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Evaluation of toxicity profile, analgesic anti-inlfammatory and ant-pyretic potency of seeds of *Meyna laxiflora Robyns*

Janarthanan L[1*], Venkateswarlu BS[2]

[1]*Research Scholar, Sunrise University, Bagad Rajput Tech. Ramgarh Dist, Alwar 301030, Rajasthan-1, India.*
[2]*Principal, Vinayaga Mission's College of Pharmacy, VMRF(DU), Salem-8, Tamil Nadu, India.*

Corresponding author: Janarthanan L
Email: jana_loganathan@yahoo.co.in

ABSTRACT

Narcotics (e.g., opioids) or non-narcotics (e.g., salicylates and corticosteroids) are used in the management of pain and inflammation, both of which have side effects, thereby emphasizing the search for natural substances. The objective of the present study was to evaluate the acute toxicity study, analgesic, anti-inflammatory, and antipyretic activity of *Meyna laxiflora* Robyns seeds. In hot plate method, The tail immersion method and acetic acid-induced writhing method were used for the evaluation of analgesic activity. The carrageenan induced paw edema method, cotton pellet-induced granuloma methods were used for the evaluation of anti-inflammatory activity. Brewer's yeast-induced pyrexia model was used to evaluate antipyretic activity. The aqueous and ethanolic seeds extract of *M.laxiflora* showed significant analgesic activity at 200 and 400 mg/kg. Analgesic activity was comparable with standard drug pentazocine. The maximum inhibitory effect of ethanolic extract of *M.laxiflora* showed significant ($p< 0.01$) at 90 min post dose in 400 mg/kg. Injection of acetic acid into control mice produced 51.4±6.4 writhes. Pre-treatment of extract, in this ethanolic extract significant effect of *M.laxiflora* reduced the number of writhes 39.4±2.4 (23.34 % protection) and 31.2±2.1 (39.29 % protection) respectively. The extract showed significant effect against Brewer's yeast induced pyrexia method. The extract showed maximum decreased formation of granuloma tissue, which indicate that *M.laxiflora* produced a significant decrease in the weight of granuloma 38.16±0.04 (7.4% inhibition) and 34.58±0.04 (16.1% inhibition) respectively. The seeds of *M.laxiflora* Robyns exhibit significant analgesic, anti-inflammatory and antipyretic activity.

Keywords: Analgesic, Anti-inflammatory, Antipyretic, *Meyna laxiflora* Robyns

INTRODUCTION

Inflammation is defined as the reaction of living tissue to injury, and its cardinal signs include pain, swelling, redness, increased local warmth, and loss of function. Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage"[1]. It is fundamentally a protective response, the ultimate goal of which is to help the organism get rid of both, the initial cause of injury (e.g., microbes and toxins) and the consequences of such injury (e.g., necrotic cells and tissues). Inflammation is of acute and chronic types[2]. Acute inflammation is the immediate and early response to an injurious agent, while chronic inflammation is the inflammation of prolonged duration (weeks or months) in which there are active inflammation, tissue destruction, and attempts at repair, which proceed simultaneously. However, inflammation, if uncontrolled, can become a cause of suffering, leading to disabilities, contractures, disfiguring of body, and chronic pain. In such situations, the inflammation needs to be controlled or suppressed [1].

Fever, on the other hand, is defined as the elevation of core body temperature above normal; in healthy adults, the average oral temperature is 37°C (98.6°F). Although fever is beneficial, suppression of fever may be necessary for certain conditions, such as febrile convulsions, or to help alleviate discomfort and constitutional symptoms, such as fatigue,

myalgias, diaphoresis, and chills[3]. Drugs used for the management of pain and inflammation are either narcotics (e.g., opioids) or non-narcotics (e.g., salicylates and corticosteroids), both of which are well known for their side effects, such as intestinal tract ulcers and erosions of the stomach linings[4]. As a result, there is greater interest in finding a safer and potent alternative, especially agents from natural sources.

