Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2378-2385
Received: 09-11-2017
Accepted: 10-12-2017

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Investigation of in vivo analgesic and anti-inflammatory activities of methanol extracts of *Phyllanthus reticulatus* and *Mimosa pigra*

Shammi Akhter, Simom Hasan, Md. Mehdi Hasan and Yesmin Begum

Abstract
The petroleum ether, ethyl acetate, and methanol extracts of Phyllanthus reticulatus. Poir. (Euphorbiaceae) were chosen for pharmacological screening. Comparative antioxidant, antibacterial activities and general toxicity studies on the n-hexane, dichloromethane (DCM) and methanol (MeOH) extracts of Mimosa pudicaand Mimosa rubicaulis, two Bangladeshi medicinal plants, were carried out, using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, the resazurin microtitre plate based assay, and the brine shrimp lethality assay, respectively. The research work was conducted to investigate the in vivo Analgesic and Anti-inflammatory effects of Leaves of *Phyllanthus reticulatus* and *Mimosa pigra*. The Leaves of both plants extracted with Methanol (80%). The Analgesic activity of the extracts was performed using acetic acid induced Writhing in mice and Formalin induced to have Analgesic activity of the dose 100 and 200mg/kg body weight in the tested models compared to control. The percent of inhibition of standard drug and extract (100 and 200 mg/kg) was 40.19%, 51.95%, for *Phyllanthus reticulatus* and 44.73%, 55.75% for *Mimosa pigra* respectively. The Analgesic activity of the extract using Formalin induced Biting and Licking in mice was studied MELPR and MELMP were found to have Analgesic activity of the dose 100 and 200 mg/kg body weight in the tested models compared to control. The Anti-inflammatory activity of the extract using Carrageenan induced Anti-inflammatory in mice was studied of the dose 100 and 200 mg/kg body weight in the tested models compared to control. The crude extract of the plant were found to have Anti-inflammatory activity of the dose 100 and 200 mg/kg body weight in the tested models compared to control.
So Methanolic extract of *Phyllanthus reticulatus* and *Mimosa pigra* have remarkable Analgesic and Anti-inflammatory activities.

Keywords: acetic acid-induced writhing response, antiinflammatory, carrageenan-induced rat paw edema assay, euphorbiaceae, phyllanthus reticulatus, radiant heat tail-flick method

Introduction
In traditional culture medicinal plants are used all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals (Verma KR., *et al* 2011) [1]. The word drug itself comes from the Dutch word "Droog" (via the French word Drogue), which means "Dried plant". Some examples are insulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove.
Medical plants are used as a source of drugs for the treatment of various human and livestock health disorder all over the world from ancient time to the present day. A total of 25000 species of flowering plants are referred to as medicinal plants (Kirtikar KR., *et al*, 1991) [2]. The world health Organization enlisted some 2100 plants species.
In Bangladesh, about 500 plant species have been identified as medicinal plant because of their therapeutic properties (Council SIT *et al*. 2005) [3]. In the meantime, a large number of industries have been estimated that Bangladesh has a market of about 100 core taka worth herbal products annually.
Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for their treatment of various diseases. However, Scientific studies have been conducted only a limited extent with few medicinal plants.
Due to destruction of forest and overgrazing of remote and marginal lands, expansion of industry and urbanization a will as the excessive harvesting of wild rate an endangered plants biological diversity of medicinal plants are being reduced day by day.
Efforts should be made to protect and conserve the worst affected and most economically important species in their natural habits. Controlled access to bio diversity thus conserved and will pay an important role in raising the living standard and alleviating poverty amongst the rural poor (Nadkarni KM *et al*., 2009) [5]. Mimosa pudica are rich in mimosine (N-(3-alanyl)-3-

hydroxy-4- piridone), norepinefrine, linoleneic acid, oleic acid, palmitic acid, stearic acid, phenol, amino acid, steroid/ triterpenoid, sterol, tannins, and flavonoids (Depkes RI, 1995; ASEAN Countries, 1993), 4"-hydroxymycine, dan cassiaocidentalin B (Lobstein *et al*, 2002). While in Central Java leaves of Mimosa pudica L. are being used to cure insomnia.

