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Research Article

ANTINOCICEPTIVE AND ANTIPYRETIC EFFECTS OF HERBAL PREPARATION, MOBILAX - A REVERSE PHARMACOLOGICAL APPROACH FOR ARTHRITICAL PAIN

Dutt HK[1], Lata S[2], Saxena KK[3], Singh G[4]

[1]Assistant Professor, Dept. of Pharmacology, Govt. Medical College, Haldwani, Nainital-263139, Uttarakhand, India
[2] Professor & Head, Dept. of Pharmacology, Muzaffarnagar Medical College, Muzaffarnagar-251203, Uttar Pradesh, India
[3] Professor & Head, Dept. of Pharmacology, Lala Lajpat Rai Memorial Medical College and S.V.B.P. Hospital, Meerut-250004, Uttar Pradesh, India
[4] Lecturer, Statistics, Dept. of Obstetrics & Gynaecology, Lala Lajpat Rai Memorial Medical College and S.V.B.P. Hospital, Meerut-250004, Uttar Pradesh, India

ABSTRACT

Rheumatoid arthritis is a chronic multi-system disease of unknown cause. It affects the people in their prime of life, predominantly between the ages of 20-50 years with an unpredictable course. Pain in affected joints, aggravated by movement, is the most common manifestation of established RA. In many parts 'little traditions' of indigenous systems of medicine have yet their role in chronic diseases including various types of arthritis. Thus, drug discovery based on Ayurveda follows a 'Reverse Pharmacology' path from clinics to laboratories. Mobilax is used in the Ayurvedic system of medicine for the treatment of inflammation and pain associated with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and chronic backache. The present study was aimed to evaluate antipyretic and analgesic efficacy of mobilax using respectively pyrexia and pain animal models. Antipyretic activity by Brewer's Yeast induced pyrexia and analgesic activity by Tail flick method, Writhing test and Eddy's hot plate method. Pyrexia model showed antipyretic activity of mobilax 400mgkg^{-1}b.w. which was compared to Paracetamol 150mgkg^{-1}b.w. The pain inducing model showed good analgesic activity at all graded doses of mobilax 100, 200 & 400mgkg^{-1}b. w. compared to respective standard analgesic drugs according to respective pain models. The results indicate that the formulation mobilax possesses low antipyretic and good analgesic activities in the experimental animal models.

Keywords: Antinociceptive; Antipyretic; Arthritis; Reverse Pharmacology; Rheumatoid arthritis.

INTRODUCTION

Rheumatoid Arthritis is the most distressing and disabling syndrome which is encountered in medical practice which has also been called the "great crippler" and "the king of human miseries". The exact pathogenesis is not clear, but the inflammatory process is one which is shared in common. Pain, swelling, and tenderness may initially be poorly localized to the joints. Initially, impairment in physical function is caused by pain and inflammation, and disability owing to this is a frequent early feature of aggressive RA. Pain originates predominantly from the joint capsule, which is abundantly supplied with pain fibers and is markedly sensitive to stretching or distention. For this reason, the majority of drugs, preferred for treatment are anti-inflammatory drugs having good analgesic activity. The indigenous systems of medicine present various therapeutic agents which need careful scrutiny and scientific zeal to study and evaluate their present-day status in therapy. The advances in medicine are significant and many diseases hitherto not amenable to treatment have now started to be responsive. The modern scientific world today is ready to accept in its armamentarium these tried drugs.

Corresponding Author:- **Hemant Kumar Dutt** Email:- dr.hemantkdutt@yahoo.co.in

There are some drugs from the Ayurvedic stream which have a large potential as anti-inflammatory, analgesic and antipyretic action at the same time they are also claimed to be free from side effects which limit the use of the above mentioned conventional allopathic stream drugs. There are many such ayurvedic herbal preparations available in the market which may have a solo constituent or may be mixtures of the few which may show promising effects in inflammatory conditions.

