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Experimental Evaluation of Analgesic and Anti-Inflammatory Potential of *Urai mathirai* – A Siddha Formulation

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ABSTRACT

Aim: The aim of the present study was to evaluate the analgesic and anti-inflammatory activities of *Urai mathirai*, a siddha formulation in experimentally induced pain and inflammation models in rats. **Materials and Methods**: Analgesic activity of *Urai mathirai* was assessed in tail flick model in rats and Anti-inflammatory activity of *Urai mathirai* was evaluated using carrageenan induced paw edema in rats. Diclofenac was used as standard drug in both experiments. **Results**: In analgesic activity of *Urai mathirai*, the reaction time was recorded at all doses and when compared with normal control and diclofenac treated animals, a significant increase in the reaction time in all the *Urai mathirai* treated groups was observed. For anti-inflammatory activity of *Urai mathirai* administration of carrageenan alone have shown a significant increase in paw volume whereas the animals treated with diclofenac and *Urai mathirai* have shown a significant decrease in paw volume at all doses compared to carrageenan control group and the effect was good at 50 mg/kg dose. **Conclusion**: The results of the study reveal that *Urai mathirai* has significant analgesic and anti-inflammatory activities. Further investigations are warranted in other models of pain and inflammation, which may substantiate its role in alleviating pain and inflammatory disorders.

INTRODUCTION:

Siddha medicine, traditional system of healing that originated in South India and is marked to be one of India's oldest systems of medicine. Siddha medicine is of Dravidian origin, has its entire literature in Tamil language and practitioners of Siddha medicine are known as *siddhars*.[1] This system is based on a combination of ancient medicinal practices and spiritual disciplines as well as mysticism and alchemy.[2] Unlike Ayurveda, which is another

traditional system of Indian medicine, but which gives topmost priority to herbal treatment, Siddha medicine gives importance to the conjunctive use of plants and minerals. For simple ailments, initial use of herbs is advised and if this does not prove effective, the judicious use of plants, minerals, and animal products will be recommended. [3] *Urai mathirai* is a folklore siddha formulation employed as an immune pill for the past 3 decades in Hospital pharmacopeia of all Government Siddha Hospitals. It promises to be a very good immune booster, prevents contagious diseases besides effective against *Maantham* (Indigestion), *Kanai* (Primary complex), chronic cough and fever. It is composed of *Zingiber officinale, Glycyrrhiza glabra, Anacyclus pyrethrum, Acorus calamus, Myristica fragrans, Terminalia chebula, Quercus infectoria, Allium sativum, Ferulla asafetida* and *Piper logum.* The children nursed with *Urai mathirai* are said to be almost free from pre-school and schooling age health hazards such as frequent respiratory infections /gastrointestinal infections and anorexia. Claims suggest even usage of antibiotics were minimal at the period of *Urai mathirai* administration. But the appropriate scientific validation is required to claim the traditional benefits. [4,5]

Medicines usually employed in modern medicine for alleviation of pain and inflammation like non-steroidal anti-inflammatory drugs and corticosteroids offer only symptomatic relief. Long-term use of these medications is associated with grave adverse effects. On the other side, ancient Indian systems of medicine provide potent and safe therapies for the prevention and treatment of diseases. [6] Yet they are underutilised in the management of diseases due to lack of scientific validation. Hence, the present study was designed to evaluate analgesic and anti-inflammatory activities of *Urai mathirai in* tail flick model of pain and carrageenan induced paw edema model of inflammation in albino rats.

Materials and Methods:

Preparation of *Urai mathirai*:

Zingiber officinale, Glycyrrhiza glabra, Anacyclus pyrethrum, Acorus calamus, Myristica fragrans, Terminalia chebula, Quercus infectoria, Allium sativum, Ferulla asafetida and *Piper logum* were obtained locally and the formulation was prepared as per the procedure mentioned in the Pharmacopeia of hospital of Indian medicine, Tamilnadu Siddha medical board. [4] The obtained brown coloured powder formulation was made into tablets by using tablet punching machine.

Animals:

Adult Wistar albino rats (age 8-12 weeks) of either sex were used. Animals were kept in cages in temperature-regulated rooms 22±3^{o}C and 12 hours light and dark cycle has maintained throughout the study. Animals were free access to water and food *ad libitum.* They were allowed to acclimatize to the laboratory conditions for a period of one week, and kept fasting overnight prior to the experiment. The study was approved by the Institutional Animal Ethics Committee (162/PHARMA/SCRI, 2017) and all the animal experiments were performed as per the Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines.

