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Kodasuri Veeravaippu' a sidha preparation, against Carrageenan induced paw edema and Cotton pellet induced granuloma in albino rats

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ABSTRACT

The anti-inflammatory property of Kodasuri Veeravaippu was studied on rats by inducing Carrageenan induced paw edema and cotton pellet induced granuloma. Kodasuri veeravaippu was prepared by standard sidha medical methodology. Diclofenac sodium was administered orally at a dose of 5 mg/kg to Wistar strain rats which were positive control group. To the negative control group Normal saline 5 ml/kg was given orally. 9.75 mg, 13 mg and 16.25 mg of Kodasuri veera vaippu per kg were given orally as low, medium and high dose respectively. After 30min 0.1ml 1% Carrageenan was injected into the plantar region of left paw of all groups. The paw volume was measured at 0, 60, 120, 180, 240, 300 min after Carrageenan administration. The percentage difference in left and right paw was calculated. For cotton pellet granuloma study sterile pre-weighed cotton pellets (50±1 mg) were implanted in the axillary region of each rat through a single needle incision. 'Kodasuri veeravaippu' (9.75 mg, 13 mg and 16.25 mg), positive controls (Diclofenac sodium 5 mg/kg) and negative control (Normal saline 5 ml/kg) were administered to the respective group of animals for seven consecutive days from the day of cotton pellet implantation. On the eighth day, the animals were anaesthetized again; the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 hours and dried at 60°C to obtain constant weight. The increment in the dry weight of the pellets was regarded as a measure of granuloma formation. The results clearly indicate that sidha preparation, Kudasuri Veeravaippu should be used as an effective anti-inflammatory drug which gave better results compared to diclofence sodium.

Key Words: *Kodasuri veeravaippu*, Diclofenac Sodium, edema, anti inflammatory, arthritis

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INTRODUCTION

The siddha system of medicine is one of the ancient system of medicine of India. According to siddha , there are three primary life-forces in the body or three biological humors . These are '*Vatha (Windy constitution)', 'pitha (Bilious constitution)'and 'kapha(Phlegmatic constitution)'*. Whenever there is a an imbalance among these three humors, due to aggravating factors like, diet, climate, season, life style, emotions, etc., disease process sets in. This causes weakening of the digestive capacity, resulting in the increased undigested food mass (*Ama*). The *Ama* may cause many diseases and the most significant among them is Arthritis. In Ayurveda, arthritis is known as *'Amavata'*, a toxic windy condition. In siddha, it is called *'keelvayu'*. Arthritis is a disease of the joints leading to swelling, severe pain and difficulty in movements. In Sidha a complex mixture of salts known as *'Kodasuri veeravaippu'* is known to cure this disease.

Arthritis is one of the most common chronic and degenerative diseases in the world . Yet modern medicine has little to offer for treating it with lot of adverse effect . According to the WHO, 2002 report, about 70–80%

of the world's population depends on local and alternative medicines which are available as local herbs and other salts for their primary health care [1]. As compared to the allopathic drugs these medicines have proved to be cost effective, easily available and produce very low or no side effects [2]. Some of these drugs are anti-inflammatory and are used for arthritis, muscular pain and even as analgesics and antipyretics.

With these facts taken into account, present study was planned to ascertain the anti inflammatory property of *'Kodasuri veeravaippu'* against Carrageenan induced paw edema in rats Cotton pellet induced granuloma in Wistar strain Swiss albino rats.

MATERIALS AND METHODS

2 A. Chemicals used for the preparation of Kudasuri veravaippu.
1. Veeram (Mercuric Chloride), *2. Rasam (Mercury, 3. Kariyuppu* (Sodium Chloride), 4. Kalluppu (Rock Salt), 5. *Padikaram*(Potassium alum), 6. *Navacharam* (Ammonium Chloride), 7. Pooneeru (Oxides of Calcium and Potassium), 8. *Thurusu* (Copper Sulphate) and 9. *Vediyuppu* (Potassium Nitrate).

