active constituents from plants include: Soxhlet, hydrodistillation and maceration with and alcohol –water mixture or other organic solvents. Soxhlet extraction is a general and well –established technique, which surpasses in performance other conventional extraction techniques except for, into limited fields of application, the extraction of thermolabile compounds. In a conventional Soxhlet system, as shown in Figure, plant material is placed in a thimble-holder, which is filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble—holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. Solute is left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction is achieved.

Advantages and Disadvantages of Soxhlet Extraction

Advantages

1. The displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix.
2. Maintaining a relatively high extraction temperature with heat from the distillation flask.
3. No filtration of the extract is required.

Disadvantages

1. Agitation is not possible in the Soxhlet device.
2. The possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time. World wide, most of the solvent extraction units are based on the Soxhlet principle with recycling of solvents. Basic equipment for a solvent extraction unit consists of a drug holder-extractor, a solvent storage vessel, a reboilerkettle, a condenser, a breather system (to minimize solvent loss) and supporting structures like a boiler, a refrigerated chilling unit and a vacuum unit.

Extraction procedure

1. 800gms of the air-dried powdered plant material extracted successively with the following solvents in Soxhlet extractor.

a) Petroleum ether (60-80C)
b) Chloroform
c) Methanol
d) Water

Each time before extracting with the next solvent, dry the powdered material in Hot air-oven below 50C.

2. Soxhlation of leaf powder with methanol for 24hrs to obtain the product.
3. Then the dried marc is extracted with water by decoction.
4. Concentrate the extract by distilling of the solvent and then evaporating to dryness on the water-bath.

Collection and Authentication of plant material

The fresh leaves of Salacia *fruticosa* were collected from Thirupathi, Andrapradesh, shade dried and ground powdered in a mechanical blender. The powdered is subjected to soxhlet extraction using methanol as solvent. A specimen of the plant is deposited in the herbarium of Talla Padmavathi College of pharmacy identified and authenticated by Dr. K.Madhava Chetty, botanist of Sri Venkateswara university.

Preparation of Extracts[16]

The dried and powdered leaves of Salacia *fruticosa* was extracted successively with of methanol and aqueous in soxhelt apparatus. A greenish colored methanolic extract was obtained (5.6% with respect to dry powdered plant material), then, the same has been extracted with water to yield brownish green semisolid mass (yield 4.2% with respect to dry plant material). Two extraction procedures were carried out until the solvent system becomes a colorless. All the extracts were collected and concentrated by evaporating the solvent completely. These extracts were dried and stirred in refrigerator for the future use of various chemical group identification and pharmacological evaluation.

A) PHYTOCHEMICAL INVESTIGATIONS

Preliminary Phytochemical Screening [17&18]

Preliminary tests were carried out for the presence or absence of phytoconstituents like Glycosides, Flavanoids, Saponins, Alkaloids, Sterols, Proteins, Phenolic compounds and reducing compounds. A description of methods adopted for performing the tests are summarized below.

Test for Alkaloids[19]

A. Mayer's Test: The Extract to be tested is treated with few drops of dilute 2N HCL and 0.5 ml Mayer's reagent.White precipitate was obtained which confirm the presence of alkaloids.

B. Wagner's Test: The extract is treated with few drops of 2N HCL and 0.5 ml Wagner's reagent. Brown flocculent precipitate was obtained which confirm the presence of alkaloids.

C. Dragendroff's Test: The extract is treated with few drops of dilute 2N HCL and 0.5 ml Dragendroff's reagent. Brown precipitate was obtained which confirm the presence of alkaloids.

D. Hager's Test: The extract is treated with few drops of dilute 2N HCL and 0.5 ml Hager's reagent. Yellow colored precipitate was obtained which confirms the presence of alkaloids.

Test for Flavonoids[20]

A. Shinoda test: 5-10 drops of dilute Hcl was added to 0.5 ml of extract. Small piece of magnesium was added to it. Appearance of pink, reddish pink or brown coloration was considered as positive test.

B. A small portion of extract is treated with neutral ferric chloride solution. Appearance of green to blue color was taken as positive test for flavonoids.

C. Small portion of extract is treated with few drops of 10%lead acetate solution. Appearance of yellow precipitate was considered as positive test for flavonoids.

Test for Glycosides[21]

A. The dried extract was dissolved in glacial acetic acid and few drops of ferric chloride were added followed by concentrated sulphuric acid. A bluish green precipitate was considered to be a positive test for glycosides.

Test for reducing Sugar[22]

A. Fehling's test: 5ml of solution of extract was heated with equal volumes of Fehling's solution A & B. Transition of color from blue through green to reddish orange confirms the presence of reducing sugars.

B. Benedict's test: 5 ml of solution of the extract was heated with 5 ml of Benedict's reagent.A green, yellow or orange red precipitate was considered as a positive test for reducing sugars.

Test for Saponins

- **Foam's test:** A small amount of dry extract was boiled with water and allowed to cool. It was then shaken vigorously for a minute. The formation of persistent honey comb like froth was considered as a positive test for saponins.

Test for Sterols[23]

A. Liebermann–Burchard test: A small portion of extract was dissolved in chloroform and 2ml of Liebermann-burchard reagent was added. Appearance of bluish green was considered as positive test for sterols and pink or violet coloration was considered as positive test for Terpenoids.

B. Salkowski test: A small portion of extract was dissolved in chloroform and treated with an equal volume of concentrated sulphuric acid. A Red to purple color formation was considered as a positive test for Terpenoids.

Test for Tannins

A. A small portion of extract was treated with 5%ferric chloride solution. Appearance of green to blue color was taken as a positive test for tannins.

B. Small portion of extract was treated with lead acetate. Appearance of creamy precipitate was considered as a positive test for tannins.

