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In vivo Evaluation of Analgesic, Antipyretic and Anti-inflammatory potential of *Siddha* Formulation Natural and Synthetic *Pooraparpam* in selective Rodent Model

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ABSTRACT

Siddha system of medicine is one of the oldest medical systems of India existed separately in early times. Pooram is one among the Panchasootham (five mercurial compounds) which is widely used in Siddha preparation. The main component of the Pooram is Mercury. The mercurial compound has been in use in Siddha since many centuries and it is identified and indicated for the treatment of many diseases in ancient Siddha literature. The present study was undertaken to assess the in vivo evaluation of analgesic, antipyretic and anti-inflammatory potential of Siddha formulation Natural and Synthetic Pooraparpamin rodents. The analgesic activity were evaluated through thermal (Eddy's hot plate test) and mechanical method (Tail clip method) of pain induction in mice, whereas antipyretic activity by yeast induced pyrexia in rats. On the other hand, anti-inflammatory activity evaluated by carrageenan and cotton pellet induced inflammation in rats. Both the test drug was administered orally at the dose of 1.15 and 2.30 mg/kg, the activity was compared with a standard reference drug Indomethacin 20 mg/Kg and Paracetamol 150mg/kg. The result obtained from the study clearly demonstrates that the Siddha formulations Natural and Synthetic Pooraparpam has promising analgesic, anti-inflammatory and antipyretic activity in tested animals. Hence it was concluded that Pooraparpamis considered to be one of the safe medicine for the clinical management of fever, pain and inflammation associates medical conditions.

Key words: *Pooraparpam*, Analgesic, Anti-inflammatory, Antipyretic, Eddy's hot plate, Tail clip, Carrageenan, Cotton pellet, Yeast.

INTRODUCTION

Siddha system has flourished well in India for many centuries. Although this system has declined in later years, in the wake of changing mode of life and modern medicine, it continues to sustain its influence on the masses because of its incomparable intrinsic merits. Siddha medicine can combat all types of diseases, especially the chronic diseases, which baffles and eludes even the modern sophisticated medicine.

Medicinal ingredients in Siddha Vaidya are classified into three main groups: Thavaram (medicines derived from plants), Jangamam (those derived from animals), and Thatu (those derived from earth and organic toxins). Thavaram includes the thousands of whole plants and plant products [1].The National Siddha Formulary of India lists more than 10000 well practiced Siddha formulations described in Gunavagadam (Siddha pharmacology) [2].

This system has enormous pharmacopoeia containing vegetable, animal and mineral products. Mineral drug usage should be viewed before and after Bogar's period. All Siddhars are well versed in using mineral drugs [3]. Silver, gold, zinc, copper and other metals which are effect in modern medicines are used as wonderful life saving drugs against infectious diseases for thousands of years without any adverse effects [4].

Inflammation is a complex response intended to minimize the effects of injury or infection, removes the damaged tissue and generate new tissue. It accomplishes by diluting, destroying or otherwise neutralizing the harmful agents. Inflammation is the reaction of vascularized tissues to cell injury or death. It is characterized by the elaboration of inflammatory mediators and the movement of fluid and leukocytes from the vascular system into the extra vascular tissues [5,6].

Inflammation can be acute or chronic. Acute inflammation is the adaptive response that is triggered by noxious stimuli and conditions, such as infection and tissue injury and is of relatively short duration, lasting from a few minutes to several days. It is characterized by the exudation of fluid and plasma proteins and emigration of leukocytes, predominantly neutrophils. Chronic inflammation is of a longer duration, lasting for days to years and is associated with the proliferation of blood vessels, tissue necrosis and fibrosis (scarring). Acute and chronic inflammation may co-exist, with episodes of acute inflammation being superimposed on chronic inflammation [7]. The purpose of Inflammation is to defend against injurious agent and start healing and repair of injured tissue. Inflammation brings together defense forces such as WBC, antibodies and other chemicals and also bringing more nutrients and healing factors to the site of injury. However, inflammation may also be potentially harmful (Rheumatoid Arthritis, Lung fibrosis, Atherosclerosis, etc). Anti–inflammatory drugs ideally control the harmful effect of inflammation without affecting its beneficial effects [8].

Suram or Pyrexia is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF-α) are formed in large amount under this condition, which increase Prostaglandin E2 (PGE2) which in turn triggers hypothalamus to elevate body temperature [9].