Medicinal plant: plants used as natural medicines. This practice has existed since prehistoric times. There are three ways in which plants have been found useful in medicine. First, they may be used directly as teas or in other extracted forms for their natural chemical constituents. Second, they may be used as models for synthetic drugs.

Definition

"Medicinal plants are plants that provide people with medicines-to prevent disease, maintain health or cure ailments.

Importance of Medicinal Plants

The use of plants medicine is by farther biggest use of plants in term of the number of species specially targeted. Plants provide the predominant ingredients of medicines in most traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs.

According to plant life international, the scale of trade in maps ranges from local to international. Much of this trade is unrecorded or poorly documented in official statistics. Due to the poor documentation, decision makers usually have little awareness of the significance of trade and consumption of medicinal plants, or of the problems of un-sustainability and the sometimes deleterious impacts on natural habits of wild collection (Chatterjee A *et al*., vol 3) [6].

According to the International Standard for the sustainable Wild collection of medicinal and Aromatic plants (ISSC-MAP), themain threats for the MAP populations are over-harvesting and habits loss, including through land conversion.

Aim and objective of the work

Plants have been the basis of much traditional medicine system throughout the world for thousands of years and continue to provide humankind with new remedies.

Many drugs used in contemporary medicine have been derived from plants were originally discovered though the traditional use by indigenous people. The use of plants as medicine isolation and characterization of pharmacologically active compounds from medicinal plants continue today (Shruthi SD *et al*., 2010, Jamal AK *et al*., 2008) [7, 8].

Bangladesh has a vast resource of medicinal plants and majority of our population has to rely upon indigenous system of medication from economic point of view.

So the aim of study to evaluate the analgesic and anti-inflammatory activities.

Materials and Methods

Selection of plants

The fresh leaves of this plant *phyllantus reticulatus* and *Mimosa pigra*were selected for the study.

Collection and identification of the plants

The fresh leaves of these plants *phyllantus reticulatus* and *Mimosa pigra* were collected from the area of Bandorban in Bangladesh.

Drying and pulverization

The fresh leaves were first washed with water to remove the adhering dirt and then cut into small pieces, sun dried for 12-15days. After complete drying, the entire portions were pulverized into coarse powder with the help of grinding machine and were stored in an air tight container for further use (Begum T *et al*., 2006).

Extraction of plant materials

The powdered 100mg powders were extracted with methanol (85%) of their in a flat bottom glass container, through occasional shaking and stirring. After 7 days the extract was then filtered through the cotton at first and then through the filter paper.

Drying of extract

Then the liquid extract was dried by solvent evaporation method, afford a greenish mass.

Analgesic activity by acetic acid induced writhing method: Principle

In this method acetic acid is administered intra-peritoneal to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval of pain. This squirms of contraction of the body is termed as writhing. As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indicated pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing inhibition of positive control was taken as standard and control. As positive standard control any NSAIDS drug can be used. In the present study, formalin was used to serve the purpose.

Experimental animal

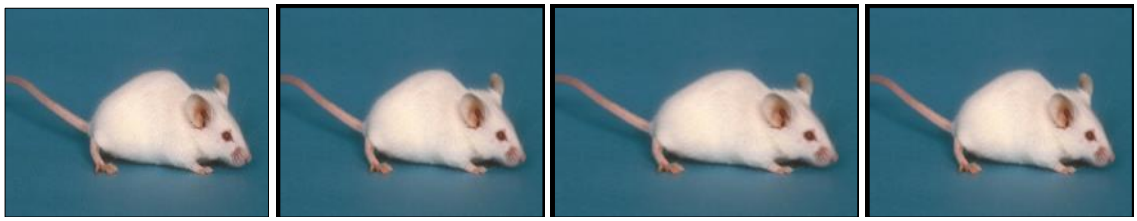
Swiss Albino mice of either sex obtained from the animal Resource Branch of the International center for Diarrheal Disease and Research, Bangladesh (ICDDR, B) were used for the experiment. They were kept in standard environmental condition and fed the ICDDR; B formulated rodent food and water.