Test for Proteins

- **Biuret test:** A small portion of extract was treated with Biuret reagent.

Appearance of violet color indicates positive test for proteins.

5.0. B) PHARMACOLOGICAL INVESTIGATION[24&25]

Experimental Animals

Animals were procured from Central animal house, Talla padmavathi college of Pharmacy, Warangal (CPCSEA Reg. No: SURA15/po/a/2024). Male wister albino rats weighing between 150 and 2000gm were selected for the study. Female albino mice weighing between 25gm and 30gm were selected for the acute oral toxicity studies. The were maintained on 12hr/12hr light and dark cycle at ambient room temperature and relative humidity (50%). They were kept in propylene cages in a well-ventilated room under hygienic conditions throught the study. The animals were fed with commercial rst feed pellets and were given water ad libitum. Maintenance of animals was as per CPCSEA guidelines. All animals were carried out only after approval of IAEC.

Determination of LD50

The herbal preparation ***Salacia fruticosa Wall*** have been subjected to toxicity studies according to OECD guidelines and no death was found up to 3g/kg body weight, so 10% and 20% of it i.e. 300mg/kg and 600mg/kg are taken for carrying pharmacological activities.

Acute oral toxicity study (acute toxic class method)

A prilimenary pharmacological study was condected to assess the acute pharmacological effects and safety of the drug. Acute toxicity was conducted to determine the median lethal dose (LD_{50}) of the methanolic extracts of the leaves of *Salacia fruticosa*. The procedure was followed by OECD (organization for ethical and cooperative development) guidelines, 423(acute toxic class method). The acute toxic class method was step wise procedure with 3 animals of a single sex per step. The method uses defined doses (5, 50, 300, 2000 mg/kg body weight).

It was observed that the extract was not mortal for mice even at 2000mg/kg dose. Hence one tenth $1/10^{th}$ of the dose (200mg/kg) and one fifth $1/5^{th}$ of the dose (400mg/kg) selected for further study.

C) STUDY OF INFLAMMATORY ACTIVITY

IN-VITRO METHOD

Egg albumin induced paw oedema[26]

Method

Test solutions (2ml) containing different concentrations (25-800 µg/ml) of drug was mixed with 2ml of egg albumin solution (1mM) and incubated at 27±1^{o}c for 15min. Denaturation was induced by keeping the reaction mixture at 70^{o}c in a water bath for 10min. After cooling, the turbidity was measured at 660nm. Percentage inhibition was calculated from control where no drug was added.

IN-VIVO METHOD

1. Carrageenan induced paw oedema[27-31]

Animals: Wistar albino rats weighing 180-200 gm of either sex were used in the study. Animals were procured from Laboratory Animal House of Talla Padmavathi College of Pharmacy, urus (CPCSEA Reg NO: 1505/po/a/11). All animal experiments strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12 h light–12 h dark cycle. They were acclimatized for seven days. Food was provided in the form of dry pellets and water ad libitum.

Equipment: Plethysmometer

Method

All the animals received their respective doses of test drugs 1hr prior to the administration of the phlogistic agent. After 1hr, 0.1ml of 1% solution of freshly prepared carrageenan in normal saline was injected into the plantar surface of the right hind paw of the rats. The paw volume was measured before and each hour afterwards for a period of 5hrs using mercury displacement plethysmograph. The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group:

$$\% \text{ inhibition of edema} = \frac{\text{paw vol. of control- paw vol. of treated}}{\text{paw vol. of control}} * 100$$

Treatment Protocol: The animals were numbered, weighed and then divided into 6 groups with 6 animals in each as follows:

- Group I: Animals received carrageenan alone (0.1 ml of 1% solution.)
- Group II: Animals received carrageenan and 200mg/kg body weight METO (p.o.)
- Group III: Animals received carrageenan and 400 mg/Kg body weight, METO (p.o.)
- Group IV: Animals received carrageenan and 200mg/kg body weight, AETO(p.o.)
- Group V: Animals received carrageenan and 400mg/kg body weight, AETO(p.o.)
- Group VI: Animals received carrageenan and 10 mg/Kg body weight, diclofenac sodium (p.o).

2. Egg albumin induced paw oedema[27-31]

Materials and Method

Animals: Wistar albino rats weighing 180-200 gm of either sex were used in the study. Animals were procured from Laboratory Animal House of Talla Padmavathi College of Pharmacy, urus (Reg. no.:/ac/CPCSEA).. All animal

experiments strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12 h light–12 h dark cycle. They were acclimatized for seven days. Food was provided in the form of dry pellets and water ad libitum.

Equipment: Plethysmometer.

Method

Acute inflammation was induced by the injection of 0.1ml of fresh egg albumin into the sub plantar surface of the right hind paw of the rats. Test samples were administered 1hr prior to the phlogistic agent. Oedema was assessed for 5hrs at 1hr intervals, an increase in paw volume of the albumin injected paw compared with the non-injected paw. The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group:

$$\% \text{ inhibition of edema} = \frac{\text{paw vol. of control- paw vol. of treated}}{\text{paw vol. of control}} * 100$$

Treatment Protocol: The animals were numbered, weighed and then divided into 4 groups with 5 animals in each as follows:

- Group I: Animals received Egg albumin alone(0.1ml of 1% solution)
- Group II: Animals received egg albumin and 200mg/kg body weight METO (p.o.)
- Group III: Animals received egg albumin and 400 mg/Kg body weight, METO (p.o.)
- Group IV: Animals received egg albumin and 200mg/kg body weight, AETO(p.o.)
- Group V: Animals received egg albumin and 400mg/kg body weight, AETO(p.o.)
- Group VI: Animals received egg albumin and 10 mg/Kg body weight, diclofenac sodium (p.o.)

