International Journal of Psychosocial Rehabilitation, Vol.24, Issue 9, 2020
ISSN: 1475-7192

The Anti-nociceptive activity of a herbo-mineral and Siddha Nano formulation *Linga Chenduram* (LC) in selective pain induced models in Swiss albino mice

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Abstract
Background: The present study was aimed to investigate the anti-nociceptive activity of the *Linga Chenduram* in laboratory using the in vivo method so as to justify its traditional use in the above mentioned pathological conditions.
Methods: In vivo anti-nociceptive activity was performed employing the hot plate method, test on Swiss albino mice at doses of 3 and 6 mg/kg body weight. Finally, data were analyzed by one-way analysis of variance (ANOVA) and Dunnett's t-test was used as the test of significance. P value < 0.001 was considered as the minimum level of significance.
Results: *Linga chenduram* at dose of 3 mg/kg is statistically not significance when compared to Vehicle control group. At 6 mg/kg, the drug showed anti-nociceptive activity with significance (P<0.05) at 60mins and 90 mins when compared to the Vehicle control group.
Conclusions: The results obtained from the tests indicate that the *Linga Chenduram* having central and peripheral anti-nociceptive activity.

Keywords: *Linga chenduram*, Anti-nociceptive activity, Siddha formulation, Nano

Introduction

Siddha medicine is one of the ancient medicine among the southern part of North and East part of Sri Lanka and South part of India (1). Siddha classical textbooks set out to explain and elucidate the minute details of the subjects like diseases, preventive measures, methods of treatment, herbal usage in the medication, methods of manufacturing drugs, *Naadi saastram* and astrological science (2).
According to the Siddha system, the three biological humors of the human body are *Vatham*, *Pitham* and *Kabam*, in normal state are called as Life constituent (*Uyir Thathukkal*). (3) They are considered as three pillars of health and support, the structure and functions of the body. They are involved in regulating all the functions of the body and maintaining balance in the physical, emotional and mental spheres. These "*Uyir thathukkal*" co-exist in all the cells of the body. They function in a harmonious manner to create balance. *Vatham* is formed by Air (*Vayu*) and Space (*Agayam*). It controls the sensory and motor functions of the nervous system such as sensation and movement,(3-5) *Linga Chenduram* (*LC*) from the classical *Siddha* literature of *Anuboga vaithiya navanitham* is a herbo-mineral preparation, which was traditionally used in the treatment of Uterine Infection, and *Alkul Putru* (Cervical Carcinoma) (6), (7).
So, the objective of the present study is to analyze the *Linga Chenduram* where its therapeutic properties are utilized for the management of pain. The hypothesis of the research is to prove that *Linga Chenduram* possess therapeutic property like Anti-nociceptive which has shown better results in cancer treatment compared to some of the standard modern drugs for the same.

Aim

To study the Anti-nociceptive effect of ***Linga chenduram*** in Swiss albino mice by Eddy's Hot plate method.

Materials and Methods:

Preparation of *Linga chenduram.*

- **Ingredients**

Purified *Lingam* (Cinnabar) - 17.5g (5 *varaganedai*) *Thirugukalli* Latex (*Euphorbia Tortilis* Rottler ex Ainslie) - Sufficient, *Utthamani* Flowers (*Pergularia Daemia* (forsk.) Chiov., 70g (2 *Palam*) and *Vellaierukkam* Flowers (*Calotropis Procera* (Aiton) W. T. Aiton) 70g (2 *Palam*). (6)

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• Identification and Authentication of drugs
The collected raw materials were identified and authenticated by the experts of Gunapadam Department, National Institute of Siddha, Chennai 47.

• Preparation process
Purified *Lingam* was measured and made into powder form with mortar and pestle. Then *Euphorbia tortilis* (*Thirugukalli*) latex was poured into it and ground well by stone motor and pestle for 12 hours (4 *Saamam*). The mixture of *Lingam* was then made into small disc (*villai*) and spread in a suitable pot for drying in sun light. Flowers of *Pergularia daemia* (*Utthamani*) and *Calotropis procera* (*Vellarukkam*) were ground together and made into paste (*karkam*). Dried disc of *Lingam* was covered with prepared *karkam* then placed into pot with lid and sealed with clay smeared cloth (*Seelai mann*). Weight of clay pot with lid containing mixture was measured. Then it was subjected into incineration process (*Pudam*) by cow dung cake (4 times the weight of the measured clay pot weight). After the incineration process clay pot was allowed to cool itself. Processed medicine was taken from the clay pot and ground into fine powder. (6)