Drugs and chemicals:

Carrageenan was procured from Sigma-Aldrich, India, Diclofenac from Zydus Cadilla Pvt. Ltd.,

Urai mathirai dose calculation:

The clinical dose of *Urai mathirai* employed in children is 50 mg (HED = 2.5mg/kg body weight). The Rat doses are

calculated based on the FDA guidelines [7] and based on that therapeutic dose was calculated to be 10mg/kg of body weight. In the present study to estimate the dose related effects, 5 times TD and 10 times TD i.e. 50 mg/kg & 100 mg/kg of bodyweight were chosen respectively.

Evaluation of analgesic activity:

Tail-flick method using immersion of tail:

The animals were divided into five groups of six animals each. Group I served as normal control and treated with RO water. Group II served as standard control and diclofenac -150 mg/kg was administered orally. Group III, IV & V were treated orally with single dose of *Urai mathirai* 10 mg/kg, 50 mg/kg & 100 mg/kg body weight respectively. Before drug administration and recoding of reaction time of removal of tail was noticed at 0 hour, followed by 30min, 1 hour, 2nd hour & 4th hour, 5cm of the tail was dipped into hot water maintained at 58°C. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups.
The time required for flicking off the tail, was recorded, to assess response to noxiou s stimuls [8].

Evaluation of anti-inflammatory activity:

Carrageenan-induced paw edema model:

Anti-inflammatory activity of *Urai mathirai* was carried out as per the method carrageenan-induced edema in hind paw of the rat described by Winter CA et al. [9] Acute inflammation is induced by injection of 0.1ml of 1% carrageenan in normal saline into the sub plantar route of rat's left hind paw. The animals were divided into five groups of six animals. Group I served as carrageenan control. Group II served as standard control and diclofenac (150 mg/kg) was administered orally. Group III, IV & V were treated orally with single dose of *Urai mathirai* 10 mg/kg, 50 mg/kg & 100 mg/kg body weight respectively, 30 minutes prior to carrageenan injection. Paw thickness were measured just before the carrageenan injection, that is, at 0 hour and then at 1, 2, 3, 4 and 24th hour after carrageenan injection using digital water plethysmometer (Panlab LE 7500). % Inhibition of paw edema was calculated as per the below given formula:

% Inhibition of paw edema = Vc – Vt/ Vc X 100

Where, Vc = Paw edema of control animals, Vt = Paw edema of drug treated animals

Data Analysis:

Results are expressed as mean ± Standard Error of Mean (SEM). Data was analyzed using Graph pad prism software version 5.01. Comparison between different groups was done by One-Way Analysis of Variance (ANOVA) followed by Tukey's test. P value less than 0.05 was considered statistically significant.

RESULTS:

Analgesic Activity: Tail-Flick Method

Table 1 shows that reaction time of *Urai mathirai* at all doses was compared to that of control animals a significant reaction time observed at 1 and 2 hours for the doses of 10 mg/kg & 50 mg/kg.

Anti-inflammatory activity: carrageenan-induced paw edema model

Table 2 shows that there was a significant increase in paw volume in carrageenan control group when compared with diclofenac treated animals. Whereas the animals treated with *Urai mathirai* at all doses have shown a significant decrease in paw volume when compared with carrageenan control group animals and the effect was found good at 50mg/kg dose.

DISCUSSION:

Sidhha is the first system of medicine to emphasize health as the perfect state of physical, psychological, social and spiritual components of a human being. The fundamental principle of this medicine successfully eliminates the evil side effects without losing the beneficial medicinal properties. [1] Siddha formulations are being prescribed to patients of all ages from time immemorial by the traditional healers and they have shown substantial therapeutic effectiveness with insignificant side effects. The only drawback is the lack of scientific evidence in authenticating the potential of these medicines among scientific fraternity. *Urai mathirai*, is one of the poly herbal siddha formulation commonly prescribed to numerous children attending the OPD of all Government Siddha Hospitals and PHCs for boosting up of the immune status of the individuals. This formulation has earned good trust among the siddha physicians and public of Tamilnadu region for its potency and safety in treating common ailments of paediatric population. [5] Hence, the aim of the present study was to validate the analgesic and anti-inflammatory potential of *Urai mathirai* in tail flick method of algesia and carrageenan-induced hind paw edema model of inflammation in experimental animals. Heat is as suitable stimulus for activating cutaneous receptors. Tail flick method of algesia is effective in estimating the efficacy and potency of centrally acting analgesics. [10] Diclofenac was used as a reference drug in the current study as it has both central, peripheral actions and can significantly treat nociceptive pain as in this model. [11] In the current study, pain threshold increased significantly during the period of observation in all the drug treated groups, with maximum effect observed in the *Urai mathirai* at a dose of 50mg/kg as shown in table 1. The analgesic activity of *Urai mathirai* was comparable to diclofenac at 60 & 120 minutes appears to be a significant finding and suggests that this drug has a slow onset of analgesic action.