Experimental Design

Experimental animals were randomly selected and divided into 6 group-I, group-II, group-III, group-IV, group-V, and group-VI consisting of 4 mice in each i.e.; control, standard; and the doses of the exact (100 and 200 mg/kg) of plant respectively. Prior to any treatment, each mice was weighted properly and the dose of the test sample and control material was adjusted accordingly.

Method of identification of animals

Each group considered of four mice. As it was difficult to observe the biologic response of four mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized and marked as M1=Mice1, M2=Mice2, M3=Mice3, M4=Mice4,



M1=Mice M2=Mice M3=Mice M4=Mice

Fig 1: Identification of mice for study of Analgesic Activity

Reagents, Chemical and equipment are used for Analgesic activity
Cv

Table 1: Reagents, chemical and equipment's are used for Analgesic activity: Reagents and Chemicals

Reagents and Chemicals	Source
Indomethacin	Square pharmaceutical Ltd. Bangladesh
Acetic acid	Merck, Germany
Tween-80 (as suspending agent)	BDH chemical Ltd.
Normal saline water (0.9%) NaCl	Beximco Infusion Ltd.

Equipment's

Equipment's	source
Sterile disposal syringe (1ml, 100 division)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electric and digital balance	Denver Instruments M-220

Preparation of test Materials
In order to administer the crude extract at a dose of 100 and 200 mg/kg body weight of mice appropriate amount of extracts was measured and was triturated unidirectional way by the addition of small amount of suspending agents Tween-80. The final volume was made 5ml by saline water. To stabilize the suspension it was stirred well by vortex mixer.
For the preparation of Indomethacin, at a dose 10mg/kg body weight, 12.5mg of Indomethacin was taken and a suspension of 2.5 ml was made.

Table 2: Test samples use in the evaluation of analgesic activity of MEPR and MEMP by acetic acid induced writhing method

Test samples	Group	Purpose	Dose(mg/kg)	Route of Administration
1% Tween-80 in saline	1	Control group	0.1ml/10gm of body weight	oral
Indomethacin	2	Standard group	10	Oral
MEPR	3	Test sample	100	
MEPR	4	Test sample	200	
MEMP	5	Test sample	100	
MEMP	6	Test sample	0.1ml/10mg of body weight	Intra-peritoneal
Acetic acid	……	writhing	0.1ml/10mg of body weight	Intra-peritoneal

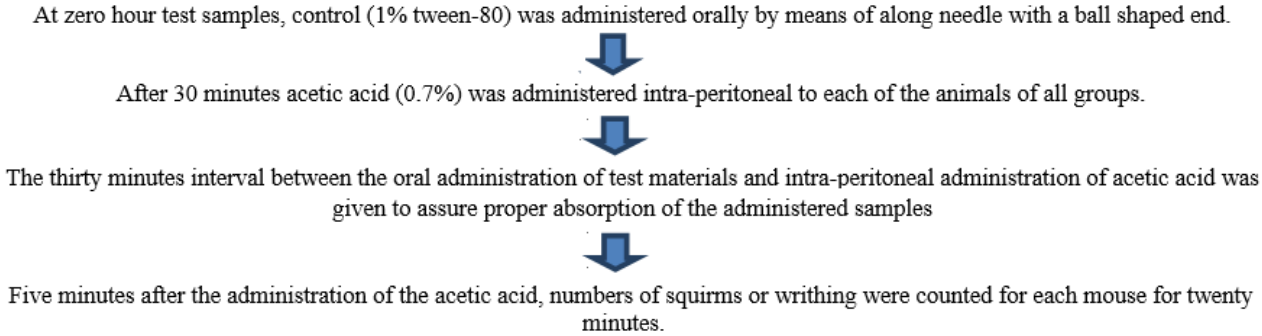


Fig 2: Schematic representation of procedure for screening f analgesic property on mice by acetic acid induced method for *Phyllanthus reticulatus* and *Mimosa pigra.*

Counting writhing
Each mouse of all groups were observed individually for counting the numbers of writhing they made in 20 minutes commencing just 5 minutes after administration of acetic acid solution. Full writhing was not accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half- writhing. Accordingly two half writhing was taken as one full writhing.

