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Original Research Article

Screening of Indhuppu Bhavanai for Anti - inflammatory and Analgesic activity animal model.

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Abstract

To evaluate Anti inflammatory and analgesic activity of "Indhuppu bhavanai" in cotton pellet induced granuloma method and Eddy's hot plate method respectively in animal model.
Indhuppu bhavanai was prepared by standard operative procedure mentioned in the siddha text.
Materials and Methods: Indhuppu Bhavanai was prepared by standard operative procedure mentioned in the siddha text. Anti inflammatory activity was evaluated by cotton pellet induced granuloma model in Wistar albino rats treated with 200mg/kg and 400mg/kg compared with control of dexamethasone 0.5mg/kg. Analgesic activity was evaluated by Eddy's hot plate method in Swiss albino mice treated with 200mg/kg and 400mg/kg compared with control of pentazocine 5mg/kg. The statistical analysis was carried out by One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests using GraphPad In Stat 3.0 software.
Results: The results indicate that *Indhuppu bhavanai* at the dose level of 200mg/kg and 400mg/kg produced a decrease in wet granuloma weight154.66±3.78 (20.15% inhibition) and 152.50±3.45*(19.37% inhibition)respectively when compared to control. Similarly there was a significant decrease in dry granuloma weight 35.4±0.26*(11.94% inhibition) and 30.2±1.20*(24.87% inhibition) respectively compared to control.
Indhuppu bhavanai at the two doses 200 mg/kg showed significant (p<0.05)analgesic activity at reaction time 90 min (4.80±1.11*) and 400 mg/kg showed significant (p<0.01)analgesic activityat 120 min (8.22±0.24**) was slightly lower than the standard drug Pentazocine 11.63±0.39**.
Conclusion: Thus it was concluded that administration of *Indhuppu Bhavanai*at the dose of 400 mg/kg exhibited significant (p<0.01) anti -inflammatory activity in Cotton pellet induced granuloma model of inflammation in rats. It also showed significant analgesic activity at the dose level of 400mg/kg.

Keywords: Siddha, anti inflammatory, analgesic, cotton pellet induced granuloma, Eddy's hot plate, Indhuppu Bhavanai

Introduction

The inflammation is a sequence of events that occurs in response to noxious stimuli, infection, trauma, or injury in the living tissues [1]. The inflammation is initiated by a cascade of events including enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair processes[2].

Many of the diseases in the modern world are thought to be due to inflammation; therefore, anti-inflammatory agents, anti-inflammatory food and food products are of great interest to contain or reduce inflammation-induced health disorders [3]. Fossil records indicate the use of natural products, especially

the plants as medicine since Middle Paleolithic (approximately 60,000 years) age[4]. The modern allopathic drugs are single active chemical molecules and target one specific pathway, whereas herbal medicines contain pleiotropic molecules that work on an orchestral approach which are able to target many elements of the complex cellular pathway[5]. Pain and inflammation go hand by hand. Initially anti inflammatory drugs were prepared by decoction of salicylates. There were many more anti-inflammatory drugs since ancient days but some ill effects were reported. Research has been going on still for anti-inflammatory drugs causing nil side effects. This is a prelimenary step up for the Siddha medicine regarding in-vivo studies for anti-inflammatory and analgesic activity. The researcher has selected Indhuppu Bhavanai for screening of anti-inflammatory and analgesic activity.

Materials and Methods

Collection And Authentication Of Raw Drug:

The raw drugs were procured from raw drug store in Chennai. All the plant materials were identified and authenticated by the Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai. Mineral drug was authenticated by the chemist in Central Research Institute of Siddha, Arumbakkam, Chennai.