Pain is probably one of the most dreaded aspects of inflammation, cancer and other degenerative conditions. Pain management is one of the major concern for persons with incurable inflammatory disease. Pain is a multidimensional experience that is essential for the maintenance and preservation of an individual. It warns the danger of bodily harm and alerts to trauma and injury. The experience of pain has a distinctly unpleasant character, that is, an affective or motivational aspect that can be distinguished from its discriminative sensory aspects and from the long-term emotional experience of 'suffering' [10].

Mercury and its compounds are considered among the most poisonous medicines in Modern scientific world because of the various toxic effects produced by them. Mercurial preparations were widely used in modern medicine in the past till the middle of the twentieth century. But after the discovery of antibiotics and other advancements in the field of medicine, use of mercury as medicine was no more in practice.

Pooram is one among the Panchasootham (five mercurial compounds) which is widely used in Siddha preparation. The main component of the *Pooram* is Mercury. Mercury is considered as Eesan in Siddha practice ie. Lord Siva who performs all the three actions of Aakkal, Kaaththal & Azhiththal (Creation, Preservation & Destruction) through his different incarnation. Mercury destroys almost all the diseases of mankind. The mercurial compound has been in use in Siddha since many centuries. *Pooram* is identified and indicated for many diseases in ancient Siddha Literatures.

Siddha system also has mentioned in detail about the toxic effects of mercury and its compounds. But it also has explained in depth about the procedures for purification and detoxification of the same. Moreover, before it is being administered as a medicine, it undergoes a series of processes which change the total physical and chemical nature of the medicine and make it a much safer one. And when prescribed as per the dose, adjuvant and duration as mentioned in the texts, it is a completely safe medicine for the treatment of fever, pain management and also effective in treating acute and chronic inflammation.

The main aim of the present investigation is to evaluate the analgesic, antipyretic and anti-inflammatory efficacy of Natural and Synthetic *Pooraparpam* by using standard pharmacological screening models in mice and rat.

EXPERIMENTAL SECTION

Procurement and authentication of raw drugs
The drugs were appropriately collected from country drug merchant shop, Chennai and were authenticated by Department of Geology, V.O. Chidambaram College, Tuticorin, Tamil nadu, India.

Purification of Pooram

Pooram (raw)	–	35 gm
Vettrilai (Piper bettle) leaves	–	8.75gm
Milagu (Piper nigram)	–	8.75gm

Method of purification
Piper bettle leaves and Piper nigram seeds were ground together and made into a poultice. Then one liter of water was taken in a mud pot and the poultice was mixed in that water. *Pooram*(raw) was covered with a piece of clean dry cloth, so that it was not exposed outside. One end of the cloth was tied to a bamboo stick and placed horizontally over the opening of the mud pot. The raw drug *Pooram* in cloth was suspended in the above water. The vessel was constantly heated till water reduced by three fourth of its volume. Finally the Pooram was taken out from the cloth, washed with clean water and dried in sunlight [11].

Preparation of *Pooraparpam*
Preparation of the study drugs was carried as per the GMP guidelines of the Drugs and cosmetics act 1940 and the Rules 1945.

Materials and methods
Ingredients
1. Purified Natural *Pooram* / Synthetic *Pooram*(Calomel)

Preparation of study drug
Take 50 g of the purified natural *Pooram* and put in the *kalvam*. Ground for seven days continuously. Then collect into the container. This was a study drug Natural *Pooraparpam*.

The above method of preparation was followed for Synthetic *Pooram* (Calomel).

Route of Administration: Oral

Dosage	**:**	1/2 *ulunduedai* (32 milligram) to 3 *ulunduedai*(195 milligram)
Anubanam	**:**	*KarumbuVellam* (Cane sugar)
Duration of Treatment	**:**	Twice a day for seven days after morning and night meal.

Study Approval
The experimental protocol was approved by The Institutional Animal Ethics Committee of National Institute of Siddha, Chennai, Tamil nadu, India.