Meyna laxiflora Robyns also known as Vangueria spinosa, belongs to the family of Rubiaceae. In tamil its known as Manakkarai. It is a genus of about 6500 species tree, shrubs plant belonging to the family Rubiaceae natives of Northern Bengal to Burma from the thick rain forest region. The fruit and the leaves are edible. The leaves are used as fodder, but are of an inferior quality. The dry fruits of *M.laxiflora* Robyns believed to be narcotic and are reported to be used for boils and dysentery. The powdered leaves are used by ethnopharmacists for curing diphtheria. *M.laxiflora* Robyns mainly is used in Ayurveda; Pinditaka. Madana or Mainphala are misleading synonyms. It is equated with randia dumetorum Poir[5]. The present paper deals with the standardization of seeds on the basis of various Pharmacognostic, physicochemical and phytochemical parameters. The determination of these characters will aid future investigators in their Pharmacological analysis of this species.

MATERIALS AND METHODS

Plant collection and authentification

The plant specimens for the proposed study were collected from the trees carefully because they have thorns. The plant parts of *Meyna laxiflora* Robyns was collected from the dense forest in the Bhimashankar range (The three lakes-Tansa, Vaitarna and Bhatsa) in Maharastra and it was authenticated by Dr. P.JAYARAMAN, Plant Anatomy Research Centre (PARC), Sakthi Nagar, West Tambaram, Chennai.

Preparation of extracts

Preparation of the extract of seeds of *Meyna laxiflora* Robyns was done by using methanol and water. The extraction process is done by percolation process. Preliminary phytochemical investigations for secondary metabolites were conducted on different extracts of *M.laxiflora* Robyns and examined for metabolites like carbohydrates, alkaloids, glycosides, tannins, protein and amino acid, saponins.

Animals

Male Swiss albino mice weighing 25-30g were used in the present study. All rats were kept at room temperature of 22-25°C in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. All animal procedures were performed after approval from the institutional ethics committee.

Acute oral toxicity study

By following OECD (Organization of Economic Co-operation and Development) guidelines 420- Fixed Dose Procedure (FDP), acute oral toxicity was evaluated. This involves the identification/calculation of the doses level that becomes evidence of non-lethal toxicity (termed Evident toxicity), which gives clear signs and symptoms of toxicity of a test drug/substance. When dose where increase to next level of highest fixed dose, which would result in the development of severe toxicity sign or even death. Next highest fixed dose producing, Evident toxicity was assumed and was also calculated on ones experiences. These doses also provide information that lead to a similar classification, to that based on the LD_{50} value[6-9].

Procedure

Five animals (Wister Albino rats, 150-200 gm) and Swiss mice, 20-25 gm/kg) were selected for studies. Then the defined or fixed dose levels of ethanolic and aqueous extracts in 50, 100, 200, 500 and 1000 mg/kg was given to identify a dose producing evident toxicity. After giving different doses, the toxicity signs were observed within 48 hrs. Food was withheld for 3-4 hours after drug administration. Further, last highest fixed dose 2000 mg/kg body weight was given and again sign of toxicity and mortality was observed. Most of the crude extracts possess LD_{50} value more than 2000 mg/kg of the body weight of animal used. Dose volume administered was 0.1ml/100gm body weight of the animal orally. Following was observed: body weights of the rats before and after drug administration, onset of toxicity and sign of toxicity like changes in skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity, behavior pattern, sign of tremors, convulsions, salvation, lethargy, sleep and coma was also noted if any.

Observation

No toxicity or death was observed for these given dose levels, in the selected and treated animals. So, the LD_{50} of the ethanolic and aqueous extracts as per OECD guidelines-420 is greater than 2000 mg/kg (LD_{50}>2000 mg/kg). Hence, the biological dose was fixed at100 and 200 mg/kg for both the extracts[10-13].

Analgesic activity
Hot plate method in mice

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally medicated analgesic effects of aqueous and ethanolic extract of *Meyna laxiflora* Robyns . The central analgesic drug pentazocine was used for positive control group. In this experiment, four groups (n=6) of Swiss albino mice (20-25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group I- Normal Control received CMC (0.5%), and Group II- standard treated with pentazocine (3 mg/kg i.p), where as 200 mg/kg (Group III and VI) and 400 mg/kg group (V and VI) animals were

treated orally with aqueous and ethanolic extract of *Meyna laxiflora* Robyns.