3. Adjuvant induced arthritis in rats[32]

Arthritis was induced by injecting 0.05ml of 0.5%w/v suspension of killed mycobacterium tuberculli in paraffin oil in to the right hind limb. Paw volume was measured till 12th day using mercury displacement plethysmograph. During treatment was started on day 13 and terminated on day 21. The difference in paw volume on day 3 and day 21 were considered, as oedema volume and % inhibition of oedema was determined.

Treatment protocol: Animals were numbered, weighed and divided into 5 groups with 6 animals in each group:

- Group I – Normal animals received normal saline.
- Group II – Arthritis induced animals received normal saline (10ml/kg p.o.)
- Group III - Arthritis induced animals treated with METO at dose of 200 mg/kg in normal saline.
- Group IV Arthritis induced animals treated with AETO at a dose of 200mg/kg in normal saline.
- Group V - Arthritis induced animals treated with indomethacin at a dose of 10mg/kg in normal saline.

D) Anti pyretic Activity

Yeast induced Pyrexia[33]

Material & Methods

Animals: Wistar albino rats weighing 180-200 gm of either sex were used in the study. Animals were procured from Laboratory Animal House of Talla Padmavathi College of Pharmacy, uru s(Reg. no.:/ac/CPCSEA). All animal

experiments strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12 h light–12 h dark cycle. They were acclimatized for seven days. Food was provided in the form of dry pellets and water ad libitum.

Equipment: Digital Clinical Thermometer (Hartmann, Germany).

Method

Rectal temperature was measured by inserting a thermister probe 3-4cm deep into the rectum. After measuring the basal temperature, animals were given a subcutaneous injection of 10ml/kg of 15%w/v yeast suspended in normal saline solution. After 19hrs of yeast injection, the animals were treated with their respective doses of test drugs and their rectal temperatures were recorded till 23rd hour per every day.

Treatment Protocol: The animals were numbered, weighed and then divided into 6 groups with 6 animals in each as follows:

Group I: Animals received yeast (10ml/kg 15%w/v of yeast s.c.)

Group II: Animals received yeast and Paracetamol (150 mg/kg weight s.c.)

Group III: Animals received yeast and 200mg/kg body weight, METO (p.o.)

Group IV: Animals received yeast and 400mg/kg body weight, METO (p.o.)

Group V: Animals received yeast and 200mg/kg body weight, AETO (p.o.)

Group VI: Animals received yeast and 400mg/kg body weight, AETO (p.o).

Vaccine induced pyrexia[34]

Material & Methods

Animals: Wistar albino rats weighing 180-200 gm of either sex were used in the study. Animals were procured from Laboratory Animal House of Talla Padmavathi College of Pharmacy, uru s(Reg. no.:/ac/CPCSEA).. All animal experiments strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12 h light–12 h dark cycle. They were acclimatized for seven days. Food was provided in the form of dry pellets and water ad libitum.

Equipment: Digital Clinical Thermometer (Hartmann, Germany).

Method: Animals were fasted and grouped. Basal rectal temperatures of all the animals were recorded. Pyrexia was induced by injecting typhoid- paratyphoid vaccine A and B (TAB) at a dose of 1ml/kg body weight (i. p.). six hours after injection of TAB, the temperature was again recorded and the animals that did not show a minimum rise of 1.5^{o}C in temperature were discarded. Thus 24 animals were selected and grouped accordingly. After 6grs, the animals were treated with their respective test compounds and their rectal temperatures were recorded till 11th hr pert every 1 hr.

Treatment Protocol: The animals were numbered, weighed and then divided into 4 groups with 5 animals in each as follows:

- Group I: Animals received vaccine alone (1ml/kg body weight i.p.)
- Group II: Animals received vaccine and Paracetamol 150 mg/kg weight (i.p.)

- Group III: Animals received vaccine and 200mg/kg body weight, METO (p.o.)
- Group IV: Animals received vaccine and 400mg/kg body weight, METO(p.o.)
- Group V: Animals received vaccine and 200mg/kg body weight, AETO(p.o.)
- Group VI: Animals received vaccine and 400mg/kg body weight, AETO(p.o).

ANALGESIC ACTIVITY

Principle

Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damage. Various types of pain are seen in humans for example somatic pain, visceral pain referred pain, cancer pain etc. Chemical mediators of pain are numerous. These mediators come from sources intrinsic to the neuron, including neurotransmitters such as 5-HT, Substance-P and extrinsic to the nervous system, including substances from inflammatory immune cells and red blood cells such as Prostaglandins, Kinins, cytokinins, chemokinins and ATP that are released following injury to the tissue. Pain is produced by the excitation of particular receptors, the nociceptors or of their afferent fibers. These remarkable cells respond to broad spectrum of physical or chemical noxious stimuli.

Pain can be classified as acute or chronic. The distinction between acute and chronic pain is not based on its duration of sensation, but rather the nature of the pain itself. The primary distinction is: acute pain serves to protect one after an injury where as chronic pain does not serve this or any other purpose. Acute pain is the symptom of pain. Chronic pain was originally defined as pain that lasts for long time. The most common causes of chronic pain include cancer pain, neuropathic pain and arthritic pain.

Methods

1) Eddy's hot plate method
2) The tail immersion method
3) Acetic acid induced writhing

Eddy's hot plate method

Animals

Studies were carried out using Wistar albino tats of both sexes weighing 175-200g. They were obtained from the Mahaveer enterprises, Hyderabad. All animal experiments strictly complied with the approval of Institutional Animal Ethical Committee (15/SURA/2024/11/CPCSEA).The animals were grouped and housed in polyacrylic cages (38cm*23cm*10cm) with not more than six animals per cage and maintained under standard laboratory condition (temp.25c) with dark and light cycle(12/12h). They were allowed free access to standard dry pellet diet and water *ad libitum*. The rats were acclimatized to laboratory condition for 10days before commencement of experiment.