Mechanism of pain induction in acetic acid Writhing method of Analgesic activity screening
Acetic acid Induced Method
Intra-peritoneal administration of acetic acid (0.7%) causes

localized inflammation in mice.following inflammation, there is biogenesis of prostaglandin (from cyclooxygenase pathway) and leukotrienes (lipoxygenase pathway). The released prostaglandins, mainly prostacyclin's (PGI_2) and prostaglandin-E have been reported responsible for pain sensation.

The exact mechanisms by which prostaglandins produce pain are not still clear but there are number of proposed mechanism of action

1. All kinds of pain or noxious stimuli are conveyed by specific nervous called un-myelinated C-fibers and myelinated A- δ fibers, the former being slow conducing the latter being fast conducing. It has been investigate that un-myelinated C-fibers are the most usual conveyer of two.
2. The prostaglandin and another liberated product of inflammation serve as noxious stimuli. They are supposed to sensitized C fibers and subsequently reduce pain threshold. These C fibers get stimulated and cause enhanced release of tachykinins, mainly substance P and neurokinins. It is the substance P released in excessive amount following the stimulation of C fibers that has been held responsible for sensation of pain in animal.
3. The precise mechanism by which substance P arouses pain sensation is not well documented. But upon release, substances P and other tachykinins binds to specific receptors (NK1.NK2, NK3) that are G-protein. Among these receptors, substances P specially bounds to NK1. After binding of substance P to the respective receptor, there is stimulation of phospholipase C resulting in the formation of two second messengers-Inositol triphosphate (IP_3), and Diacylglycerol (DAG). IP_3 causes the exocytosis release of Ca^{++} stored intra-cellular and DAG activities protein kinase C which then causes the influx of Ca^{++} through the voltage gate Ca^{++} channel. These happening may have role in the neural processing of the pain sensation and in subsequent conveyance to higher centers of the brain.
4. Prostaglandin also potential the pain producing activity of bradykinins and other autacoids.

Flow chart: Schematic diagram of pain induction by acetic acid

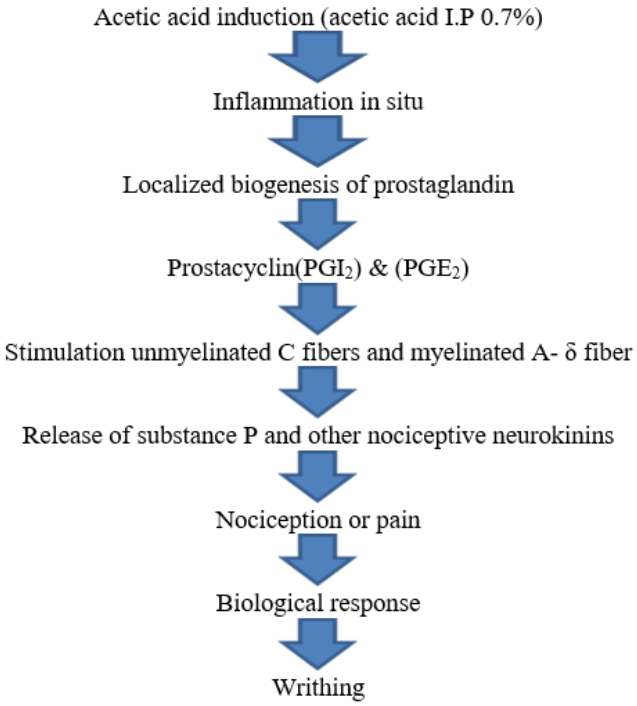


Fig 3: Schematic diagram of pain induction in writhing investigate method.