A.i. Purification of Chemicals as per Sidha methods
In the Siddha system of medicine purification of raw drugs is an important procedure in making medicine in order to reduce its toxic effects and to potentiate its therapeutic efficacy. According to the following process each chemical was purified.

i.a *Veeram* (Mercuric Chloride) This was obtained and powdered till further use.
i. b. *'Rasam'* (Mercury) Mercury was purified by grinding with appropriate quantity of 'turmeric powder' and after in red break powder for three hours each and finally squished with thick cotton cloth for one hundred times to remove foreign particles.
i. c. '***Kariyuppu'* (Sodium chloride)** *Kariyuppu* was dissolved in distilled water and heated in flame to semi solid state and was kept in sun light, well dried crystals of salt was collected.
i. d. '***Kalluppu'* (Rock salt)** *Kalluppu* was dissolved in distilled water and heated to semi solid state and well dried in sun light and collected.
i. e. *'Padikaram'* (Alum) *Padikaram* was powdered and heated in earthenware, after its crystalline water evaporated completely was collected.
i. f. *'Navacharam'* (Ammonium chloride) Powdered *navacharam* was allowed to dissolve in hot water and filtered by using thick cotton cloth and dried in hot sun light.
i. g. *'Pooneeru'* (Oxides of calcium and potassium) *Pooneeru* was dissolved in distilled water and the solution was filtered by thick cotton cloth and heated to dry powder. This process was repeated for ten times and purified *pooneeru* was collected.
i. h. *'Thurusu'* (Copper sulfate) *Thurusu* was powdered and heated to white coloured crystals then collected.
i. i. *'Vediyuppu'* (Potassium nitrate) *Vediyuppu* was powdered and dissolved in distilled water then heated, when the water boils little amount of lemon juice is added to remove impurities, after the solution was dried well, the salt was collected. This process was repeated for seven times.

2 B. Preparation of *Kodasuri Veeravaippu*.
One part of purified mercury was mixed with four parts of purified *Kariyuppu* in earthen pot. This was covered with appropriate size of another pot which was thickly coated with aqueous extract of *Acalypha indica* and dried earlier. The apparatus was tightly sealed by using clay pasted cotton cloth. The pot was heated in medium to high flame for twenty four hours. The apparatus was then allowed cool in room temperature for a day. The upper pot was carefully separated from the lower pot and the sublimate deposited inside of the upper pot was smoothly collected by using a brush. This is known as 'Rasapathangam' and was stored in a closed bottle. Eight part of this *'Rasapathangam'* was mixed with two part each of purified *'Kalluppu,(Rock salt), 'Thurusu'(Copper sulphate), 'Vediyuppu'(Potassium nitrate), 'Navacharam'(Ammonium cholride), 'Pooneeru'(Salts of Potassium and Calcium)* and *'Padikaram'(Alum).*

This combination of ingredients was ground well in a mortar pestle for three hours and filled in a thick bottle up to half mark and closed with a glass stopper. Then whole bottle was covered clay pasted cotton cloth) and dried. After drying, the bottle was placed inside a pot which was filled up to its quarter mark by fine sand. The remaining space was filled by the coarse sand up to four inch above the bottle. This apparatus was heated in a gas stove for twenty four hours continuously in medium to high flame. After cooling, the bottle was opened and the medicine was weighed. One fourth weights of each of purified *'Kalluppu', 'Thurusu', 'Vediyuppu', 'Navacharam', 'Pooneeru'* and *'Padikaram'* were mixed to above mixture.

This combination of ingredients was well ground in a mortor for three hours and filled in a thick bottle up to half mark and closed with a glass stopper. Then whole bottle was covered by the clay and covered with *'Seven seelaiman'* and dried. After drying, the bottle was subjected to the heating cooling and sublimation as mentioned above.

The same process was repeated for five times adding the above mentioned salts each time at one fourth by weight. Each cycle is known as one '*Pudam*'. After finishing five *'Pudams'* the final product is called *'Kodasuri veeravaippu.'* This was collected and well powdered in a *mortor* and stored in a tightly closed bottle till further use. '*Kodasuri veera vaippu'* is a unique formulation that is used to cure arthritis in sidha medical practice. As this study is based on Indian system of medicine i.e. Siddha , both traditional as well as chemical studies were deeply explored to find out the validity, composition, effectiveness and safety of the drug used.

2 C. Experimental Design

The experimental setup was to study the acute and chronic anti inflammatory property of '*Kodasuri veeravaippu'* against Carrageenan induced paw edema and Cotton pellet induced granuloma. Inflammation is a tissue reaction to infection or irritation due to a foreign substance. There are several mechanisms involved in inflammatory reactions such as release of histamines, prostaglandins or bradykinin. In animals, inflammatory reaction is readily produced in the form of paw edema by the help of Carrageenan, formalin or histamine or egg white. Carrageenan is a sulphated polysaccharide obtained from sea weeds which when injected produces inflammation and edema.