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. This model exhibits a high degree of reproducibility and has significant predictive value for clinically useful anti-inflammatory drugs. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve. The first phase of inflammation occurs within an hour of carrageenan injection and is mediated through the release of histamine, serotonin and kinins, whereas the second phase is due to the release of prostaglandin and slow reacting substances [12,13]. As shown in table 2, there was no significant inhibition of paw edema, in the early hours of study by *Urai mathirai*. Hence, it can be concluded that there is no inhibition of histamine and serotonin. In contrary, there was a significant ($P < 0.05$) percentage inhibition of paw edema, at doses of 50 and 100mg/kg, respectively, at 3rd & 4th hour by *Urai mathirai*. Therefore, it can be inferred that the inhibitory effect of *Urai mathirai* on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

Urai mathirai may have increased the pain threshold and inhibited the production of inflammatory mediators due to the presence of multiple therapeutically active phytoconstituents in the formulation and these phytoconstituents may have synergistically acted on multiple targets in pain and inflammatory pathways. Hence, further studies need to be carried out to identify the active phytoconstituents and their targets in pain and inflammatory pathways.

Table 1: Effect of *Urai Mathirai* on Tail Flick Response in Experimental Rats

S.no	Groups	Reaction time (Sec)				
		0 hour	**1/2 hour**	**1st hour**	**2nd hour**	**4th hour**
1	Normal control	3.45 ± 0.35	3.65 ± 0.22	5.05 ± 0.54	3.4 ± 0.70	2.85 ± 0.22
2	Diclofenac control	3.25 ± 0.03	3.5 ± 0.32	4.15 ± 1.11	2.5 ± 0.13	4.85 ± 0.35
3	*Urai mathirai* 10 mg/kg	3.25 ± 0.22	5.05* ± 0.16	7.6* ± 1.39	6.45* ± 0.73	3.125 ± 0.43
4	*Urai mathirai* 50 mg/kg	2.95 ± 0.66	3.45* ± 0.03	4.55* ± 0.66	8.45* ± 0.60	2.885 ± 0.12
5	*Urai mathirai* 100 mg/kg	2.8 ± 0.57	4.3* ± 0.70	5.55* ± 0.47	5.45* ± 0.41	3.15 ± 0.47

Results are expressed as Mean ± Standard Error of Mean (SEM). *p<0.05, when compared to control.

Table 2: Effect of *Urai Mathirai* on Paw Volume in Carrageenan Induced Paw Edema in Experimental Rats

S.no	Groups	Paw Volume in ml Mean + SEM (%Inhibition of Paw edema)			
		1st hour	**2nd hour**	**3rd hour**	**4th hour**
1	Carrageenan control	0.90 ± 0.06	0.79 ± 0.18	0.94 ± 0.18	1.02 ± 0.14
2	Diclofenac control	0.67 ± 0.07 (25.39)	0.75 ± 0.10 (5.88)	0.80 ± 0.08 (15.01)	0.73 ± 0.09 (27.84)
3	*Urai mathirai* 10 mg/kg	0.80* ± 0.14 (11.48)	0.77 ± 0.09 (2.88)	1.17 ± 0.16 (-23.68)	0.81* ± 0.14 (20.59)
4	*Urai mathirai* 50 mg/kg	0.80* ± 0.05 (11.70)	0.83 ± 0.05 (-4.63)	0.77* ± 0.12 (18.18)	0.57 ± 0.06 (43.53)
5	*Urai mathirai* 100 mg/kg	0.75* ± 0.05 (17.00)	0.57 ± 0.06 (28.66)	0.65* ± 0.05 (30.66)	0.82* ± 0.08 (18.82)

Results are expressed as Mean ± Standard Error of Mean (SEM). *p<0.05, when compared to carrageenan control.

CONCLUSION:

Urai mathirai possess significant analgesic and anti-inflammatory potential as evidenced from the present preclinical study. These findings support the use of *Urai mathirai* in traditional system of medicine for the management of pain and inflammatory conditions. Further studies are needed to be carried out in other animal models of pain and inflammatory to validate its efficacy and to identify the active phytoconstituents in the formulation and their targets in pain and inflammatory pathways.

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