Animal Source: The Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, **Animals:** Swiss albino mice (Male – 24), **Age:** 6 – 8 weeks, **Body Weight:** 20 – 25 gm., **Acclimatization:** 7 days prior to dosing, **Veterinary examination:** Prior and at the end of the acclimatization period, **Identification of animals:** By cage number, animal number and individual marking by using Picric acid. **Diet:** Pellet feed, **Water:** Aqua guard portable water in polypropylene bottles, **Housing & Environment:** The animals were housed in Polypropylene cages provided with bedding of husk, **Housing temperature:** 24 - 28° C, **Relative humidity:** Between 30% and 70%, **Air changes:** 10 to 12 per hour, **Dark and light cycle:** 12 : 12 hours. . (11, 12)

Selection of Experimental animals:
The experiment protocol was submitted and approved by Institutional Animal Ethical Committee of National Institute of Siddha, **(IAEC approved No: NIS/IAEC - 1/06/30092020/06)**. Swiss albino mice (20-25 gm) of approximate same age were employed in this investigation.
The animals kept in polypropylene cages are maintained at 24-28°C. All the mice were housed individually with free access to food, water ad libitum. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12hrs throughout the study. Mice were allowed an acclimatization period of 7 days before actual experiment. The mice were closely observed for any infection and if they show any signs of infection they were excluded from the study. The animal experiment was performed with accordance to legislation on welfare. (11, 12)

Evaluation of Anti-nociceptive activity

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contralateral spinothalamic tract, pons, hind brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain.(8)

Eddy's Hot plate method
Principle

Painful reactions can be produced in experimental animals by applying noxious stimuli such as Thermal using radiant heat as a source of pain, Chemical using irritants such as acetic acid and bradykinin Physical using tail compressions.
The Hot plate test was a test of the pain response in animals. It was used in basic pain research and in testing the effectiveness of anti-nociceptive by observing the reaction to pain caused by heat.
They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking was a response to painful thermal stimuli that was a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with latency and encompasses an emotional component of escaping. (9. 10)

Experimental design
The animals were divided into 4 groups, consisting of six animals for each group.
Group I**:** Vehicle control (Milk - 1 ml/Kg. P.O)
Group II**:** Standard drug - Pentazocine (5mg/kg/i.p)
Group III**:** *Linga Chenduram* (3 mg/kg. P.O) + Milk.
Group IV**:** *Linga Chenduram* (6 mg/kg P.O) + Milk.

Experiment Method:
Eddy's Hot plate method.

Experimental procedure

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55 ± 1°C. Licking / Jumping response was seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals, 10 seconds was kept as a cut off time. The time obtained was considered the basal / normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia.
All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30, 60, 90 and 120 minutes' interval after drug administration. (11 – 18)

Statistical analysis

Analysis was done by using One-Way ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