Recently, India has amended the Drug Act to include a category of phytopharmaceuticals to be developed from medicinal plants by Reverse Pharmacology, with evidence of quality, safety and efficacy. Mobilax is an ayurvedic formulation containing various herbs and mineral. Its main ingredients are (mg): Mahayograj Guggal (*Commiphora mukul*) 200, Maharasnadi Quath 80, Nirgundi (*Vitex negundi*) 50, Rasna (*Pluchea lanceolata*) 50, Erandmool (Riccinus cummunis) 50, Ajmoda(*Apium graveolens)* 50, Punarnava (*Boerhaavia diffusa Linn)* 50, Sigru (*Moringa oleifera*) 20 and Shudh Shilajeet 20. Its constituents have been reported to have an antipyretic, antinociceptive, antioxidant and antiulcerogenic activity, [1], [2], [3], [4]. The present study was undertaken to confirm the anti-pyretic and antinociceptive potential of mobilax in respective models and to compare with standard agents.

MATERIALS AND METHODS

The present study was conducted on adult mice (20-35 g) and adult rats (100-150 gm) of either sex in the Department of Pharmacology, LLRM Medical College, Meerut for antipyretic (assessed in rats) and antinociceptive activity (Tail flick method assessed on rats and Hot plate method and Writhing test assessed in mice). The rats and mice were acclimatized to laboratory condition for 15 days before commencement of the experiment. The animals (six per cage) were maintained under standard laboratory conditions (light period of 12 h/day and at room temperature), with access to commercial pellet diets and water ad libitum. Food was withdrawn 12 h before and during the experimental hours.

After the approval by the Institutional Animal Ethical Committee, an experimental study was undertaken according to their rules and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals [5].

Acute Toxicity studies

The acute toxicity of Mobilax was determined by using female albino mice (20-35g). The animals were fasted 3 hours prior to the experiment according to the Organization for Economic Co-operation and Development (OECD) guideline no. 425, up and down procedure [6]. Animals were administered with a single dose of extract and observed for its mortality for 48 h. Based on the drug short term profile the dose of the next animals were determined as per as OECD guideline 425. All the animals were observed for long term toxicity (14 days).

Drugs – Mobilax (S.B.Pharmaceutical Laboratories, Cochin), Solar Dry Active Yeast (Solar Sales, Delhi), Acetic acid glacial (BTL Research Lab, Vadodra), Paracetamol (Crocin, Remidex Pharma Pvt. Ltd),Aspirin (Disprin, Reckitt Benckiser Ltd), Tramadol (Taridol-50, Starry health care Pvt Ltd), Digital Thermometer, Normal Saline (Albert David Ltd).

Following standard methods were used to measure antipyretic and analgesic activities

In all the models animals were divided into five groups. Group I acted as control which received vehicle (Normal Saline 5mlkg^{-1} b.w., po). Group II, III, IV and V acted as study groups. Group II received standard agents, per oral and Group III, IV and V were treated with graded doses of Mobilax 100, 200 and 400 mgkg^{-1} b.w., per oral respectively.

Anti-pyretic study

Brewer's yeast induced pyrexia was measured by clinical thermometer before and after, at every 30min of interval for the period of 2hrs of oral administration of drugs by the method of Gujral et al. [7] The standard anti-pyretic drug used was Paracetamol (150mgkg^{-1} b.w., po).

Anti-nociceptive studies

(1) Glacial acetic acid (GAA) - induced writhing [8] 0.6% GAA solution was injected (ip) and following this typical abdominal writhing response was observed. After 5min following GAA injection, the number of writhes over a period of 10 min was counted. The standard antinociceptive drug used was Aspirin (100mgkg^{-1} b.w., po).

(2) Tail flick response - Analgesic effect was studied by Tail flick method of D'Amour and Smith [12] modified by Kulkarni et al [9] for inducing pain by heat stimulus in which tail flick latency was ranged between 6.20 - 8.10 Sec. The standard antinociceptive drug used was Tramadol (5mgkg^{-1} b.w., po).

(3) Hind paw licking/Jumping response - Analgesic effect was studied by the hot plate method of Hosseinzadeh et al. [10] for inducing pain by a heat stimulus in which hind paw licking/jumping latency was ranged between 5.09 - 6.45 Sec. The standard antinociceptive drug used was Tramadol (5mg kg^{-1} b.w., po).