Albino Wistar strain rats weighing 150-200gm were taken as experimental animals. The drug, Carrageenan 1% solution was used to induce paw edema in rats. Diclofenac sodium was used for pain relief. The equipment, Plethysmograph was used to record the results.

Albino Wister rats weighing 150-200 g of either sex were used. The animals were kept in the standard polypropylene cages and provided with food and water *ad libitum.* The animals were acclimatized for a period of 14 days prior to performing the experiments. The experimental protocols were approved by the CPCSEA/IAEC of Mohamed Sathak A.J College of pharmacy, Sholinganallur Chennai. (Regn No: AJ/IAEC/10/11).

2 c i. Acute anti inflammatory activity by Carrageenan induced paw edema method

Animals were weighed and numbered. A mark was made on both the hind paws just beyond tibia-tarsal junction with a permanent marker. The initial paw volume was noted by dipping the paws one by one and by measuring the mercury displacement. Animals were divided into

5 groups (n=6). To the positive control group Diclofenac sodium was administered orally at a dose of 5 mg/Kg., To the negative control group Normal saline 5 ml/kg was given orally, For the third, fourth and fifth groups 9.75 mg, 13 mg and 16.25 mg of *Kodasuri veeravaippu* /kg were given orally as low, medium and high dose respectively. After 30min 0.1ml 1% Carrageenan was injected into the plantar region of left paw of all groups. The other paw was kept as control for comparison. The paw volume was measured at 0, 60, 120, 180, 240, 300 min after Carrageenan administration. The percentage difference in left and right paw was calculated.

2 c ii. Chronic anti inflammatory activity by Cotton pellet induced granuloma method

The rats were divided into five groups, each group consisting of six animals. After shaving off the fur, the animals were anaesthetized using ketamine. Sterile pre-weighed cotton pellets (50±1 mg) were implanted in the axillary region of each rat through a single needle incision. '*Kodasuri veeravaippu'* (9.75 mg, 13 mg and 16.25 mg), positive controls (Diclofenac sodium 5 mg/kg) and negative control (Normal saline 5 ml/kg) were administered to the respective group of animals for seven consecutive days from the day of cotton pellet implantation. On the eighth day, the animals were anaesthetized again; the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 hours and dried at 60°C to obtain constant weight. The increment in the dry weight of the pellets was regarded as a measure of granuloma formation.

3. Statistical Analysis

The results are presented as mean ± SEM. students't'-test for multiple comparisons was used for statistical evaluation. P-values less than 0.05 were considered significant.

RESULTS

4 a. Inhibition of Carrageenan induced paw edema

Intra plantar injection of Carrageenan in the hind paw induced gradual increase in the edema paw volume in the control group. '*Kodasuri veeravaippu'* at doses of 9.75 mg, 13 mg and 16.25 mg mg/kg significantly ($p<0.01$)

inhibited edema formation in rat paw 3 h after Carrageenan challenge (Table 1) (Figure 1). The reference drug, Diclofenac sodium at a dose of 5 mg/kg markedly reduced the paw edema.

Table 1. Effect of '*Kodasuri veeravaippu*' on Carrageenan induced paw edema- percentage of inhibition.

GROUP	DRUG	PAW VOLUME (Values of paw volume are mean ± SE from 6 animals in each group)											
		0 th hour		1 st hour		2 nd hour		3 rd hour		4 th hour		5 th hour	
		L	R	L	R	L	R	L	R	L	R	L	R
Positive Control	Diclofenac sodium 5mg/kg	7.8 ±0.01	8 ±0.01	7.8 ±0.01	10.2 ±0.02	7.8 ±0.01	10.8 ±0.01	7.8 ±0.01	11 ±0.01	7.8 ±0.01	9.2 ±0.01	7.8 ±0.01	8.4 ±0.02
Negative Control	Normal saline 5ml/kg	7.4 ±0.03	7.8 ±0.01	7.4 ±0.03	10.8 ±0.01	7.4 ±0.03	11.6 ±0.01	7.4 ±0.03	12.5 ±0.01	7.4 ±0.03	12.8 ±0.01	7.4 ±0.03	13 ±0.01
Low Dose	9.75 mg of Kodasuri veera vaippu /kg rat	8 ±0.02	8.3 ±0.03	8 ±0.02	11.4 ±0.02	8 ±0.02	11 ±0.01	8 ±0.02	10.8 ±0.01	8 ±0.02	10.4 ±0.03	8 ±0.01	10 ±0.01
Medium Dose	13 mg of Kodasuri veera vaippu /kg rat	8.2 ±0.01	8.5 ±0.01	8.2 ±0.01	11.5 ±0.02	8.2 ±0.01	10.6 ±0.02	8.2 ±0.01	10.5 ±0.03	8.2 ±0.01	9.8 ±0.03	8.2 ±0.01	8.6 ±0.03
High Dose	16.25 mg of Kodasuri veera vaippu /kg rat	6.2 ±0.02	6.5 ±0.02	6.2 ±0.02	8.6 ±0.02	6.2 ±0.02	8 ±0.03	6.2 ±0.02	7.9 ±0.01	6.2 ±0.02	7.4 ±0.02	6.2 ±0.03	7.0 ±0.02