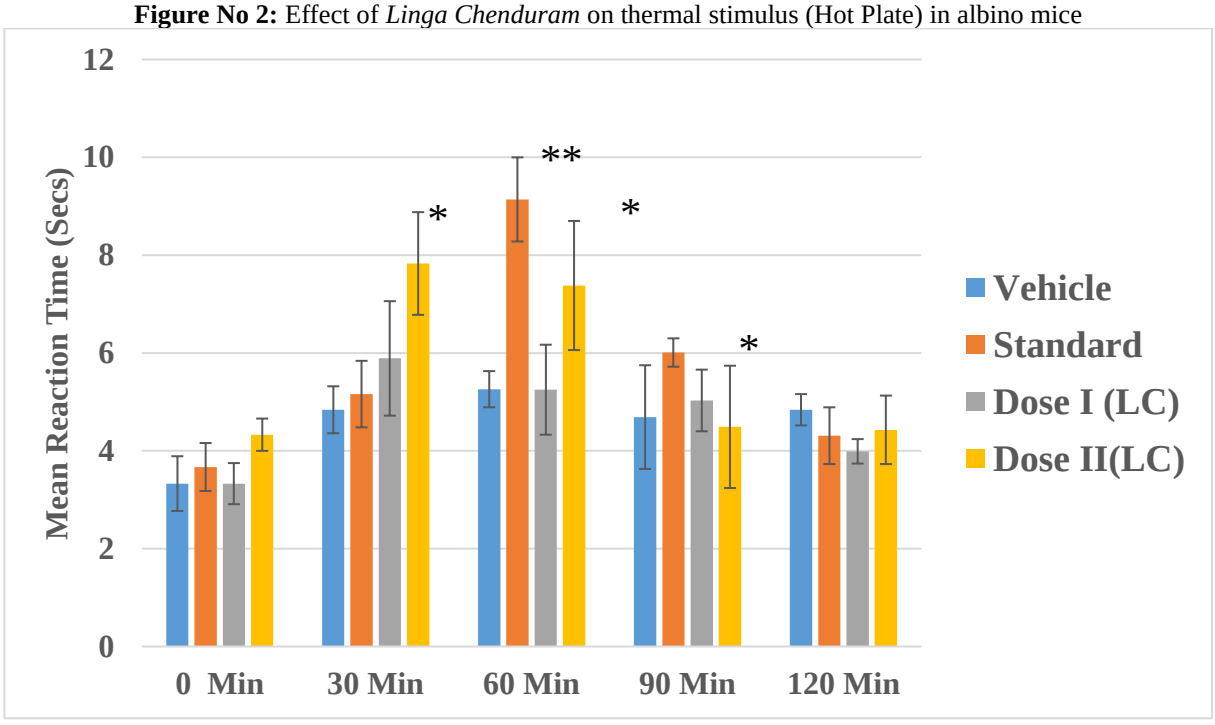
Results and Discussion

Values are in Mean ± SEM (n = 6), *P < 0.05, **P < 0.01 & ***P < 0.001 as compared with Vehicle.
Anti-nociceptive activity was carried out by Eddy's Hot plate method. *Linga chenduram* at dose of 3 mg/kg is statistically not significance when compared to Vehicle control group. At 6 mg/kg, the drug showed anti-nociceptive activity with significance (P < 0.05) at 60mins and 90 mins when compared to the Vehicle control group.

Table No. 1: Effect of *Linga Chenduram* on mean reaction time in albino mice

Treatment	Dose (mg/kg)	Mean Reaction Time (Secs)				
		0 min	30 min	60 min	90 min	120 min
Vehicle (Milk)	1ml/kg	3.33±0.56	4.84±0.48	5.26±0.37	4.69±1.06	4.84±0.32
Standard Pentazocine	5mg/kg	3.67±0.49	5.16±0.68	9.14±0.86**	6.01±0.29*	4.31±0.58
Dose 1 LC	3mg/kg	3.33±0.42	5.89±1.17	5.25±0.92	5.03±0.63	3.99±0.25
Dose 1I LC	6mg/kg	4.33±0.33	7.83±1.05*	7.38±1.32*	4.49±1.25	4.43±0.70

Figure No 2: Effect of *Linga Chenduram* on thermal stimulus (Hot Plate) in albino mice



The animals pretreated with *Linga chenduram* on (3 & 6 mg/kg) showed a dose dependent Increase in latency of response in the hot plate method (Figure No 2). The increase in the latency responses were significant (P < 0.05). The results were showed in Table No 2

Table No 2: Latency of response in the hot plate method of *Linga chenduram*

Treatment	Reaction time in Sec. before drug	% Increase in reaction time after drug treatment			
		30 min	60 min	90 min	120 min
Vehicle (Milk)1ml/kg	3.33±0.56	45.34	57.96	40.84	45.34
Standard (Pentazocine 5mg/kg)	3.67±0.49	40.59	149.05	63.76	17.44
Dose I LC 3mg/kg	3.33±0.42	76.87	57.66	51.05	19.82
Dose II LC 6mg/kg	4.33±0.33	80.83	70.44	3.69	2.31

Conclusion:

From the above results, it is concluded that *Linga chenduram* (LC) shows significant anti-nociceptive activity. *Linga chenduram* (LC) has shown a dose dependent effect and the further clinical trial need to be contacted

Acknowledgement:

We are greatly thankful to the Director, National Institute of Siddha, Chennai - 47 for providing necessary facilities and permission to carry out this work. I express my sincere gratitude to the In-Charge and Veterinarian of the Animal House at National Institute of Siddha, Chennai - 47. Last but not the least heart full thanks to my colleagues of our Department of Gunapadam, National Institute of Siddha and DR. T. N. MGR Medical University, Tamil Nadu, India for their kind support and cooperation.

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