Requirements

Animals: Albino mice (18-22g)

Drugs: Tramadol (10mg/kg)

Methanolic and aqueous extracts of *Salacia fruticosa* Wall (50mg and 100mg/kg)

Equipment: Eddy's hot plate

Treatment protocol

The animals were numbered, weighed and then divided into six groups with six animals in each as follows:

Group-1: Control (CMC10ml/kg.p.o.)

Group-2: Standard (Tramadol 10mg/kg.p.o)

Group-3: METT (50mg/kg) is suspended with CMC (0.5%w/v)

Group-4: METT (100mg/kg) is suspended with CMC (0.5%w/v)

Group-5: AETT (50mg/kg) is suspended with CMC (0.5%w/v)

Group-6: AETT (100mg/kg) is suspended with CMC (0.5%w/v)

Procedure

Animals were derived into six groups. Each group consisting of six animals, one group served as Negative control (CMC 10ml/kg) and second groups served as positive control (received Tramadol 10mg/kg) by oral route. Third group received Methanolic extract of *Salacia fruticosa* (50mg/kg) by oral route. Fourth group received methanolic extract of *Salacia fruticosa* (100mg/kg) by oral route. Fifth group received aqueous extract of *Salacia fruticosa* (50mg/kg) by oral route and the sixth group received aqueous extract of *Salacia fruticosa* (100mg/kg) by oral route.

Animals are placed on the hot plate, which consists of electrically heated surface Temperature of the hot plate was maintained at 55c+-0.5c.Responses such as jumping, withdrawal of the paws and licking of the paws are seen.The time period (latency period), when animals are placed and until responses occur, was recorded on stop watch. Test compounds are administered subcutaneously and latency period was recorded after 30, 60, & 90min. These values are compared with the values before the administration of the test drug by using t-test. The results were shown in table and figure.

Table no. 5: Mean reaction time in mice by Eddy's Hot Plate method.

Before administration of drug:

Group	Response Time(in seconds)			
	0min	10min	20min	30min
Group I	10.25±0.22	10.43±0.15	10.26±0.11	10.31±0.14
Group II	9.9±0.2	10.2±0.08	10.35±0.15	10.6±0.16
Group III	10.28±0.1	10.53±0.18	10.46±0.16	10.43±0.22
Group IV	10.2±0.1	10.41±0.14	10.48±0.17	10.55±0.16
Group V	10.41±0.2	10.4±0.14	10.7±0.15	10.55±0.18
Group VI	10.43±0.15	10.46±0.16	10.5±0.17	10.56±0.18

After administration of drug:

Group	0min	30min	60min	90min
Group I	10.38±0.09	10.58±0.2	10.48±0.18	10.55±0.2
Group II	10.28±0.16	15.11±0.2(42.8%)	15.73±0.11(51%)	11.66±0.22
Group III	10.7±0.16	11.41±0.21*(68.8%)	13.11±0.1(76.9%)	10.98±0.24
Group IV	10.5±0.18	13.35±0.17**(81.7%)	13.76±0.18(81.1%)	11.43±0.15
Group V	10.48±0.18	13.05±0.19(79.7%)	11.53±0.14(66.9%)	10.55±0.14
Group VI	10.53±0.15	13.8±0.16*(84.7%)	12.78±0.19(74.8%)	10.59±0.18

Values are expressed as mean ± S.E.M.(n=6);*p<0.05,**p<0.01 vs.control.one way ANOVA followed by Dunnets't' test. METT: methanolic extract; AETT: aqueous extract of *Salacia fruticosa Wall*. (): Parenthesis indicates the percentage of Inhibition.

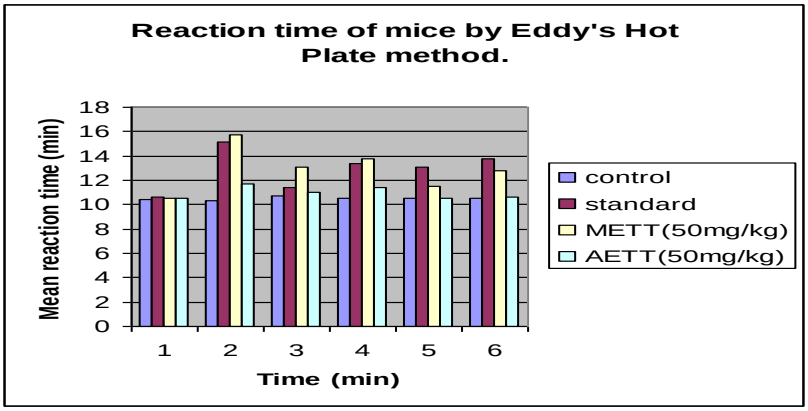


Figure 5: Effect of different leaf extracts of *Salacia fruticosa* on reaction time of mice by Eddy's Hot plate method.

RESULTS AND DISCUSSION

The results obtained in the present study shown that the Methanolic extract exert concentration or dosed-dependent antinociceptive effects, the rank order of relative potencies in the Eddy's Hot plate test was: ME>AE. The reaction time was found to be 11.4, and 10.4sec respectively, while the standard drug Tramadol was 11.6sec. The methanol extract shows most significant increased reaction time for mice than Tramadol.

The analgesic activities were evaluated by using both chemical and thermal methods of nociception in mice. These methods are used to detect central and peripheral analgesics Nociception reaction towards thermal stimuli is a well validated model for detection of opiate like analgesic drugs where in pain response is from spinal origin. In present study it was found that extract does shows significant analgesic activity in Heat model i.e., Eddy's Hot plate method test are affected by non-steroidal anti-inflammatory drugs (NSAIDs), but they are sensitive to the analgesic effects of opioid agents. Centrally acting drugs such as narcotics could inhibit both early and late phases equally, the peripherally acting drugs like NSAIDS (Aspirin, Diclofenac and Ibuprofen etc.) only inhibit the late phase indicating a possible development of an inflammatory response and the release of analgesic mediators.