Group I: Normal Control (CMC), Group II: Standard (Pentazocine 3 mg/kg), Group III: Test Drug I (Aqueous seed extract of *Meyna laxiflora* Robyns 200 mg/kg), Group IV: Test Drug II (Aqueous seed extract of *M.laxiflora* Robyns 400 mg/kg), Group V: Test Drug I (Ethanolic seed extract of *M.laxiflora* Robyns 200 mg/kg), Group VI: Test Drug II (Ethanolic seed extract of *M.laxiflora* Robyns 400 mg/kg). Each animal was then individually placed gently on Eddy's hot plate at 55ºC. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate were determined at 30, 60, 90 and 120 min after administration of the drugs or vehicle[11,14].

Tail immersion test

This method assessment was used to evaluate the centrally medicated analgesic effects of aqueous and ethanolic extract of *Meyna laxiflora* Robyns. The wistar rats were divided into four groups each consists of six animals. They were placed into individual restraining cages leaving the tail hanging out freely. The lower 5cm portion of the tail is marked and this part of the tail was immersed in a water bath containing water at a temperature of 55± 0.5 ºC. Withdrawing the tail from the hot water showed the analgesic effect. The reaction time was noted on a stop-watch. Each animal served as control. The average of the two values was the initial reaction time. Group –II served as standard and received pentazocine (3 mg/kg, i.p) where as 200 mg/kg (Group III and VI) and 400 mg/kg group (V and VI) were treated orally with aqueous and ethanolic extract of *M.laxiflora* Robyns respectively.

Group I: Normal control (CMC), Group II : Standard (Pentazocine 3 mg/kg), Group III: Test Drug I (Aqueous seed extract of *M.laxiflora* Robyns 200 mg/kg), Group IV: Test Drug II (Aqueous seed extract of *M.laxiflora* Robyns 400 mg/kg*),* Group V: Test Drug I(Ethanolic seed extract of *M.laxiflora* Robyns 200 mg/kg), Group VI: Test Drug II (Ethanolic seed extract of *M.laxiflora* Robyns 400 mg/kg*)*. The reaction time of the groups was taken at 0, 30, 60, 90 and 120min. The cut off time of the immersion was 15seconds. The reaction time was measured [11,15].

Acetic acid induced writhing response in mice

This method was used to preferentially evaluate possible peripheral analgesic effects of aqueous and ethanolic extract of *Meyna laxiflora* Robyns.. Four groups of Swiss albino male mice (n=6) were fasted overnight prior to start the experiment with free access to water. The peripheral analgesic drug Diclofenac sodium (10 mg/kg) was used as a positive control. Group-I Normal Control received CMC (0.5%) Group-II was treated with Diclofenac Sodium (10mg/kg), where as 200 mg/kg (Group III and VI) and 400 mg/kg group (V and VI) were treated orally with aqueous and ethanolic extract of *M.laxiflora* Robyns respectively.

Group I: Normal control (CMC),Group II : Standard (Pentazocine 3 mg/kg), Group III: Test Drug I (Aqueous seed extract of *M.laxiflora* Robyns 200 mg/kg), Group IV: Test Drug II (Aqueous seed extract of *M.laxiflora* Robyns 400 mg/kg), Group V: Test Drug I(Ethanolic seed extract of *M.laxiflora* Robyns 200 mg/kg), Group VI: Test Drug II (Ethanolic seed extract of *M.laxiflora* Robyns 400 mg/kg). After 30 min of treatment, the mice were injected intra peritoneally with 0.1 ml of 1% acetic acid solution to induce the characteristic writhing. The mice were then placed in an observation box and the numbers of writhing were counted in a 5min period. The response of the extract and Diclofenac sodium treated groups was compared with those of animals in the control group[16,17].