Approval reference number- No.1248/AC/09/CPCSEA – 9 / DEC -2013 /1- dated 05.12.2013

Experimental Animal
Healthy adult Swiss albino mice weighing between 20-25 g were used for the analgesic study and adult albino Wistar rats weighing between 150-175 g were used for the anti-inflammatory and antipyretic study. The animals were purchased from Laboratory Animal Medicine Unit, TANUVAS, Chennai -600 051. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air conditioning. A 12 hr light / dark cycle was maintained. Room temperature was maintained between 20 $\pm$ 2^{o} C and relative humidity 40–65%. They were provided with food (Nutrilab Rodent feed, Provimi animal nutrition India Pvt Ltd, Bangalore) and water *ad libitum*. All the animals were acclimatized to the laboratory condition of about 7 days prior to experimentation.

Analgesic Activity
Analgesic activity of natural and synthetic *Pooraparpam* were evaluated in mice model by using two different methods

1. Thermal method: Eddy's hot plate test
2. Mechanical method: Tail clip method

Thermal method: Eddy's hot plate test

Animals were divided in 6 groups of 6 animals each and received

GROUP I :*Distilled water (10ml/kg) (p.o)*
GROUP II:*Natural Pooraparpam 0.128 mg / kg b.w (p.o)*
GROUP III:*Natural Pooraparpam 0.256 mg / kg b.w (p.o)*
GROUP IV:*Synthetic Pooraparpam 0.128 mg / kg b.w (p.o)*
GROUP V :*Synthetic Pooraparpam 0.256 mg / kg b.w (p.o)*
GROUP VI:*Standard drug Indomethacin 20 mg/Kg b.w(p.o)*

Evaluation of analgesic activity of the *Pooraparpam* was carried out by using hot plate method (Thermal method of inducing pain). The mice were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in sec) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before and at 15^{th}, 30^{th}, 45^{th}, and 60^{th} min after giving the study drugs. The maximum reaction time was fixed at 45^{th} sec to prevent any injury to the tissues of the paw [12].

The Maximum Possible Analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction Time of Treatment} - \text{Reaction Time of Control}}{\text{Cut off time (20 Sec)} - \text{Reaction time of Control}} \times 100$$

Mechanical method: Tail clip method

Animals were divided in 6 groups of 6 animals each and received

GROUP I :*Distilled water 10ml/kg (p.o)*
GROUP II:*Natural Pooraparpam0.128 mg / kg b.w (p.o)*
GROUP III:*Natural Pooraparpam0.256 mg / kg b.w (p.o)*
GROUP IV:*Synthetic Pooraparpam0.128 mg / kg b.w (p.o)*
GROUP V :*Synthetic Pooraparpam0.256 mg / kg b.w (p.o)*
GROUP VI:*Standard drug Indomethacin (20 mg/Kg b.w). (p.o)*

A metal artery clip was applied to root of the mice tail to induce pain. A sensitivity test was carried out and animals that were not attempted to dislodge the clip within 10^{th} sec were discarded. The responsive mice were allotted to 6 groups of 6 animals each. The tail clip was applied 60^{th} min after oral administration of both Natural and Synthetic *Pooraparpam* (0.128 & 0.256 mg/kg, per oral). Distilled water (10ml/kg) was served as the control [13].Time taken by mice to react to remove the clip in seconds before and 15^{th},30^{th} and 60^{th} minutes after test drug administration was calculated.

Antipyretic Activity

Yeast induced pyrexia

Animals were divided in 7 groups of 6 animals each

GROUP I : *Distilled water 10ml/kg (p.o)*
GROUP II: *Animals injected with yeast via subcutaneous injection 10ml/kg (s.c).*
GROUP III: *Animals injected with yeast 10ml/kg (s.c) and treated with standard drug paracetamol 150mg/kg b.w. (p.o).*
GROUP IV: *Animals injected with yeast 10ml/kg (s.c) and treated with Natural Pooraparpam 1.15 mg / kg b.w (p.o)*
GROUP V : *Animals injected with yeast (10ml/kg (s.c) and treated with Natural Pooraparpam 2.30 / kg b.w (p.o)*
GROUP VI: *Animals injected with yeast (10ml/kg (s.c) and treated with Synthetic Pooraparpam 1.15 mg / kg b.w (p.o)*
GROUP VII: *Animals injected with yeast (10ml/kg (s.c) and treated with SyntheticPooraparpam 2.30 mg / kg b.w (p.o)*

Pyrexia was induced by subcutaneous injection of 20 % w/v of Brewer's yeast (10ml/kg) in distilled water. Basal rectal temperature was measured before injection of yeast, by inserting digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 19 h after yeast injection. The animals were given drug treatment as above. Paracetamol 150 mg/kg b.w. was used as the standard antipyretic drug. Rectal temperature of animals was noted at regular intervals following the respective treatments. The temperature was measured at 1^{st}, 2^{nd}, and 3^{rd} h after drug administration [14].