Analgesic Activity by Formalin Induced Method

Principle

On day of experiment non fasted animals were weighted and weighted dosed as per randomization; test compounds were dosed orally one hr. before formation challenge. Formalin was injected into the dorsal lateral surface of the left hind paw and the time spent for licking and biting in seconds by each animal at 5 min interval is recorded for 40 min immediately after formalin injection in the following order of time intervals.

Experimental animal

Swiss Albino mice of either sex obtained from the animal Resource Branch of the International center for Diarrheal Disease and Research, Bangladesh (ICDDR, B) were used for the experiment. They were kept in standard environmental condition and fed the ICDDR; B formulated rodent food and water.

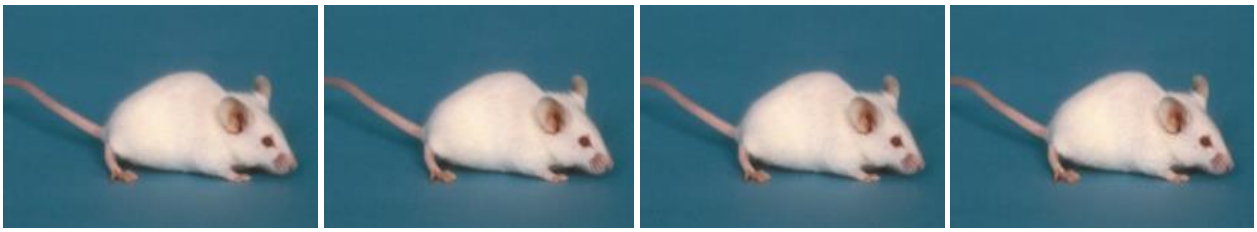
Experimental Design

Experimental animals were randomly selected and divided into 6 group-I, group-II, group-III, group-IV, group-V, and group-VI consisting of 4 mice in each i.e.; control, standard; and the doses of the exact (100 and 200 mg/kg) of plant respectively. Prior to any treatment, each mice was weighted properly and the dose of the test sample and control material was adjusted accordingly.

Method of identification of animals

Each group considered of four mice. As it was difficult to observe the biologic response of four mice at a time receiving same treatment, it was quite necessary to identify individual

animal of a group during the treatment. The animals were individualized and marked as M1=Mice1, M2=Mice2, M3=Mice3, M4=Mice4,



M1=Mice M2=Mice M3=Mice M4=Mice

Fig 4: Identification of mice for study of Analgesic Activity

Reagents, Chemical and equipment are used for Analgesic activity

Table 3: Reagents, chemical and equipment's are used for Analgesic activity: Reagents and Chemicals

Reagents and Chemicals	Source
Indomethacin	Square pharmaceuticals Ltd. Bangladesh
Acetic acid	Merck, Germany.
Tween-80 (as suspending agent)	BDH chemicals Ltd.
Normal saline water (0.9%)NaCl	Beximco Infusion Ltd.

Equipment's

Equipment's	Source
Sterile disposable syringe (1ml,100 divisions)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electric and digital balance	Denver Instruments M-220

Preparation of test Materials

In order to administer the crude extract at a dose of 100 and 200 mg/kg body weight of mice appropriate amount of extracts was measured and was triturated unidirectional way by the addition of small amount of suspending agents Tween-80. The final volume was made 5ml by saline water. To stabilize the suspension it was stirred well by vortex mixer.

For the preparation of Indomethacin, at a dose 10mg/kg body weight, 12.5mg of Indomethacin was taken and a suspension of 2.5 ml was made.