Anti-pyretic activity

The estimation of anti-pyretic efficacy of aqueous and ethanolic extract was carried out using brewer's yeast induced pyrexia method. Fever was induced by means of subcutaneously injecting 10.0 ml/kg of a 20% w/v suspension of brewer's yeast in normal saline. Only animals whose rectal increased by at least 1.0º C after subcutaneously injecting 10.0 ml/kg of a 20% w/v suspension of brewer's yeast in normal saline. Only animals whose rectal temperature increased by at least 1.0ºC after 18 h of this yeast injection were included for the study. The normal rectal temperature of each animal was measured by using a flexible tail thermostat probe coated with lubricant, and temperature was recorded using a digital tele thermometer. The experimental animals were randomly divided into four groups containing six animals in each group. The control group (I) was orally administered 0.5ml saline while the standard group (II) was given 150 mg/kg Paracetamol and where as 200 mg/kg (Group III and VI) and 400 mg/kg group (V and VI) were treated orally with aqueous and ethanolic extract of *M.laxiflora* Robyns respectively[11,15].

Group I: Normal control (CMC), Group II: Standard (Pentazocine 3 mg/kg), Group III: Test Drug I (Aqueous seed extract of *M.laxiflora* Robyns 200 mg/kg), Group IV: Test Drug II (Aqueous seed extract of *M.laxiflora* Robyns 400 mg/kg), Group V :Test Drug I (Ethanolic seed extract of *M.laxiflora* Robyns 200 mg/kg), Group VI :Test Drug II (Ethanolic seed extract of *M.laxiflora* Robyns 400 mg/kg). The rectal temperature was recorded at time intervals of 1, 2, 3and 4 h after drug administration. Animals are rehabilitated with standards anti-pyretic drugs.

Anti-inflammatory activity

Carrageenan-induced paw edema in rats

For this experiment, the rats (120-150 g) were divided into four groups (n=6). The group I received 0.5% CMC (10 ml/kg), while the Group received Indomethacin (10 mg/kg). Whereas 200 mg/kg (Group III and VI) and 400 mg/kg group (V and VI) were treated orally with aqueous and ethanolic extract of *M.laxiflora* Robyns respectively.

Group I: Normal control (CMC), Group II: Standard (Pentazocine 3 mg/kg), Group III: Test Drug I (Aqueous seed extract of *M.laxiflora* Robyns 200 mg/kg), Group IV: Test Drug II (Aqueous seed extract of *M.laxiflora* Robyns 400 mg/kg*),* Group V: Test Drug I (Ethanolic seed extract of *M.laxiflora* Robyns 200 mg/kg), Group VI: Test Drug II (Ethanolic seed extract of *M.laxiflora* Robyns 400 mg/kg). Acute inflammation was produced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the sub planter region of the right hind paw of the rats. The animals were pre treated

with the drug 1hour before the administration of carrageenan [18].The paw thickness was measured at 1, 2, 3 and 4 h after carrageenan injection by using digital vernier calipers.

Cotton pellet induced granuloma method in rats

Cotton pellets, weighing 5 mg each were sterilized. Under ether an aesthesia, the pellets were introduced subcutaneously through a skin incision on the back of the animals. Starting from 30 min after the implantation of cotton pellet for all the rats, Group-I normal control received CMC (0.5%) orally. Group-II was treated with Dexamethasone (1 mg/kg), where as 200 mg/kg (Group III and VI) and 400 mg/kg group (V and VI) were treated orally with aqueous and ethanolic extract of *M.laxiflora* Robyns respectively [19,20].

Group I: Normal control (CMC), Group II : Standard (Pentazocine 3 mg/kg), Group III: Test Drug I (Aqueous seed extract of *M.laxiflora* Robyns 200 mg/kg), Group IV: Test Drug II (Aqueous seed extract of *M.laxiflora* Robyns 400 mg/kg), Group V :Test Drug I (Ethanolic seed extract of *M.laxiflora* Robyns 200 mg/kg), Group VI :Test Drug II (Ethanolic seed extract of *M.laxiflora* Robyns 400 mg/kg). The test drugs were administered daily for 7days. On the 8th day, the animals were sacrificed with diethyl ether. The granulomas were removed and the weighed.

STATISTICAL ANALYSIS

All the values estimations were expressed as mean ± standard error of mean and was analyzed for significance by ANOVA and groups were compared by Turkey-Kramer multiple comparison test, using In Stat v. 2.02 software (Graph Pad Software Inc.). Differences between groups (p Value) were considered significant at P <0.05 level.