Results are expressed as mean ± SEM. Statistical differences between the control and the treated groups were tested by one way analysis of variance (ANOVA) followed by Dunnett's post hoc multiple comparisons. p values were calculated referring to the appropriate tables. Values of $P<0.05$ were considered as statistically significant.

RESULTS

Acute Toxicity studies

In the acute toxicity assay no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsions, ataxia, diarrhoea or diuresis. The median lethal dose (LD_{50}) of mobilax was determined to be higher than the highest dose tested i.e., 2.0 gkg-1 b.w. and 1/5th , 1/10th and 1/20th of this dose was used for evaluation of antipyretic and anti-nociceptive effect.

Antipyretic activity of mobilax in animal model is shown in [Table - 1]

Table 1. EFFECT OF MOBILAX AND PARACETAMOL ON BREWER'S YEAST INDUCED PYREXIA IN ALBINO RATS

Drug	Dose (mg/kg, oral	Temperature °C±SEM					
		Initial	Pyretic	30 min	60 min	90 min	120 min
Saline	5 ml	38.11±0.09	39.48±0.11	39.65±0.09	39.85±0.09	39.96±0.09	40.03±0.09
Paracetamol	150	38.08±0.15	39.53±0.15	37.93±0.15**	38.13±0.15**	38.18±0.14**	38.11±0.15**
Mobilax	100	38.10±0.08	39.50±0.13	39.50±0.09	39.65±0.09	39.70±0.08	39.80±0.05
Mobilax	200	38.05±0.16	39.46±0.16	39.43±0.09	39.58±0.10	39.66±0.09	39.70±0.08
Mobilax	400	38.25±0.15	39.73±0.13	39.43±0.09	39.45±0.08	39.43±0.08*	39.40±0.05**

Values are mean± SEM (n=6), *p<0.05, **p<0.01 (p value as compared to control group)

Table 2. EFFECT OF MOBILAX AND TRAMADOL ON TAIL FLICK METHOD IN ALBINO RATS

Drug	Dose (mg/kg, oral)	Reaction time (seconds) ± SEM			
		Before treatment	After treatment		
			30 min	60 min	90 min
Saline	5 ml	7.12±0.24	6.98±0.18	7.25±0.18	7.20±0.19
Tramadol	5	6.85±0.28	17.13±0.31*	23.32±0.30*	>30**
Mobilax	100	7.64±0.21	11.65±0.30*	19.53±0.34*	28.20±0.31*
Mobilax	200	7.23±0.26	17.07±0.31*	25.69±0.33*	>30**
Mobilax	400	7.24±0.25	21.43±0.27*	29.19±0.19*	>30**