Results and Discussions

The present study was designed to evaluate the analgesic, Anti-inflammatory and Anti-pyretic activity of Salacia *fruticosa* leaf extracts in rat model. In this study, increase in the paw volume and body temperature were used for the assessment of Anti-inflammatory and Anti-pyretic activity of *Salacia fruticosa.*

Literature review conducted on this plant indicated the presence of various active constituents like Flavonoids, Saponins, and Alkaloids etc. since these compounds were soluble in alcohol, it was used as a solvent for extraction.

Preliminary phytochemical screening

The preliminary phytochemical analysis of methanolic leaf extract of "Salacia *fruticosa*" indicate the presence of various chemical constituents like Steroids, Proteins, Glycosides, Tannins, Saponins and Alkaloids.

Preliminary Phytochemical Tests Results

Sl. No.	Tests	Methanolic Extract	Aqueous Extract
1	Alkaloids Mayer's test Wagner's test Dragendroff's test Hagner's test	 +ve +ve +ve +ve	 +ve +ve +ve +ve
2	Flavonoids Shinoda's test	 +ve	 +ve
3	Saponins foam test	+ve	+ve
4	Steroids Liberman's test Salkowski's test	 +ve +ve	 +ve +ve
5	Tannins	+ve	+ve
6	Proteins	+ve	+ve
7	Glycosides	+ve	+ve

Acute Toxicity Study

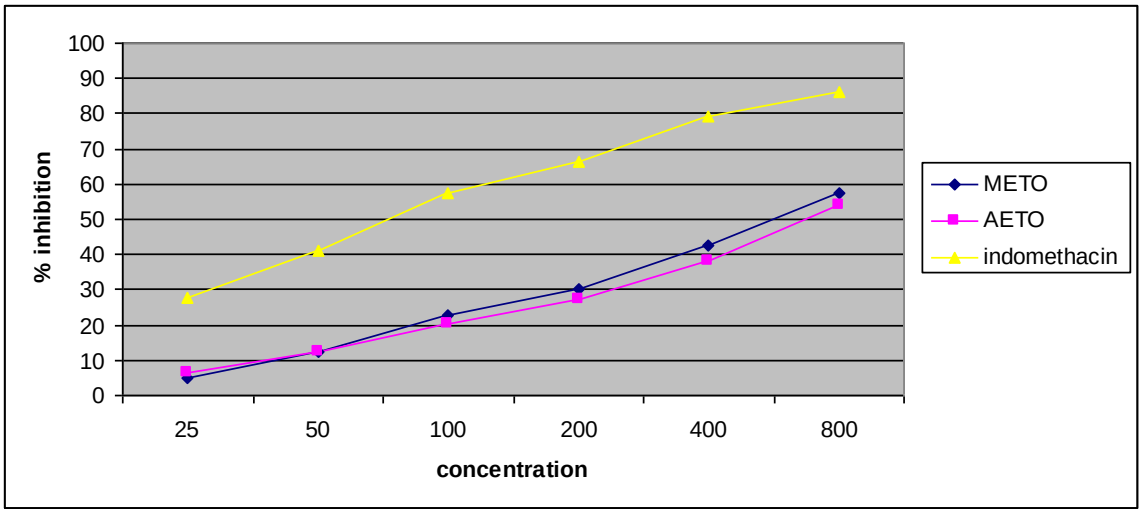
Since no mortality was observed in the mice in acute toxicity study at 2000mg/kh body weight., and based on the literature available, tha dose of methanolic extract and aquoes extract of Salacia *fruticosa* was fixed as 200mg/kg and 400mg/kg b.w., for further study.

Anti-inflammatory activity

In-vitro method

Egg induced paw oedema

	% INHIBITION		
Concentration (µg/ml)	**METO**	**AETO**	**Indomethacin**
25	4.75 ±0.03	6.25 ±0.01	27.91 ±0.02
50	12.24 ±0.01	12.55 ± 0.01	40.85 ± 0.02
100	22.58 ±0.01	20.27 ±0.01	57.50 ±0.01
200	30.29 ±0.01	27.29 ±0.12	66.23 ±0.02
400	42.66 ±0.01	38.29 ±0.09	79.39 ±0.01
800	57.37 ±0.01	54.03 ±0.01	86.34 ±0.01