Anti-Inflammatory Activity

Anti-inflammatory activity of Natural and Synthetic *Pooraparpam* will be evaluated by acute and chronic phases of

inflammation in rats.

1. Acute inflammatory model-Carrageenan induced paw edema method in rats
2.Chronic inflammatory model -Cotton pellet induced granuloma pouch model in rats.

Carrageenan induced paw edema method in rats

Animals were divided in 7 groups of 6 animals each and received

GROUP I : *Normal saline 10ml/kg (p.o) and injected with 0.1 ml of 1% solution of carrageenan (s.c)*
GROUP II:*Anubanam only [KarumbuVellam (Cane sugar)] mixed with water (p.o) and injected with 0.1 ml of 1% solution of carrageenan (s.c)*
GROUP III: *Standard drug Indomethacin 20 mg/Kg b.w (p.o) and injected with 0.1 ml of 1% solution of carrageenan (s.c)*
GROUP IV:*NaturalPooraparpam 1.15 mg/kg (p.o) and injected with 0.1 ml of 1% solution of carrageenan (s.c)*
GROUP V :*Natural Pooraparpam2.30 mg/kg (p.o) and injected with 0.1 ml of 1% solution of carrageenan (s.c)*
GROUP VI: *Synthetic Pooraparpam1.15 mg/kg (p.o) and injected with 0.1 ml of 1% solution of carrageenan (s.c)*
GROUP VII: *Synthetic Pooraparpam2.30 mg/kg (p.o) and injected with 0.1 ml of 1% solution of carrageenan (s.c)*

Left paw of the rat was marked with ink at the level of lateral malleolus. Basal paw volume was measured Plethysmographically by volume displacement method using Plethysmometer (UGO Basile 7140) by immersing the paw till the level of lateral malleolus. The animals were given drug treatment. One hour after the study drug administration, the rats were challenged by a subcutaneous injection of 0.1 ml of 1% solution of carrageenan into the sub plantar side of left hind paw. The paw volume was measured at 1,2,3,4 & 5 th h after challenge. The increase in paw volume is calculated as percentage and compared with the basal volume. The difference of average values between treated and carrageenan control group is calculated for each time interval and evaluated statistically [15].
The Inhibition was calculated using the formula

Percentage Edema Inhibition = [1- (V_t / V_c)] x 100

Where V_t and V_c were edema volume in the drug treated and control groups respectively.

Cotton pellet induced granuloma pouch model in rats.

Animals were divided in 7 groups of 6 animals each

GROUP I : *Normal saline 10ml/kg (p.o)*
GROUP II:*Anubanam only [KarumbuVellam (Cane sugar)] mixed with water (p.o)*
GROUP III: *Standard drug Indomethacin 20 mg/Kg b.w (p.o)*
GROUP IV:*Natural Pooraparpam 1.15mg/kg (p.o)*
GROUP V :*Natural Pooraparpam2.30 mg/kg (p.o)*
GROUP VI: *Synthetic Pooraparpam 1.15 mg/kg (p.o)*
GROUP VII: *Synthetic Pooraparpam 2.30 mg/kg (p.o)*

1 h after the first dosing of trial drugs, the animals were anesthetized with Sodium Thiopentone 50 mg / kg b.w and 10 mg of the sterile cotton pellet was inserted one in each hind groin of rats by making small subcutaneous incision. The incisions were sutured by sterile catgut. The drugs were given continuously for 8 days. After the 8 th day the animals were sacrificed by excess anesthesia. The cotton pellets were removed surgically. Pellets were separated from extraneous tissue and dried at 60^0 C until weight became constant. The net dry weight, i.e after subtracting the initial weight of the cotton pelletwas determined. The average weights of the pellet of the control group as well as of the test groups were calculated. The percent change of the granuloma weight relatively with vehicle control was determined and statistically evaluated. The percentage inhibition increase in the cotton pellet was calculated [16].

Percentage Inhibition = [Wc –Wt / Wc] X 100

Wc – Weight of the granuloma of Control group
Wt - Weight of the granuloma of Test group

Statistical analysis

The statistical analysis was carried by one way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett's multiple comparison test. Probability P values < 0.05 were considered as significant.