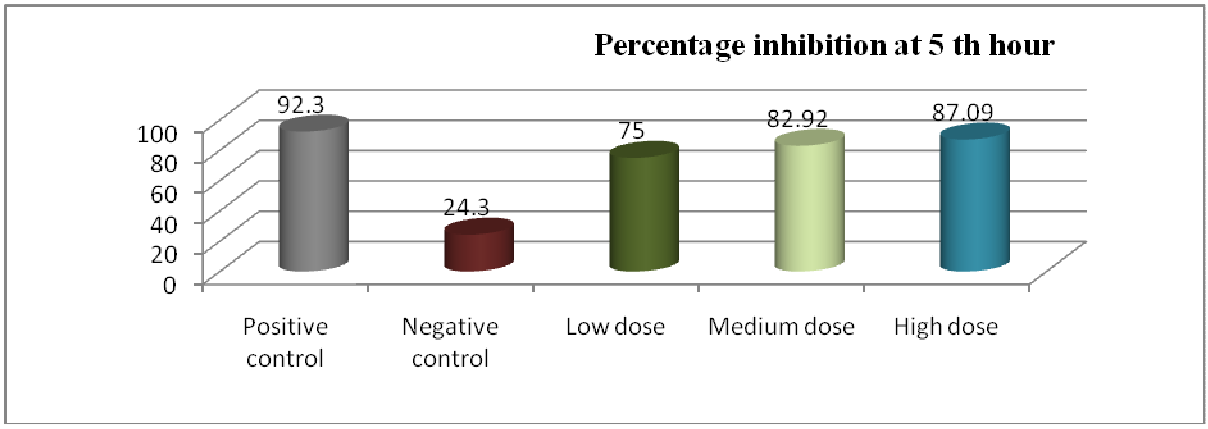
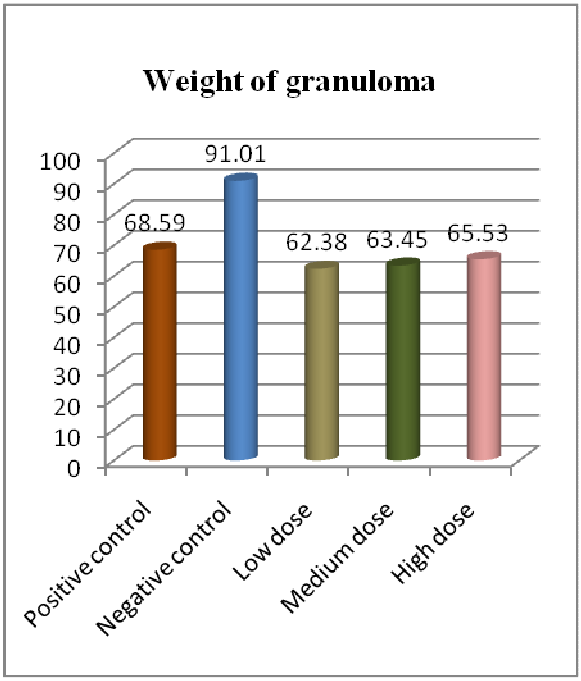


Fig. No: 1- Bar diagram chart representing percentage inhibition at 5 th hour in Carrageenan induced Paw edema.