In-Vitro Anti-Inflammatory Activity

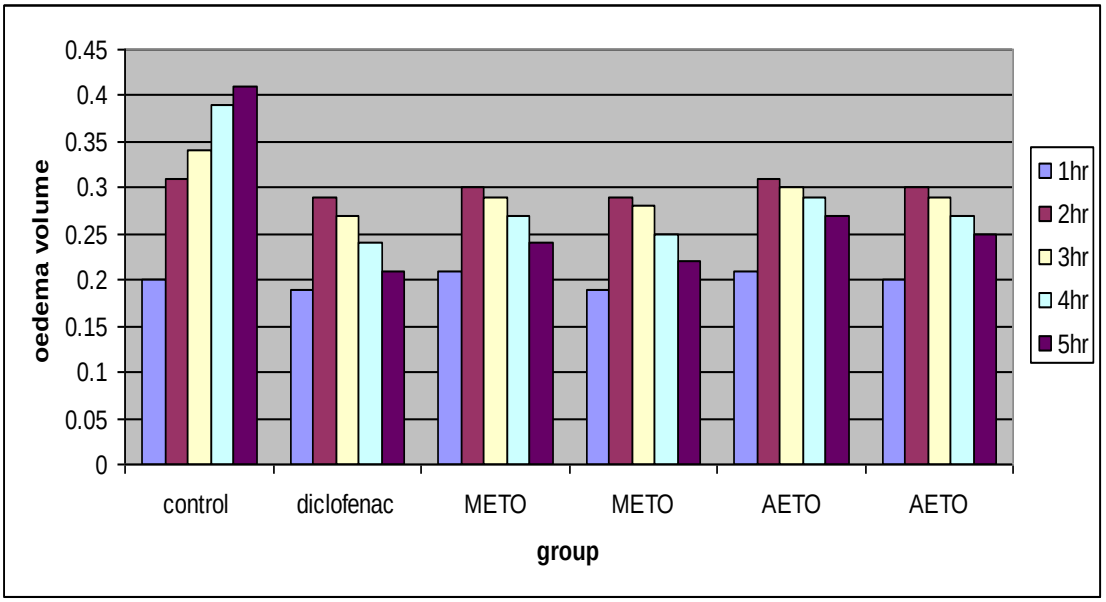
In-vivo method

Carrageenan Induced Paw Oedema

Treatment	1hr	2hr	3hr	4hr	5hr
Control	0.20±0.04	0.31 ± 0.06	0.34± 0.01	0.39± 0.01	0.41± 0.01
Diclofenac Sodium	0.19± 0.04	0.29 ±0.08*	0.27± 0.01*	0.24± 0.08**	0.21± 0.08***
METO	0.21± 0.01	0.30± 0.04	0.29± 0.09*	0.27± 0.08*	0.24± 0.08**
METO	0.19 ±0.02	0.29± 0.01*	0.28 ±0.04*	0.26±0.06**	0.22±0.06***
AETO	0.21±0.01	0.31 ±0.04	0.30±0.09	0.29±0.08*	0.27 ±0.08*
AETO	0.20±0.01	0.30± 0.02	0.29 ±0.04*	0.27 ±0.01*	0.25 ±0.01**

Values are mean ± SEM of 6 animals per group; (n=6); *p < 0.05, **p < 0.01 ***p<0.001 Vs control (ANOVA) with Dunnet's t-test.

EFFECT OF METO AND AETO ON CARRAGEENAN INDUCED PAW OEDEMA



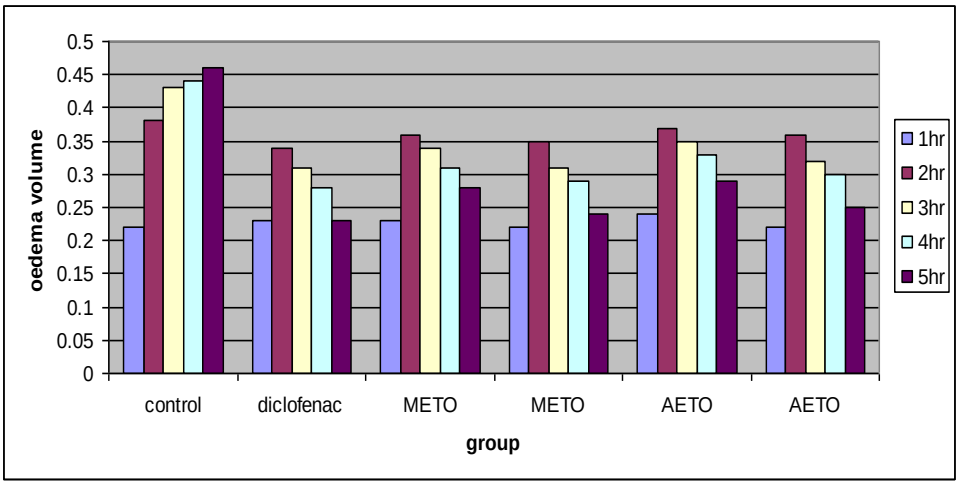
Egg albumin induced paw oedema

Treatment	1hr	2hr	3hr	4hr	5hr
Control	0.22 ± 0.01	0.38 ±0.02	0.43± 0.02	0.44± 0.03	0.46± 0.03
Diclofenac	0.22± 0.03	0.34± 0.01*	0.31± 0.04**	0.28± 0.01**	0.23± 0.02***
METO	0.23± 0.01	0.36± 0.03*	0.34± 0.03*	0.31± 0.03**	0.28± 0.02**
METO	0.22± 0.04	0.35± 0.04*	0.31± 0.04**	0.29± 0,03**	0.24± 0.03***
AETO	0.24 ±0.05	0.37± 0.02	0.35 ±0.03*	0.33± 0.02**	0.29± 0.01**
AETO	0.22± 0.01	0.36± 0.04*	0.32± 0.02*	0.30± 0.03**	0.25± 0.03 ***