RESULT

Acute Toxicity Study

LD50 of *M.laxiflora* Robyns was done as per OECD guidelines (Revised graft 423). The drug *M.laxiflora* Robyns falls under class 4 (LD50>2000 mg/kg). The animal did not show any signs of acute toxicity and behavioral changes.

Analgesic activity
Hot plate Method in Mice

The analgesic activity of aqueous and ethanolic seeds extract of *M.laxiflora* Robyns was assessed using hot plate method in Swiss albino mice. The aqueous and ethanolic seeds extract of *M.laxiflora* Robyns showed significant analgesic activity at 200 and 400 mg/kg. Analgesic activity was comparable with standard drug pentazocine. Among the two doses, 400 mg/kg showed maximum analgesic activity at reaction time 120 min (7.2±0.44) is slightly lower than the standard drug pentazocine (9.9±0.34) in this analgesic testing model, pentazocine significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally active drugs. In the present study, all extracts showed significant ($p<0.05$ and $p< 0.01$) analgesic activity but among the two doses, 400 mg/kg showed highest analgesic activity at reaction time120 min. (Fig:1)

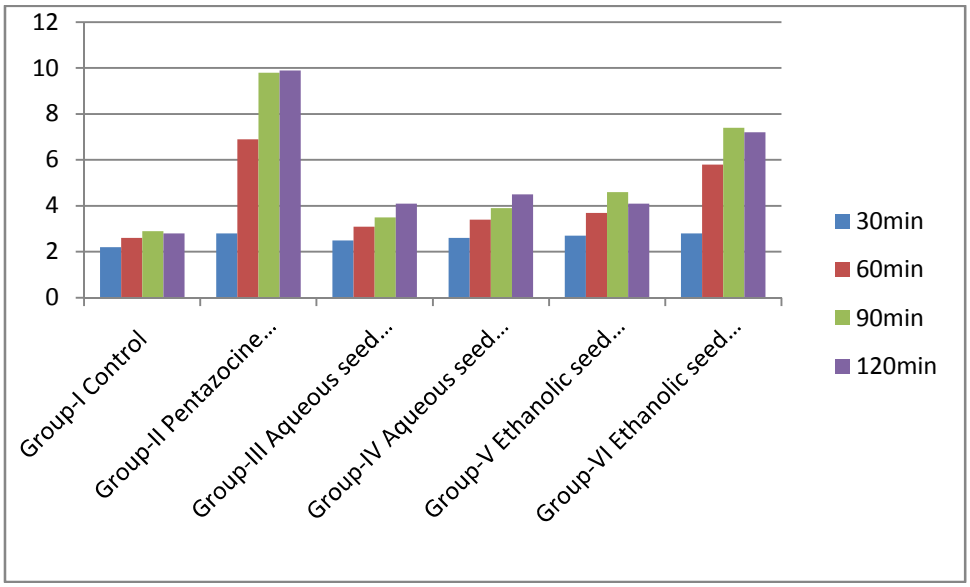

Fig 1: Analgesic effect of aqueous and ethanolic seeds extract of *M.laxiflora* Robyns on hot plate method in mice.

Tail Immersion Method

There was a significant reduction of pain full sensation due to tail immersion in warm water. The maximum inhibitory effect of ethanolic seeds extract of *M.laxiflora* Robyns showed significant ($p< 0.01$) at 90 min post dose in 400 mg/kg. The maximum anti- nociceptive properties of the plant extract (3.5±0.04) were not as effective as that of pentazocine 3 mg/kg (5.8±0.06). (Table:1)

Table: 1 Analgesic effect of aqueous and ethanolic seeds extract of *M.laxiflora* Robyns *on tail immersion method in rats*

GROUP	Mean latency to tail immersion in seconds			
	30min	**60min**	**90min**	**120min**
Group-I Control	1.5±0.04	1.4±0.02	1.6±0.01	1.7±0.04
Group-II Pentazocine (3mg/kg)	1.8±0.06	2.6±0.04**	4.2±0.02**	5.8±0.06**
Group-III Aqueous seed extract (200mg/kg)	1.1±0.25	1.4±0.11*	1.5±0.02*	1.7±0.15**
Group-IV Aqueous seed extract (400mg/kg)	1.6±0.28	1.9±0.13*	2.2±0.33**	2.5±0.41**
Group-V Ethanolic seed extract (200mg/kg)	1.2±0.02	1.9±0.01*	2.1±0.04*	2.4±0.02
Group-VI Ethanolic seed extract (400mg/kg)	1.4±0.01	2.0±0.04*	2.6±0.01**	3.5±0.04**