RESULTS

Effect of Natural and Synthetic *Pooraparpam* on Eddy's hot plate test in mice

Analgesic effect of Natural and Synthetic *Pooraparpam* evaluated by using Eddy's hot plate test. In which the basal reaction time of Natural and Synthetic *Pooraparpam* treated groups were significantly increased when compared the control group. Similarly there was a significant increase in the basal reaction time of mice treated with standard drug Indomethacin 20mg / kg b.w. The results were tabulated in Table 1 and illustrated in Figure 01.

Table 1 : Effect of Natural and Synthetic *Pooraparpam* on Eddy's hot plate test in mice

Group	Dose	Basal Reaction Time in Sec									
		Before Treatment		After Treatment							
				15 min		30 min		45 min		60 min	
		P.L	J	P.L	J	P.L	J	P.L	J	P.L	J
I	Dis.water (10ml/kg)	2.5± 0.3	3.8± 0.4	2.1± 0.16	3.8± 0.47	3.1± 0.40	4.3± 0.33	2.6± 0.31	5.1± 0.36	2.1± 0.16	4± 0.25
II	Natural *Pooraparpam* 0.128 mg / kg b.w	2.2± 0.07	5.7± 0.10	4.4± 0.14*	7.08± 0.10	5.3± 0.13*	8.2± 0.06*	7.35± 0.08*	10.1± 0.10	9.3± 0.10*	11.4± 0.11*
III	Natural *Pooraparpam* 0.256 mg / kg b.w	2.2± 0.10	5.6± 0.10	5.2± 0.13*	7.8± 0.07*	6.4± 0.17*	10± 0.08*	8.3± 0.11*	11.4± 0.11*	10.7± 0.09*	12.0± 0.10*
IV	Synthetic *Pooraparpam* 0.128 mg / kg b.w	2.3± 0.05	5.3± 0.11	3.2± 0.11*	6.3± 0.06*	4.3± 0.13*	7.4± 0.11*	6.3± 0.12*	8.5± 0.08*	8.2± 0.08*	9.4± 0.11*
V	Synthetic *Pooraparpam* 0.256 mg / kg b.w	2.4± 0.06	6.2± 0.09	3.9± 0.02*	6.9± 0.05*	5.0± 0.04*	7.8± 0.01*	7.1± 0.06*	8.6± 0.05*	8.9± 0.01*	9.7± 0.04*
VI	Indomethacin 20mg/kg b.w	2.2± 0.10	5.7± 0.15	6.5± 0.13*	9.4± 0.10*	8.3± 0.11*	11± 0.13*	10± 0.15*	13.4± 0.13*	12.4± 0.14*	14.3± 0.13*

P.L – Paw Licking , J- Jump

- *Values are expressed as mean ±SEM (n = 6) and units are in sec.*
- *Symbols represent statistical significance: *p<0.05, **p<0.01, ***p<0.001.*
- *One way ANOVA followed by Dunnett's test.*

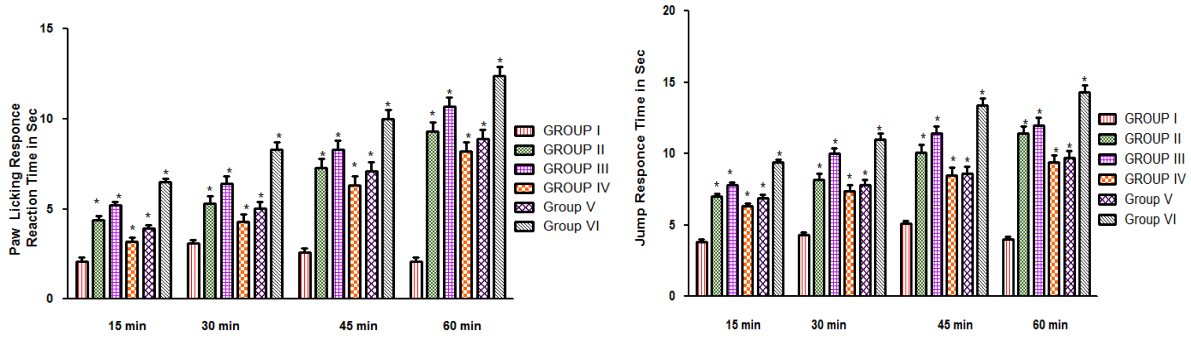

Figure 01: Effect of Natural and Synthetic *Pooraparpam* on Eddy's hot plate test in mice