Values are mean ±SEM of 6 animals per group; (n=6);*p<0.05, **p<0.01,

***p<0.001 Vs control (ANOVA) with Dunnet's t-test.

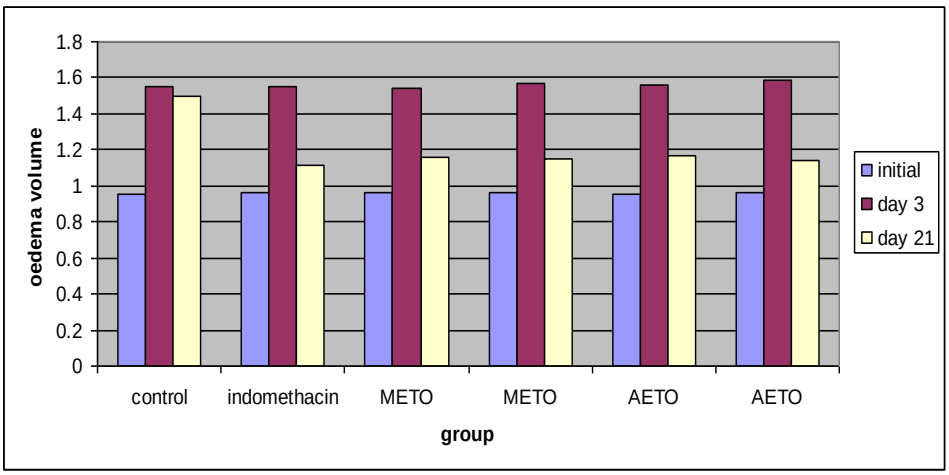
EFFECT OF METO AND AETO ON EGG ALBUMIN INDUCED PAW OEDEMA



Adjuvant induced arthritis

Treatment	Initial	After day 3	After day 21
Control	0.95 ±0.05	1.55± 0.05	1.50 ± 0.05
Indomethacin	0.95 ±0.09	1.55 ±0.06	1.11 ±0.09***
METO	0.96 ±0.06	1.54 ±0.07	1.16 ±0.05**
METO	0.96 ±0.08	1.57 ±0.08	1.14 ±0.05***
AETO	0.95 ±0.05	1.56 ±0.08	1.17 ±0.04**
AETO	0.96 ±0.06*	1.59 ±0.05	1.15 ±0.06**

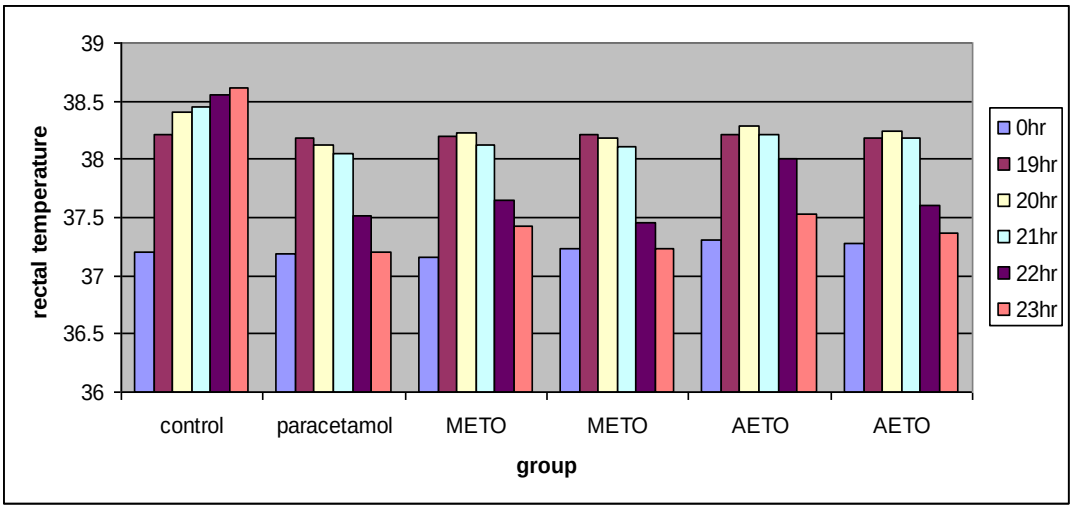
Values are mean ± SEM of 6 animals per group; (n=6); *p<0.05, **p<<0.01, ***p<0.001 Vs control (ANOVA) with Dunnet's t-test.



Yeast Induced Pyrexia

Treatment	0hr	19hr	20hr	21hr	22hr	23hr
Control	37.21 ±0.08	38.21±0.11	38.41±0.08	38.45±0.06	38.56±0.07	38.62 ±0.06
Paracetamol	37.19± 0.17	38.19± 0.15	38.13±0.14*	38.05±0.12*	37.51±0.15**	37.21±0.18***
METO	37.16± 0.10	38.20± 0.16	38.23±0.16*	38.13±0.16*	37.65±0.16**	37.42 ±0.12**
METO	37.24± 0.01	38.21± 0.05	38.19±0.02*	38.11±0.03*	37.46 0.05**	37.24±0.01***
AETO	37.30± 0.12	38.22 ±0.14	38.28±0.14*	38.22±0.16*	38.00 ±0.04*	37.53 ±0.16**
AETO	37.27± 0.06	38.18± 0.05	38.25±0.06*	38.19±0.02*	37.61±0.02**	37.36± 0.02**

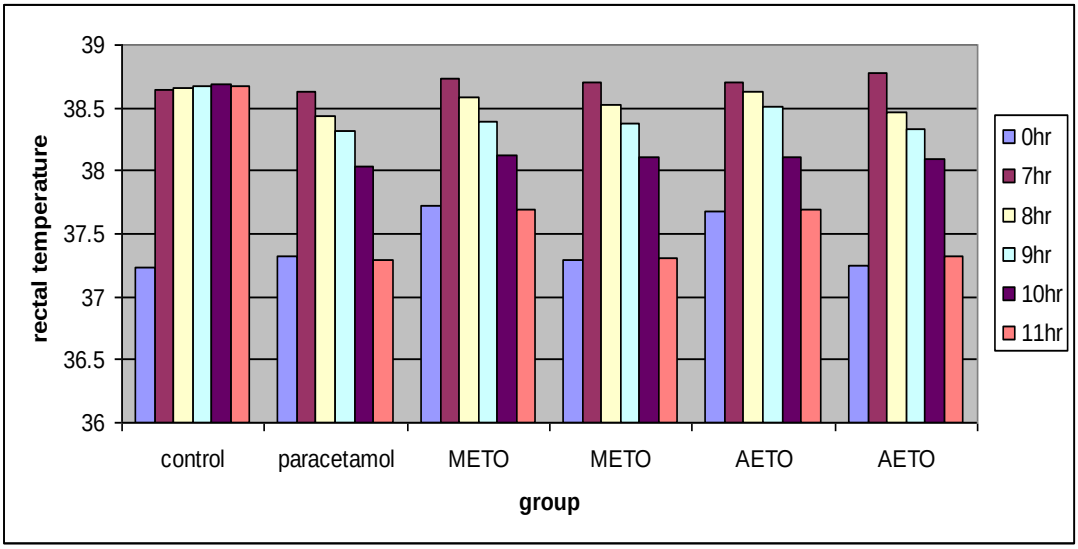
Values are mean ± SEM of 6 animals per group; (n=6); *p<0.05, **p<<0.01, ***p<0.001 Vs control (ANOVA) with Dunnet's t-test.