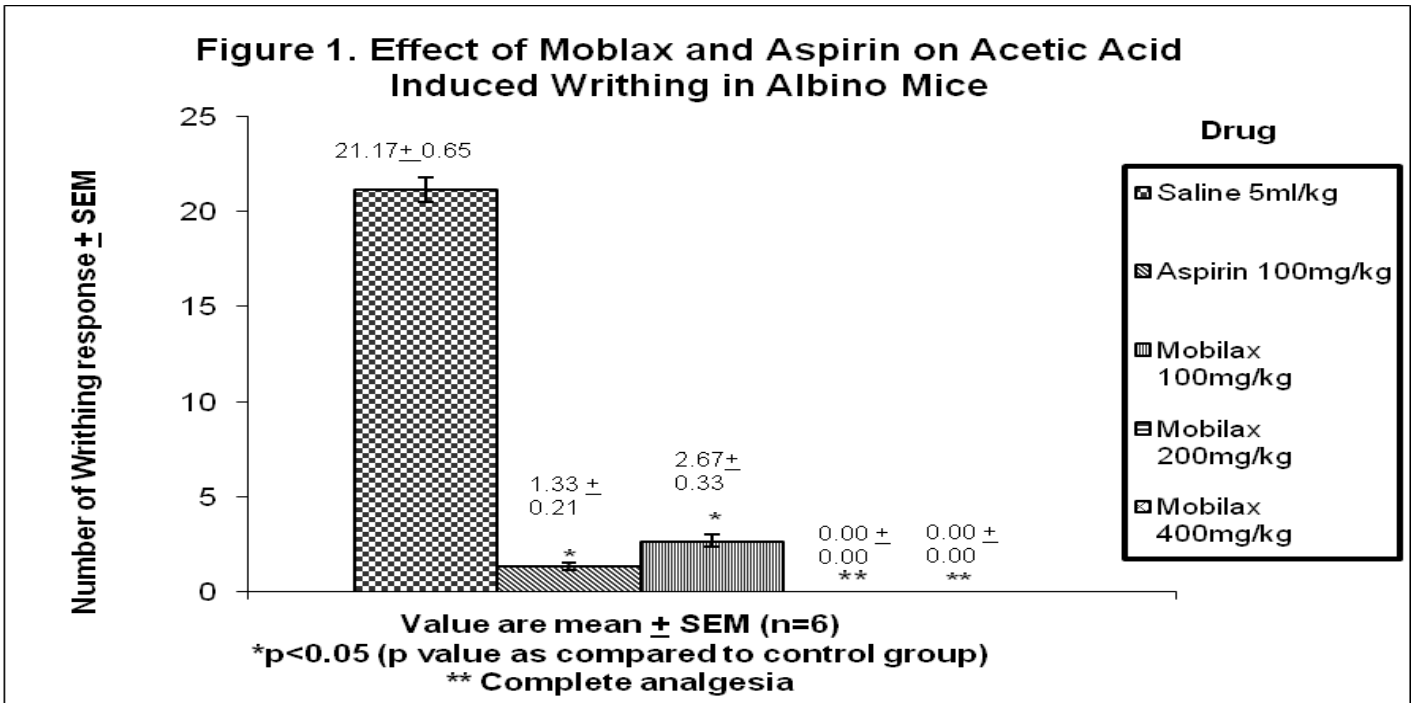
Values are mean± SEM (n=6), *p<0.05 (p value as compared to control group),**Complete analgesia

Table 3. EFFECT OF MOBILAX AND TRAMADOL ON HOT PLATE METHOD IN ALBINO MICE

Drug	Dose (mg/kg, oral)	Reaction time (seconds) ± SEM			
		Before treatment	After treatment		
			30 min	60 min	90 min
Saline	5 ml	5.86±0.17	5.79±0.22	5.72±0.18	5.83±0.16
Tramadol	5	5.88±0.13	8.80±0.31*	11.30±0.26*	>15**
Mobilax	100	5.86±0.15	6.46±0.17*	8.27±0.17*	9.11±0.17*
Mobilax	200	5.81±0.13	7.38±0.21*	9.49±0.18*	11.38±0.24*
Mobilax	400	5.86±0.15	8.97±0.22*	12.11±0.18*	>15**

Values are mean± SEM (n=6), *p<0.05 (p value as compared to control group),**Complete analgesia

Figure 1. Effect of Mobilax and Aspirin on Acetic acid induced writhing in albino mice



DISCUSSION

Mobilax is a polyherbal ayurvedic preparation which is formulated as per the ayurvedic system of medicine and claimed to be effective in the treatment of various types of arthritis. A preliminary pharmacological study of this polyherbal formulation, with special emphasis on exploring the presence of anti-pyretic and antinociceptive activity in it and to compare with respective standard agents, was therefore taken up.

Using Organization for Economic Co-operation and Development (OECD) guideline No. 425 [6], acute oral toxicity assay, the median lethal dose, LD_{50} was determined to be higher than 2.0gkg^{-1} b.w. These results indicate the relative safety of mobilax for the treatment of conditions associated with inflammation.