Preparation of the medicine:

The Ingredients of *Indhuppu Bhavanai*[2]

Purified Indhuppu (*Sodium chloride impura*)
                                           :5 palam (175 g)
Vallarai leaf juice*(Centella asiatica)*
                                           : Required volume.
Lemon pulp juice *(Citrus limon)*
                                           : Required volume
Purified fresh Ginger juice *(Zingiber officinale)*
                                           :Required volume
Nellikai pulp juice *(Phyllanthus emblica)*
                                           : Required volume.

Purification process:

Indhuppu[8]

Indhuppu was dissolved in vinegar and kept for 3 days and dried in sunlight.

Preparation of the drug [7]

Indhuppu was placed in *Kalvam* and powdered. *Vallarai* juice was poured up to the level when *Indhuppu* got immersed. Then it was ground slowly till it loses moisture content and became dry and the same process was repeated for 7 times. Then the above mentioned process was carried out with the following juices respectively Lemon juice,Ginger juice,Goose berry juice.Finally the powder was dried & preserved.

Preparation of drug and stock solution[8]:

The suspension of siddha drug *Indhuppu bhavanai* in 2% (w/v) CMC was prepared for oral administration by gastric intubation method.

Animals:

Selection of Experimental animals:

The experimental protocol was submitted and approved by institutional Ethical Committee, (IAEC approval No KKCP/2015/026). Wistar albino rats (150- 200grams) of approximate weight, same age were employed in this investigation. The animals were obtained from animal house, K.K college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2˚C and relative humidity of 30-70% at 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet.

Assessment of anti-inflammatory activity:

Anti inflammatory activity was evaluated by cotton pellet granuloma model[9].

Experimental Design for Cotton pellet Granuloma model:

The animals were divided into four groups each group consists of 6 animals.

Group 1: Treated with Normal saline
Group 2: Standard drug - Animals treated with Dexamethasone (dose: 0.5 mg/kg).
Group 3: Animals treated with Indhuppu bhavanai (200 mg/kg).
Group 4: Animals treated with Indhuppu bhavanai (400 mg/kg).

Experimental procedure

Inflammation was induced by cotton pellet granuloma model. This method was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia by using blunted forceps, subcutaneous tunnel was made and sterilized cotton pellets (10 ± 1 mg) were implanted in axilla and groin region of the rat. After recovering from anaesthesia, animals were treated orally with vehicle control (Distilled water 10 ml / kg), Dexamethasone 0.5 mg/kg, low dose (200mg/kg) and high dose (400mg/kg) of *Indhuppu bhavanai* for consecutive 7 days, once per day. They were sacrificed on day 8th by cervical dislocation and the pellets were removed and immediately the wet weight was taken, freed from extraneous tissue and dried at 600 C for 24 hrs. The percentage inhibition of wet weight and dry weight of the granuloma were calculated and compared.

Percentage inhibition (%) =

$$\frac{Control - Treated}{Control} \times 100$$

Assessment of analgesic activity:

To evaluate the Analgesic activity of *Indhuppu bhavanai* in Swiss albino mice by ***Eddy's Hot plate method***.[10]

Selection of Experimental animals:

Healthy Swiss albino mice of either sex weighing (20-25gms) were used for this study. The animals were obtained from animal house, K.K college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of $24\pm2c$ and relative humidity of 30-70%. At 12 :12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) InstitutionalAnimal Ethics Committee KKCP/20/026 of K.K college of Pharmacy and were in accordance with the guidelines of the IAEC.

Evaluation of Analgesic activity:

Analgesic activity of Indhuppu bhavanai was evaluated by Eddys hot plate method

Animals

Mice 20-25 g were grouped in four groups, six animals in each group.

Grouping:

Group I	:	**Control** - distilled water (10ml/kg, p.o.)
Group II	:	**Standard drug** Pentazocine (5mg/kg, p.o.)
Group III	:	**Received** *Indhuppu bhavanai* (200mg/kg)
Group IV	:	**Received** *Indhuppu bhavanai* (400mg/kg)

Procedure:

Eddy"s Hot plate test is a test of the pain response in animals. It is used in basic pain research and in testing the effectiveness of analgesics 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 is a rapid response to painful thermal stimuli that is a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with a latency and encompasses an emotional component of escaping.