Values were mean ± SEM,(n=6), *P<0.05 **P<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

Acetic Acid- Induced Writhing Response in Mice

The oral administration of aqueous and ethanolic seeds extracts *of M.laxiflora* Robyns showed a dose dependent analgesic activity. Injection of acetic acid into control mice produced 51.4±6.4 writhes. Pre-treatment with aqueous and ethanolic extract, in this ethanolic extract significant effect of *M.laxiflora* Robyns at doses of 200 and 400 mg/kg reduced the number of writhes 39.4±2.4 (23.34 % protection) and 31.2±2.1 (39.29 % protection) respectively. Among the two doses 200, 400 mg/kg ethanolic extract showed the slightly lower analgesic activity than standard drug Diclofenac Sodium 22.8±1.9 (55.64 % protection) it was observed that the onset of writhing was delayed and duration of writhing was shortened. (Fig:2).

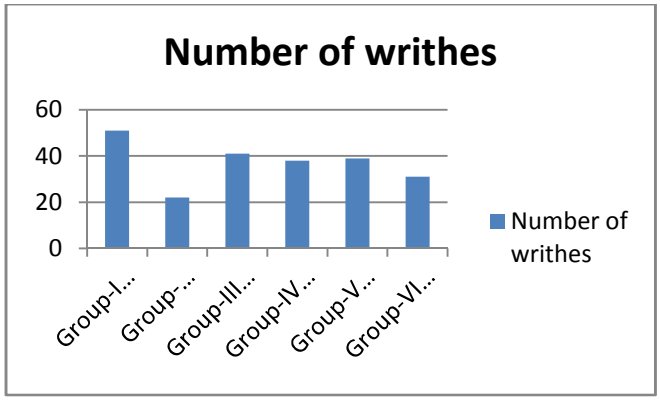

Fig 2: Analgesic effect of aqueous and ethanolic seeds extract of *Meyna laxiflora* Robyns on acetic acid induced writhing response in mice.

Anti-pyretic activity

Brewer's Yeast Induced Pyrexia in Rats

The anti-pyretic activity of aqueous and ethanol seeds extract of *M.laxiflora* Robyns against yeast induced pyrexia is shown in Table 6.The ethanolic seed extract of *M.laxiflora* Robyns at a doses of 200 and 400 mg/kg showed significant effect against Brewer's yeast induced pyrexia method. There was a progressive dose dependent reduction in the temperature of rats treated with the extract. The reduction caused by the extract was significant when compared to control. (Table:2)

Table:2 Anti-Pyretic Activity of aqueous and ethanolic Extract of *M.laxiflora* Robyns on Brewer's Yeast Induced Pyrexia in Rats

GROUP	18 h after yeast administration	Rectal temperature (°C)			
		Temperature after treatment			
		1h	**2h**	**3h**	**4h**
Group-I Control	38.1±0.1	38.4±0.2	38.0±0.1	38.3±0.2	38.2±0.1
Group-II Standard Paracetamol (150mg/kg)	40.2±0.1	40.4±0.3**	40.1±0.2	39.8±0.1	39.1±0.3
Group-III Aqueous seed extract (200mg/kg)	38.1±0.25	38.2±0.23	38.4±0.15	38.5±0.22	38.7±0.15
Group-IV Aqueous seed extract (400mg/kg)	38.6±0.28	38.4±0.2	38.4±0.32	38.2±0.32	38.3±0.25
Group-V Ethanolic seed extract (200mg/kg)	40.2±0.1	39.4±0.3	39.1±0.1	39.0±0.2	38.9±0.3
Group-VI Ethanolic seed extract (400mg/kg)	40.1±0.1	38.8±0.1	38.5±0.2	38.4±0.1	38.5±0.2

Values were mean ± SEM, (n=6), ***P*<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dennett's test.