Moringinine and alkaloids are responsible for the antipyretic effect of the sigru (*Moringa oleifera)* seeds extract [11]. In the present study the effects of various concentrations of mobilax, with that of paracetamol at different times are compared. This anti-pyretic effect appears to be dose dependent as 100mgkg^{-1} b.w. and 200mgkg^{-1} b.w. dose did not produce any pyrexia lowering effect but at the 400mgkg^{-1} b.w. dose showed the anti-pyretic effect on and after 90 min, comparable to that of paracetamol (standard drug) and was then sustained during the whole observation period (Table 1). This may be due to ineffective content of sigru which amounts to be 14mg/kg approximately in 400mgkg^{-1} b.w. dose of mobilax while Sutar et al., 2009 [1] found out the effective antipyretic dose of sigru in rats to be 100mgkg^{-1} b.w.

The thermal stimuli in tail flick method & hot plate method and the writhing response of the animals to an intra-peritoneal injection of noxious chemical such as acetic acid is used to screen both centrally and peripherally acting analgesic activity respectively. The tail Flick method is predominantly a spinal response and Hot Plate method are predominantly supraspinal [12]. Acetic acid induced writhing is used to evaluate drugs acting on pain produced by inflammation and local irritation which involves the release of mediators of inflammations like prostaglandins, histamine, serotonin, substance P etc. i.e. acting through the peripheral level with exciting the pain nerve endings [13]. From the results it is apparent that mobilax showed a significant anti-nociceptive effect in tail flick method (Table 2) and writhing response (Figure 1) at all three doses of mobilax (100, 200, 400mgkg^{-1} b.w.) which are comparable to that of the standard while in hot plate method (Table 3), mobilax in the dose of 100mgkg^{-1} b.w. didn't show a significant effect at 30min but at 60min and thereafter showed significant effect during the whole observation period. Studies demonstrate that various flavonoids such as rutin, quercetin, luteolin, hesperidin and biflavonoids produce significant antinociceptive and anti-inflammatory

activities [14]. NSAIDs can inhibit cyclo-oxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors [15]. The mechanisms of anti-nociceptive action of mobilax could be due to the presence of flavonoids and may be mediated through central and peripheral mechanisms. The other constituents of mobilax: rasna (*Pluchea lanceolata),* nirgundi *(Vitex negundo)* and erandmool *(Ricinus communis)* possess analgesic effect and also are effective in both acute and chronic inflammation [16,17]. Mobilax in the dose of 100, 200 and 400mgkg^{-1} b.w., significantly increased the reaction time in tail flick & the hot-plate test and also reduced the writhing response in mice injected with acetic acid. Hence, it is speculated that apart from inhibition of chemical mediators of inflammation, mobilax may also modulate the pain response centrally.

Although the result of present pharmacological screening can be thought of establishing the antipyretic and antinociceptive activity at some or the other doses, the exact mechanism of action needs further exploration. However, if the pure active principle could be isolated and evaluated, the constituents of mobilax may be used more rationally in different conditions of inflammatory disorders.

CONCLUSION

Arthritis is the most distressing and disabling syndrome which is encountered in medical practice. This has been called the “great crippler” and “the king of human miseries”. India with its wealth and variety of medicinal herbs are available in their different combinations through the ages, a great mass of popular remedies, many of which are in common use even today and among one of the them is mobilax. However, perusal of literature reveals that no planned and systemic efforts have been done to confirm the presence of such activity in mobilax. Therefore, the present study was conducted on mobilax.

The present study was, therefore, designed to ascertain the antipyretic and analgesic properties of mobilax in rats and mice by oral route. The antipyretic effect was significant in the dose of 400mg/kg when compared to reference standard drug Paracetamol (150mgkg^{-1} b.w.). Mobilax (100mgkg^{-1} b.w.) produced significant analgesic effect on tail flick and insignificant in hot plate method at 30min while at 400mg/kg was comparable to Tramadol (5mgkg^{-1} b.w.). On Acetic Acid induced writhing test mobilax produced significant analgesic effect at a dose of 100mgkg^{-1} b.w. which was comparable to aspirin (100mgkg^{-1} b.w.).

The mobilax comprises antipyretic and anti-nociceptive activities. It is worthwhile to isolate the bioactive principles, which are responsible for these activities, which is in process. However, further studies are essential to elucidate the detailed and other possible mechanisms of action for antipyretic and antinociceptive activities.

To conclude, the poly-herbal formulation 'mobilax' possess good analgesic.

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