Table 4: Test samples use in the evaluation of Analgesic activity of MEPR and MEMP by Formalin induced Writhing method

Test samples	Group	Purpose	Dose(mg/kg)	Route of administration
1% Tween-80 in saline	1	Control group	0.1ml/10gm of body weight	oral
Indomethacin	2	Standard group	10	oral
MELMP	3	Test sample	100 & 200	oral
MELPR	4	Test sample	100 & 200	oral
Formalin	……..	Biting and licking	0.1ml/10gm of body weight	Left hind paw

Formalin Induced Method

The pain in the early phase of formalin test was due to the direct stimulation of the sensory nerve fibers by formalin while the pain in the late phase was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins (Srivastava S *et al*, 2012) [10]. This test is believed to be a more valid analgesic model which is better correlated with clinical pain (Sharma B *et al*., 2009) [11]. In this study, the exact caused a dose-dependent effect of the extract. But no significant licking activity of ethanol and aqueous activity were found against both phases in formalin test in previous studies.

Anti-inflammatory activity by Carrageenan Induced inflammatory method:

Principle

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Several indigenous drugs have been described in Ayurveda for the management of inflammatory diseases. Combinations of pharmacology active principles in different plant families and species often exhibit remarkable potency and tolerance, particularly inthe long term treatment of chronic disorders, like rheumatic diseases. Joint care B is a herbal formulation contains Alpinia galangal, Commiphora weighty, Boswellia serrata, Foeniculum vulgare, Glycyrrhiza glabra, Vitex negundo and Anethum graveolens which are known for their anti-inflammatory activity. Joint Care B, o polyherbal formulations, was evaluated for its anti-inflammatory activity in acute and chronic models of inflammation

Experimental Animal

Swiss Albino mice of either sex obtained from the animal Resource Branch of the International center for Diarrheal Disease and Research, Bangladesh (ICDDR, B) were used for the experiment. They were kept in standard environmental condition and fed the ICDDR; B formulated rodent food and water.

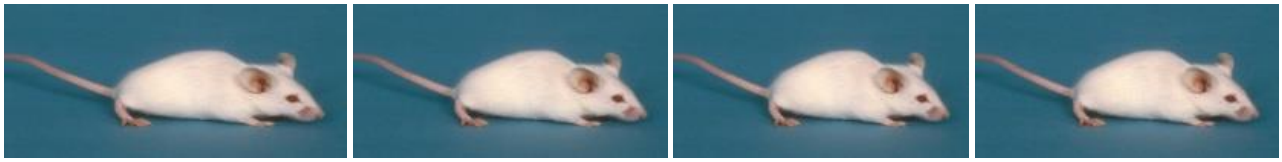
Experimental Design

Experimental animals were randomly selected and divided into 6 group-I, group-II, group-III, group-IV, group-V, and

group-VI consisting of 4 mice in each i.e.; control, standard; and the doses of the exact (100 and 200 mg/kg) of plant respectively. Prior to any treatment, each mouse was weighted properly and the dose of the test sample and control material was adjusted accordingly**.**

Method and Identification of Animals

Each group considered of four mice. As it was difficult to observe the biologic response of four mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized and marked as M1=Mice1, M2=Mice2, M3=Mice3, M4=Mice4,



M1=Mice M2=Mice M3=Mice M4=Mice

Fig 5: Identification of mice for study of Anti- inflammatory Activity.

Reagents, Chemicals and equipment's used for Anti-inflammatory activity

Table 5: Reagent, Chemicals and equipment's used for Anti-inflammatory activity: Reagents and Chemicals

Reagents and Chemical	Source
1. carrageenan	
2. tween-80	BDH chemical Ltd.
3. normal saline water (0.9% NaCl)	Beximco Infusion Ltd.

Equipment's

Equipment's	Source
Sterile disposable syringe (1ml, 100 divisions)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electric and digital balance	Denver Instruments M-220

Preparation of test materials

In order to administer the crude extract at a dose of 100 and 200 mg/kg body weight of mice appropriate amount of extracts was measured and was triturated unidirectional way by the addition of small amount of suspending agents Tween-80. The final volume was made 5ml by saline water. To stabilize the suspension it was stirred well by vortex mixer. For the preparation of Indomethacin, at a dose 10mg/kg body weight, 12.5mg of Indomethacin was taken and a suspension of 2.5 ml was made.