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at $55\pm1°$ C. 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 ,120 and 150 mins interval after drug administration.

Statistical analysis:

Results of anti-inflammatory and analgesic activity were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied.

Results and Discussion

Result of Anti-inflammatory activity of *Indhuppu bhavanai* in rats:

The results of anti-inflamatory activity by cotton pellet granuloma method shown in the Table 1.

Table 1 : Effect of *Indhuppu bhavanai* on cotton pellet induced granuloma model

Groups	Treatment	Mean wet weight of pellet(mg)	Percentage inhibition	Mean dry weight of pellet(mg)	Percentage inhibition
I	Control	191.16±1.60	0	40.60±0.12	0
II	Dexamethasone (0.5 mg/kg)	92.5±3.39*	51	22.4±1.64*	44.2
III	Indhuppu bhavanai (200mg/kg)	154.66±3.78	20.15	35.4±0.26*	11.94
IV	Indhuppu bhavanai (400mg/kg)	152.50±3.45*	19.37	30.2±1.20*	24.87

N= 6, values are expressed as mean± SEM P<0.05 when compared with control. The results were analyzed by ANOVA followed by Dunnet's test (P value less than 0.05 was considered as statistically significant).

The results indicate that *Indhuppu bhavanai* at the dose level of 200mg/kg and 400mg/kg produced a decrease in wet granuloma weight154.66±3.78 (20.15% inhibition) and 152.50±3.45*(19.37% inhibition)respectively when compared to control. Similarly there was a significant decrease in dry granuloma weight 35.4±0.26*(11.94% inhibition) and 30.2±1.20*(24.87% inhibition) respectively compared to control. Among the two doses 400 mg/kg showed slightly lower reduced weight of granuloma than standard drug. Thus it was concluded that administration of *Indhuppu bhavanai* at the dose of 400 mg/kg exhibited significant (p<0.05) anti-inflammatory activity in Cotton pellet granuloma model of inflammation in rats.

Result of Analgesic activity of *Indhuppu bhavanai* in Swiss albino mice:

The results of analgesic activity by Eddys hot plate method in Table 2.

Table 2: Analgesic activity of *Indhuppu bhavanai* in Swiss albino mice

Groups	Treatment	Reaction time in sec					
		0Min	30Min	60Min	90Min	120Min	150Min
I	Control	2.32±0.11	2.52±0.03	2.42±0.04	2.40±0.01	2.39±0.01	2.38±0.04
II	Pentazocine (5mg/kg)	2.04±0.18	4.01±0.81	8.41±0.36	11.52±0.37 **	11.63±0.39**	10.87±0.63**
III	Low dose(200mg/kg)	2.18±0.46	3.63±0.36	4.80±1.11	4.80±1.11*	5.22±0.24	5.15±0.42
IV	High dose 400mg/kg).	2.24±0.21	3.40±0.77	5.42±0.85	6.01±1.13**	8.22±0.24**	7.80±0.26

N= 6, values are expressed as mean± SEM P<0.05 ,P<0.01 when compared with control. The results were analyzed by ANOVA followed by Dunnet's test (P value less than 0.01was considered as statistically significant

Indhuppu bhavanai at the two doses 200 mg/kg showed significant (p<0.05)analgesic activity at reaction time 90 min (4.80±1.11*) and 400 mg/kg showed significant (p<0.01)analgesic activityat 120 min (8.22±0.24**) was slightly lower than the standard drug Pentazocine 11.63±0.39**.

From these results it was obvious that *Indhuppu bhavanai* has significant analgesic activity.

Conclusion

These studies conclude that Indhuppu Bhavanai has potential anti-inflammatory and analgesic activity in dose dependent manner. It may be used for inflammatory conditions like rheumatoid arthritis.

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