Anti-Inflammatory Activity

Carrageenan-Inuced Paw Edema in Rats

The anti-inflammatory effect of the aqueous and ethanol seeds extract of *Meyna laxiflora* Robyns on carrageenan–induced hind paws edema as shown in Fig 4. The ethanolic seed extract of *M.laxiflora* Robyns at doses 200 and 400 mg/kg produced a significant effect against carrageen an induced inflammatory effect. The dose of 400 mg/kg exhibited a significant inhibition of 48 % after 3 h, the effect increased after 3h (52%). Anti-inflammatory activity of ethanolic extract of *M.laxiflora* Robyns showed significant and similar to that of indomethacine (10 mg/kg).

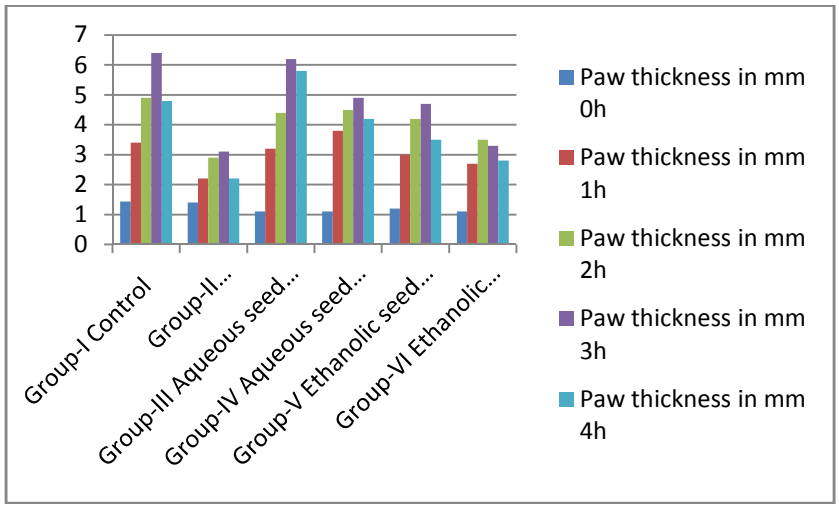

Fig 3:Anti inflammatory activity of aqueous and ethanolic extract of *M.laxiflora* Robyns on Carrageenan induced paw edema method in Wistar rats.

Cotton Pellet-Induced Granuloma Method in Rats

The anti-inflammatory effect of the aqueous and ethanol seeds extract of *M.laxiflora* Robyns assessed by using cotton pellet induced granuloma method in Wistar rats. The ethanolic seed extracts of *M.laxiflora* Robyns showed significant anti-inflammatory activity at200 and 400 mg/kg dose. After 7 days, the mean weight of granulomatous tissue surrounding the threads was significantly lower for the group treated with *M.laxiflora* Robyns extract as compared to the control group. Among the two doses 400 mg/kg showed maximum decreased formation of granuloma tissue. The results indicate that *M.laxiflora* Robyns at dose level of 200 mg/kg and 400 mg/kg produced a significant decrease in the weight of granuloma 38.16±0.04 (7.4% inhibition) and 34.58±0.04 (16.1% inhibition) respectively. Among the two dose 400 mg/kg showed the slightly lower reduced weight of granumola than standard drug dexamethazone 28.92±0.04 (29.8% inhibition).

Table 3: Anti inflammatory activity of aqueous and ethanolic extract of *Meyna laxiflora* Robyns cotton pellet induced granuloma pouch model Wistar rats

Treatments	Granuloma weight (mg)	% Inhibition
Group-I Control	41.24±0.04	___
Group-II Dexamethazone (1mg/kg)	28.92±0.04**	29.8
Group-III Aqueous seed extract (200mg/kg)	41.1±0.25	___
Group-IV Aqueous seed extract (400mg/kg)	39.6±0.28	5.2
Group-V Ethanolic seed extract (200mg/kg)	38.16±0.04**	7.4
Group-VI Ethanolic seed extract (400mg/kg)	34.58±0.04**	16.1

Values were mean ± SEM, (n=6), ***P*<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dennett's test.