Table 6: Test samples use in the evaluation of Anti-inflammatory activity of MEPR and MEMP by Carrageenan induced inflammatory method

Test samples	group	purpose	Dose(mg/kg)	Route of administration
1% Tween-80 in Saline	1	Control group	0.1ml/10mg of body weight	oral
Indomethacin	2	Standard group	10	oral
MELPR	3	Test sample	100	oral
MELPR	4	Test sample	200	oral
MELPR	5	Test sample	100	oral
MELPR	6	Test sample	200	oral
Carrageenan	…….	Inflammatory induced	0.1ml/10mg of body weight	Right hind paw

Procedure

In the day of the animals were divided into 6 groups of fine mice in each. They were weighted for accurate administration of drug. Group I received distilled water while Group- III and Group-IV a Group-V and Group-VI receive *Phyllanthus reticulatus* and *Mimosa pigra* extract at two different doses respectively. Group II acts as a positive control where any Anti-inflammatory drug may be used. In this model we used Indomethacin as standard

1. After 30 minutes, all the animals were injected 0.1 ml of 1% Carrageenan in the sub planter region of the right hind paw. The paw was marked with permanent marker at the level of the lateral alveolus. The initial reading just after injection and subsequent paw volumes were taken by plethysmograph. The readings of paw volumes were taken at 0, 30, 60, 90 and 120 hrs. After injecting Carrageenan. Edema volume was calculated by subtracting initial volume from the volume of respective time points.

Result and Discussion

Effect of Methanolic extracts of the extracts of *Phyllanthus reticulatus* and *Mimosa pigra* on acetic acid induced writhing in mice.

Table 7-shows Effect of Methanolic extracts of the extracts of Phyllanthus reticulatus and Mimosa pigra on acetic acid induced writhing in mice. The doses of extracts of *Phyllanthus reticulatus* and *Mimosa pigra* showed reduction of writhing induced by the acetic acid after oral administration in a dose dependent manner. After administration of 2 different doses (100 and 200mg/kg body weight), the percent inhibition was 40.19%, 51.95%, for *Phyllanthus reticulates*and 44.73%, 55.75% for *Mimosa*

pigra. The reference drug Indomethacin was found potent than the plant extracts at the entire dose level.

Table 7: Effect of *Phyllanthus reticulatus* and *Mimosa pigra* extract on acetic acid induced writhing method on mice.

Groups	Treatment	Dose, Route	No. of Writhing	Percent Inhibition
Group I	1% Tween-80 in saline water	0.1% ml/10gm body weight	26.33	-
Group II	Indomethacin	10mg/kg	10.83	58.86
Group III	MELPR	100mg/kg	15.75	40.19
Group IV	MELPR	200mg/kg	12.65	51.95
Group V	MELMP	100mg/kg	14.55	44.7
Group VI	MELMP	200mg/kg	11.65	55.75

MELPR= Methanolic extract of leaves of *Phyllanthus reticulatus.*
MELMP= Methanolic extract of leaves of *Mimosa pigra*.

Effect of Methanolic extracts of *Phyllanthus reticulatus* and *Mimosa pigra* on formalin induced licking and biting in mice.

Table 8- shows Effect of Methanolic extracts of the extracts of *Phyllanthus reticulatus* and *Mimosa pigra* on formalin induced licking and biting in mice. The doses of extracts of *Phyllanthus reticulatus* and *Mimosa pigra* showed reduction of licking and biting induced by formalin after oral administration in a dose dependent manner. After administration of 2 different doses (100 and 200mg/kg body weight), the percent inhibition was 20.1%, 36.9%, for *Phyllanthus reticulates* and 28.2%, 48.1% for *Mimosa pigra*. The reference drug Indomethacin was found potent than the plant extracts at the entire dose level.

Table 8: Effect of *Phyllanthus reticulatus* and *Mimosa pigra* extract on formalin induced licking and bitingmethod on mice.