Percentage protection of Natural and Synthetic *Pooraparpam* on Eddy's hot plate test in mice

The results obtained from the study showed that indomethacin treated group exerted maximum 63.37 % inhibition in Eddy's hot plate at the dose of 20 mg / kg, whereas mice treated with Natural*Pooraparpam*exhibit 46.37 and 50.12 % inhibition at the dose of 0.128 and 0.256mg / kg. Similarly mice treated with Synthetic *Pooraparpam*exhibit 34.06 and 36.04% inhibition at the dose of 0.128 and 0.256mg / kg respectively. The results were tabulated in Table 2 and illustrated in Figure 02.

Table 2: Percentage protection of Natural and Synthetic *Pooraparpam* on Eddy's hot plate

Group	Treatment and Dose	Percentage Protection							
		15 min		30 min		45 min		60 min	
		P.L	J	P.L	J	P.L	J	P.L	J
II	Natural *Pooraparpam* 0.128 mg / kg b.w	12.89	20.11	13.16	25.2	27.02	34.46	40.44	46.37
III	Natural *Pooraparpam*0.256 mg / kg b.w	17.38	25.06	19.60	38.29	32.98	42.8	48.33	50.12
IV	Synthetic *Pooraparpam* 0.128 mg / kg b.w	5.97	15.26	7.02	19.90	21.15	23.66	33.89	34.06
V	Synthetic *Pooraparpam* 0.256 mg / kg b.w	10.18	19.49	11.38	22.66	25.77	24.22	38.29	36.04
VI	Indomethacin 20mg/kg b.w	24.29	34.75	30.49	46.27	45.77	56.2	57.88	64.37

P.L – Paw Licking, J- Jump - Values are expressed as percentage and units (%)

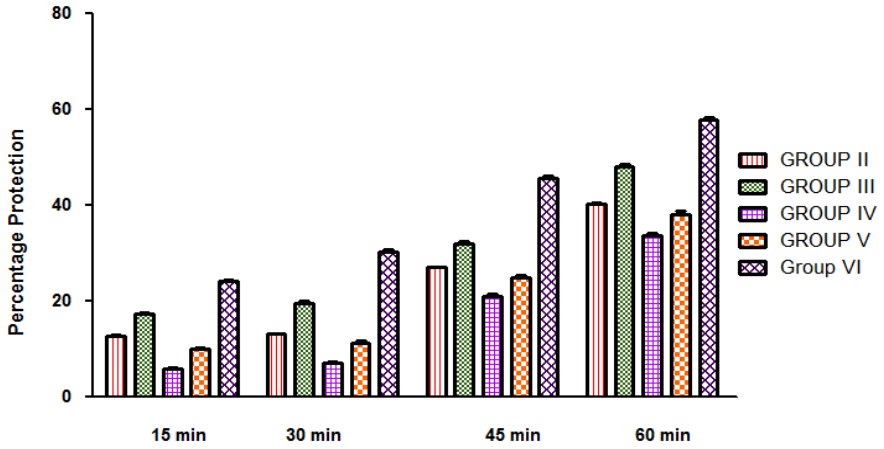

Figure 02: Percentage protection of Natural and Synthetic *Pooraparpam* on Eddy's hot plate

Effect of Natural and Synthetic *Pooraparpam* on Tail clip test in mice

The effect of Natural and Synthetic *Pooraparpam*on mechanical method of inducing nociceptive pain in mice was determined using the tail clip method. The result obtained from the study showed that there is a significant increase in reaction time of mice treated with Natural and Synthetic *Pooraparpam* at the dose of 0.128 and 0.256mg / kg respectively when compared to the control group animals. Similarly mice treated with standard drug Indomethacin has shown maximum reaction time at the dose of 20mg / kg in clip removal time when compare to the control group. The results are tabulated in Table 3 and illustrated in Figure 03.