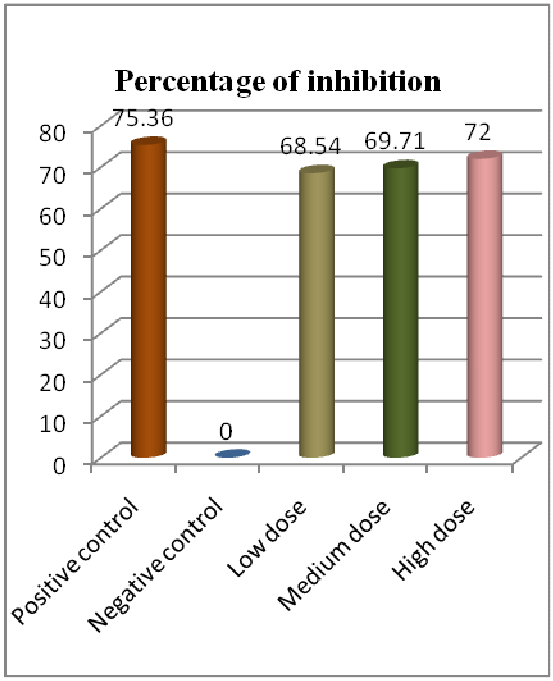


Figure 2-Chronic Anti inflammatory activity- Figure 3- Chronic Anti inflammatory activity- Weight of granuloma against Percentage of inhibition Cotton pellet.

4 b. Inhibition of cotton pellet-induced granuloma

'Kodasuri veeravaippu' at doses of 9.75 mg, 13 mg and 16.25 mg /kg significantly ($p<0.01$) inhibited granuloma formation (Table 2) (Figure 2 and Figure 3). Diclofenac sodium (5 mg/kg, p.o.), a reference drug, elicited marked reduction in granuloma formation.

Table 2. Effect of '*Kodasuri veeravaippu*' on cotton pellet-induced granuloma in rats.

Groups	Drug/ dose	Weight of granuloma (mg)	% inhibition
Positive control	Diclofenac sodium 5mg/kg	68.59 ± 0.20	75.36
Negative control	Normal saline 5ml/kg	91.07± 0.17	_
Low dose	9.75 mg of Kodasuri veera vaippu /kg rat	62.38 ± 0.16	68.54
Medium dose	13 mg of Kodasuri veera vaippu /kg rat	63.45 ± 0.14	69.71
High dose	16.25 mg of Kodasuri veera vaippu /kg rat	65.53 ± 0.19	72.00

DISCUSSION

The use of inorganic salts for treatment of diseases is an age old practice. The effect of dietary salts on gastric ulcer was described by Sonnenberg (1986) [3]. Zinc sulphate was used to gastric ulcer cure [4]. Alcohol induced ulcer was reported to be treated by Sucralfate in rats (Hollender et al, 1985) [5]. Wang et al 2000 have used low doses of ketamine to cure arthritis in rat [6].

The use of plant extracts for anti inflammatory activity was reported by number of workers [7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. There are reports of using *Lactobacillus* for anti inflammatory treatment [19, 20, 21, 22, 23]. The present report however used a preparation of natural salts, named in the sidha scriptures as "*Kodasuri veeravaippu*" for the treatment of arthritis. '*Kodasuri veeravaippu*' significantly suppressed the Carrageenan induced rat paw edema 3 hours after Carrageenan challenge. Carrageenan induced rat paw edema is commonly used as an experimental animal model for evaluation of the anti-inflammatory potential of natural products and is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after administration of Carrageenan. A more pronounced second phase is attributed to release of bradykinin, prostaglandin and lysosome.

The cotton pellet granuloma bioassay is considered as a model for studies of chronic inflammation and is considered as a typical feature of established chronic inflammatory reaction. *'Kodasuri veeravaippu'* exhibited significant reduction of granuloma formation in rats in the cotton pellet-induced granuloma. This means that *'Kodasuri veeravaippu'* may be effective in chronic inflammatory conditions.

The result of the present study indicates that *'Kodasuri veeravaippu'* possess significant anti-inflammatory activity on both acute and chronic inflammation. Since Carrageenan induced paw edema can be considered a model of prostaglandin synthesis sensitive response, the enhanced analgesic effect of *'Kodasuri veeravaippu'* may be due to inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2. The validity of this test has been shown even in the presence of substantial impairment of motor performance, and the activity is supra spinally mediated and therefore *'Kodasuri veeravaippu'* may be exhibiting its analgesic effect by involving both peripheral and central nervous mechanisms. Further researches may substantiate the pharmocodynamic properties of *'Kodasuri veeravaippu'*.

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