Vaccine induced pyrexia

Treatment	0hr	7hr	8hr	9hr	10hr	11hr
Control	37.23± 0.08	38.64± 0.11	38.78±0.09	38.64± 0.06	38.52±0.09	38.41±0.06
Paracetamol	37.32 ±0.10	38.63± 0.16	38.43 ±0.12*	38.31 ±0.16*	38.03±0.15**	37.29±0.12***
METO	37.72± 0.09	38.73± 0.11	38.58 ±0.06*	38.39± 0.05*	38.12 ±0.09**	37.69±0.12*
METO	37.29± 0.01	38.71± 0.02	38.52 ±0.06*	38.37 ±0.06*	38.09±0.06**	37.31±0.09***
AETO	37.68±0.16	38.71± 0.16	38.63±0.23*	38.51 ±0.21*	38.11±0.20**	37.69±0.26**
AETO	37.25±0.06	38.78± 0.09	38.46 ±0.08*	38.33 ±0.06*	38.10±0.06**	37.32± 0.04***

Values are mean ± SEM of 6 animals per group; (n=6); *p<0.05, **p<<0.01, ***p<0.001 Vs control (ANOVA) with Dunnet's t-test.



6.0. DISCUSSION

ANTI INFLAMMATORY ACTIVITY

Anti inflammatory activity denaturation of protein is a well documented cause of inflammation. A number of anti inflammatory agents have been shown to inhibit the protein denaturation. As a part of investigation of the mechanism of anti-inflammatory activity of METO and AETO, the ability of both the extracts to inhibit protein denaturation was studied using this in-vitro method.

Carrageenan-induced paw edema is the standard experimental model of acute inflammation and carrageenan is the phlogistic agent of choice for testing anti inflammatory drugs as it is not known to be antigenic and devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.

Carragenan-induced edema is a biphasic response, the first phase is mediated through the release of histamine, serotonin and kinins where as the second phase is related to the release of prostaglandins and mediated by bradykinin, leucotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages. The antiinflammatory activity shown by the AML (400mg/kg) in carrageenan-induced paw inflammation over a period of 3h was less significant to that exhibited by the group treated with standard Aspirin. The lower dose (200mg/kg) of AML doesn't show any significant reduction in paw edema volume. Based on the results obtained it can be concluded that the AML posses potential peripheral & centralanti-nociceptive and strong anti inflammatory activities.[35]

Sub plantar injection of fresh egg albumin produced marked, sustained, time related and progressive increase in the rat paw volume. Maximum swelling was observed approximately 2 hrs after albumin injection. Both the extracts and indomethacin showed significant activity.[36]

The first animal modal for RA is the so called adjuvant induced arthritis (AIA) in rats by injecting the complete Freund'adjuvant. CFA is a potent adjuvant that induces immune activation and granulimatouas inflammation in many organs of the body. AIA is the most frequently used chronic inflammatory model.

In the process leading to arthritis T cells are essential because the disease can be transferred by T cells and abrogated by invivo deletion of T cells. Arthritogenic structures in mycobacterium are peptidoglycon and muramyl dipeptide.[37] Both METO and AETO showed significant anti-arthritic activityin AIA model. The right hind paw volume was measured on day 0 and on day 21. Therefore in the present study, the difference between the above two volumes has been considered to be the oedema volume. Decreased paw volume on 21st day shows significant activity by both the extracts when compared to control. T lymphocytes have been reported to play acental role in the pathogenesis of RA. These cells comprise the majority of lymphoid cells found in the rheumatoid synovium. Increased lymphocyte count in arthritis rats shown the immuno suppressant nature of both the extracts. In the present study migration of leukocytes in to the inflamed area is significantly suppressed by both the extracts and indomethacin. Arthritis condition generally results in accumulation of leukocytes and release of lysosomal enzymes. Most of the NSAIDS exerts their beneficial effects by inhibiting either release of lysosomal enzymes or by stabilizing lysosomal membrane which is responsible for inflammatory process.[38]

Anti pyretic activity[39]

Subcutaneous injection of yeast suspension markedly increased the rectal temperature 19 hrs after injection. This type of fever, which is induced by yeast, is called pathogenic fever. Yeast releses high molecular weight lipopolysaccharides, which in turn cause sustainedrelease of leukocytic pyrogens. These endogenous pyrogens produce effect by activating prostaglandin synthase in the hypothalamus and the PGE1 produced cause a rise in body temperature.

Since almost all anti pyretic agents act by inhibiting PG synthesis it is logical to presume that METO and AETO also exerts their effects through the same.[40] Some of the mutant strains of gram –ve rods are used in vaccine preparations (salmonella try the cell wall of this type of bacteria consists of endotoxins (lipoplysaccharides), which activates the

macrophages thereby releasing interleukin-1 ,which causes pyrexia. The antipyretic activity of METO and AETO in vaccine induced pyrexia may be attributed to the inhibition of PGE2, which is synthesized fromIL-1.[41]

Analgesic activity

The results obtained in the present study shown that the Methanolic extract exert concentration or dosed-dependent antinociceptive effects, the rank order of relative potencies in the Eddy's Hot plate test was: ME>AE. The reaction time was found to be 11.4, and 10.4sec respectively, while the standard drug Tramadol was 11.6sec. The methanol extract shows most significant increased reaction time for mice than Tramadol.

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