Table 3: Effect of Natural and Synthetic *Pooraparpam* on Tail clip Method

Group	Dose	Time taken to react to remove the clip in Sec			
		Before Treatment	After Treatment		
			15Min	30 Min	60 Min
I	Dis.water (10ml/kg)	1.18±0.06	1.08±0.07	1.05±0.05	1.03±0.09
II	Natural *Pooraparpam* 1.28 mg / kg b.w	1.31±0.03	1.63±0.06*	2.25±008*	3.08±0.07*
III	Natural *Pooraparpam*2.56 mg / kg b.w	1.40±0.05	2.48±0.13**	3.25±0.09**	4.15±0.13**
IV	Synthetic *Pooraparpam* 1.28 mg / kg b.w	1.25±0.04	1.53±0.06*	1.93±0.04*	2.33±0.08*
V	Synthetic *Pooraparpam* 2.56 mg / kg b.w	1.35±0.05	1.93±0.06*	2.56±0.06*	3.10±0.07*
VI	Indomethacin 20mg/kg b.w	1.38±0.07	3.41±0.10**	4.90±0.18**	6.25±0.11**

- *Values are expressed as mean ±SEM (n = 6) and units are in sec.*
- *Symbols represent statistical significance: *p<0.05, **p<0.01, ***p<0.001.*
- *One way ANOVA followed by Dunnett's test*

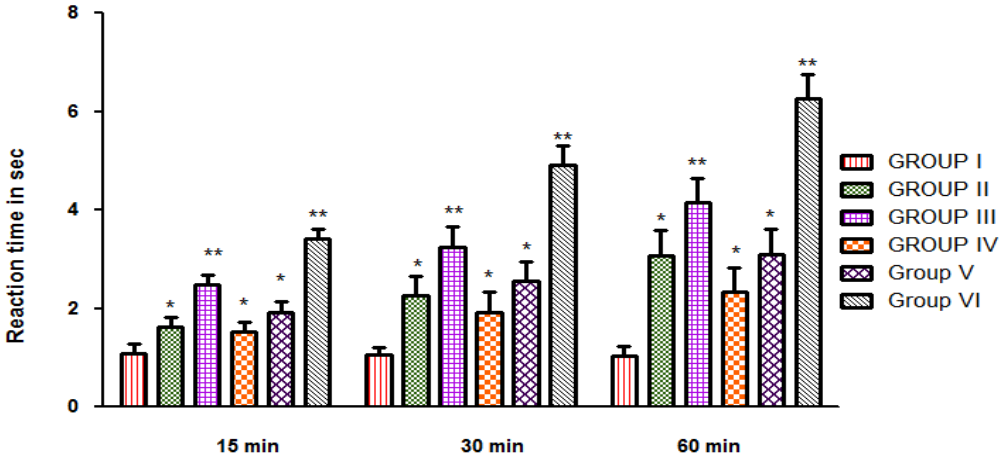

Figure 03: Effect of Natural and Synthetic *Pooraparpam* on Tail clip Method

Effect of Natural and Synthetic *Pooraparpam* on Yeast induced pyrexia in rats

Antipyretic activity of Natural and Synthetic *Pooraparpam*were evaluated using Brewer's yeast-induced hyperpyrexia in rats. The results obtained from the study showed that there was significant increase in the body temperature of rats injected only with Brewer's yeast when compared to control group animals.

Rats treated with the standard drug Paracetamol (150 mg/kg) has shown maximum reduction in rectal temperature during 4 thhour after injection of Brewer's yeast. Similar trend was observed in rats treated withNatural and Synthetic *Pooraparpam* at the dose of 1.15 and 2.30mg/kg respectively when compared to the positive control group animals. The results are tabulated in Table 4 and illustrated in Figure 04.