DISCUSSION

The inflammation is complex process, which is frequently associated with pain and involves several events, such as the increase of muscular permeability, increase of granulocytes and mono nuclear cell migration, as well as the granulomatous tissue proliferation[21]. Pain is subjective experience, which is difficult to define exactly even though we all experience it. Pain distinguished as two types, peripheral or neurogenic pain may involve the following pathological states.

The hot plate model was selected to investigate central antinociceptive activity because it has several advantages particularly the sensitivity to strong antinociceptive and

limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibits prostaglandin synthesis[22]. A number of phenolic compounds have been reported to produce analgesic activity. Other studies have demonstrated that various flavanoids such as rutin, quercetin, luteolin, biflavonoids and triterpenoids produced significant antinociceptive effect. As phytochemical test showed presence of flavonoids and tannins in ethanolic extract of *M.laxiflora* Robyns they might suppress the formation of prostaglandin and bradykinins.

Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing respons[23]. The effect of the extract against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting anti-nociceptives. This response is thought to involve local peritoneal receptors. This result indicates that the analgesic effect of ethanolic extract of *M.laxiflora* Robyns might be mediated by inhibiting the synthesis or action of prostaglandins. The centrally acting analgesic activity of the extract was also corroborated in our study by tail immersion test results. The fact that in thermal stimuli (hot plate & Tail immersion tests), the anti nociceptive effect should be shown by acting centrally on opioid receptors. Since the drugs had shown the analgesic activity in tail immersion test, it seems that the ethanolic extract can act centrally. Taking this in to consideration the ethanolic extract of *M.laxiflora Robyns* ,posses peripheral and central analgesic properties.

The ethanolic extract of *M.laxiflora* Robyns showed anti-inflammatory activity on an acute inflammatory process like in carrageen an induced paw edema in rats paw. It is well known that leukocytes migration to the injured tissues in an important aspect of the inflammatory process. Histamine and serotonin are responsible for the immediate inflammatory response, whereas kinins and prostaglandins mediate prolonged response. Anti-inflammatory activity of many plants has been attributed to their high sterol / triterpene or flavonoids content. The anti-inflammatory effect of ethanolic extract of *M.laxiflora* Robyns in rats with carrageen an-induced paw was significant[24].

It is known that the inflammatory granuloma is a typical response of a chronic inflammatory process and it has been established that the weight of the pellets is well correlated with the granulomatous tissue. The chronic inflammation occurs by means of the development of prolifereative cells. These cells can be either spread or in granuloma form. The *M.laxiflora* Robyns extract showed significant anti-inflammtory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions. It reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide during granuloma tissue formation.

Brewer's yeast was used to induce fever in albino rats. Fever was recorded 18 hrs after yeast injection since yeast takes a total of about 18 hrs to cause the elevation of body temperature. Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect. Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibitions of these mediators are responsible for the antipyretic effect.

The oral administration of *M.laxiflora* Robyns significantly attenuated rectal temperature of yeast induced albino rats. Thus it can be postulated that *M.laxiflora* Robyns contains pharmacologically active principles that interfere with the release of prostaglandins. After three hours of the test period, the ethanolic leaves extract of *M.laxiflora* Robyns produced appreciable antipyretic activity against brewer's yeast induced pyrexia in albino rat. It was revealed that the extract showed dose dependent antipyretic activity.

CONCLUSION

The *M.laxiflora* Robyns has shown a significant anti-inflammatory, anti- pyretic and analgesic effects. These effects maybe because of the presence of phytochemicals such as flavonoids, tannins and terpenoids present in the plant extract. The Present study showed that the ethanolic leaves extract of *M.laxiflora* Robyns posses peripheral and central analgesic activity inanimal model than that of aqueous extract. The *M.laxiflora* Robyns leaves extract shows anti-pyretic activity in animal model in rats and *M.laxiflora* Robyns showed anti-inflammatory activity in different animal model. Flavonoids and tannins are the major constituents of *M.laxiflora* Robyns leaves, which may be responsible for its Analgesic, Anti-pyretic and Anti-inflammatory activity. Further detailed study on *Meyna laxiflora* Robyns using different phlogestic agents in this area will enable us to understand the mechanism of action underline the above mentioned activity.

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