Groups	Treatment	Dose, Route	No. of licking & biting (0-5 min)	Percentage Inhibition	No. of licking & biting (15-30 min)	Percentage Inhibition
Group I	1% Tween-80 in saline water	0.1ml/10gm body weight	35.67	-	35.67	-
Group II	Indomethacin	10mg/kg	16.83	52.8	16.83	52.8
Group III	MELPR	100mg/kg	28.5	20.1	24.0	32.7
Group IV	MELPR	200mg/kg	22.5	36.9	24.0	32.7
Group V	MELMP	100mg/kg	25.6	28.2	23.0	35.5
Group VI	MELMP	200mg/kg	18.5	48.1	27.0	24.3

MELPR= Methanolic extract of leaves of *Phyllanthus reticulatus*.
MELMP= Methanolic extract of leaves of *Mimosa pigra*.

Anti-inflammatory effect by Carrageenan-induced Paw Edema in mice

Table 8- Shows effect of Methanolic extracts of the extracts of *Phyllanthus reticulatus* and *Mimosa pigra* on Carrageenan induced Paw Edema in mice. The doses of extracts of *Phyllanthus reticulatus* and *Mimosa pigra* showed reduction of inflammation induced by Carrageenan after oral administration in a dose dependent manner. After administration of 2 different doses (100 and 200mg/kg body weight), the percent inhibition was 20.1%, 36.9%, for *Phyllanthus reticulates* and 28.2%, 48.1% for *Mimosa pigra*. The reference drug Indomethacin was found potent than the plant extracts at the entire dose level.

Table 8: Effect of *Phyllanthus reticulatus* and *Mimosa pigra* extract on Carrageenan induced inflammation in mice.

Groups	Treatment	Dose, Route	Paw Edema (mm)			
			0 min	30 min	60 min	120 min
Group I	1% Tween-80 in saline water	0.1ml/10mg body weight	5.12	6.2	5.94	6.4
Group II	indomethacin	10mg/kg	4.61	4.49	4.33	4.21
Group III	MELPR	100mg/kg	5.08	4.82	4.66	4.49
Group IV	MELPR	200mg/kg	5	4.75	4.60	4.41
Group V	MELMP	100mg/kg	5.04	4.74	4.58	4.39
Group VI	MELMP	200mg/kg	5.02	4.75	4.6	4.38

MELPR= Methanolic extract of leaves of *Phyllanthus reticulatus*.
MELMP= Methanolic extract of leaves of *Mimosa pigra*.

Discussion

Acetic acid induced Writhing in mice attributed visceral pain finds much attention of screening analgesic drugs. The crude extracts of the plants showed analgesic drugs action compared to the reference drugs Indomethacin. *Phyllanthus reticulatus* and *Mimosa pigra* were found to exhibit the analgesic activity against acetic acid induced pain in mice at 2 dose levels, 100 and mg/kg and 200 mg/kg body weight. The acetic acid induced Writhing method was found effective to evaluate peripherally acting analgesic action. The agent reducing the number of Writhing will render analgesic effect preferably by inhibition of Prostaglandin synthesis. The percent of inhibition of plant extracts (100 and 200 mg/kg) was found 40.19%, 51.95% for *Phyllanthus reticulates* and 44.73%, 55.75% for *Mimosa pigra* respectively.

The analgesic activity of the crude extracts by formalin test was also performed to evaluate both peripheral and central activity. In the earlyphase of formalin test the pain began due to the direct stimulation of the sensory nerve fibers by formalin. In this study, the extracts caused a dose-dependent decrease in licking time by the mice injected with formalin the analgesic effect of the extracts.

The crude extracts of the plants showed Anti-inflammatory action compared to the reference Indomethacin. 5 and 4.72, 4.6 and 4.4 for *Phyllanthus reticulatus* and *Mimosa pigra* were found to exhibit Anti-inflammatory activity induced

Inflammatory activity induced Inflammatory in mice at dose level, 100 and 200 mg/kg/day body weight.

Acknowledgements: None

Conflict of interest

The authors declare that they have no conflict of interest**.**

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