Table 4: Effect of Natural and Synthetic *Pooraparpam* on Yeast induced pyrexia

Group	Treatment	Initial Rectal Temp (°F)	Rectal Temperature in °F after 18hrs of Yeast Injection (Mean± SEM)				
			0 h	1h	2 h	3 h	4 h
I	Dis. Water 10ml/kg	98± 0.25	97.67± 0.42	97.95± 0.2	98.62± 0.42	97.33± 0.55	98.08± 0.30
II	Brewer's yeast 10ml/kg	98± 0.36*	100.5± 0.40*	101.5± 0.20*	101.7± 0.21*	102.3± 0.21*	102.8± 0.32*
III	Paracetamol 150mg/kg b.w + Brewer's yeast 10ml/kg	98.33± 0.08*	99.42± 0.12*	99.65± 0.15*	99.13± 0.08*	98.73± 0.07*	98.27± 0.14*
IV	Natural *Pooraparpam* 1.15mg/kg + Brewer's yeast 10ml/kg	97.82± 0.32*	99.25± 0.19*	100.2± 0.20*	99.85± 0.16*	98.98± 0.17*	98.48± 0.16*
V	Natural *Pooraparpam* 2.30 mg/kg + Brewer's yeast 10ml/kg	98.53± 0.09*	99.72± 0.08*	99.93± 0.06*	99.58± 0.05*	99.0± 0.09*	98.53± 0.07*
VI	Synthetic *Pooraparpam* 1.15mg/kg+ Brewer's yeast 10ml/kg	98.03± 0.13*	99.30± 0.08*	99.58± 0.15*	99.03± 0.20*	98.90± 0.10*	98.25± 0.17*
VII	Synthetic *Pooraparpam* 2.30 mg/kg+ Brewer's yeast 10ml/kg	98.32± 0.09*	99.40± 0.11*	99.73± 0.81*	99.28± 0.16*	98.82± 0.10*	98.32± 0.11*

- *Values are expressed as mean ±SEM (n = 6) and units are in sec.*
- *Symbols represent statistical significance: *p<0.05, **p<0.01, ***p<0.001.*
- *One way ANOVA followed by Dunnett's test*

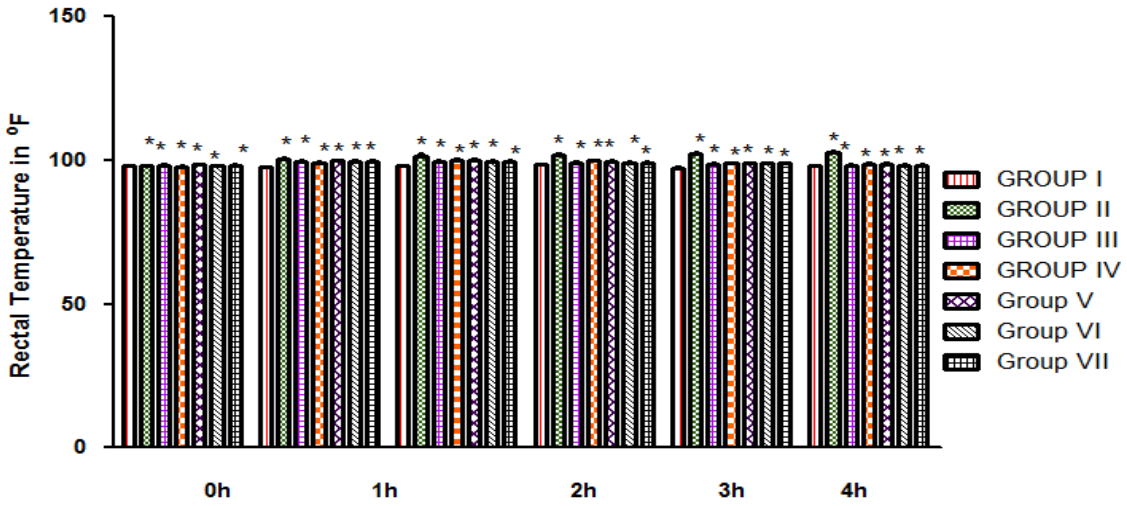

Figure 04:Effect of Natural and Synthetic *Pooraparpam* on Yeast induced pyrexia

Effect of Natural and Synthetic *Pooraparpam* on carrangeenan induced paw edema in rats

Anti-inflammatory activity of Natural and Synthetic *Pooraparpam*were evaluated using acute inflammatory model such as carrangeenan induced paw edema in rats. The results obtained from the study shows that there was a significant increase in the paw volume of rats injected with 0.1 ml of 1% solution of carrageenan.

Rats treated with Natural and Synthetic *Pooraparpam*has shown a significant reduction in paw volume at the dose of 1.15 and 2.30mg / kg respectively which is same as that of reduction volume exhibited by standard drug Indomethacin (20 mg / kg). It was further observed that vehicle control group also exhibited very minimal level of reduction in paw volume at 4^{th} and 5^{th} hour of the experiment. The results are tabulated in Table 5 and illustrated in Figure 05.

Table 5: Effect of Natural and Synthetic *Pooraparpam* on carrageenan induced paw edema

Group	Treatment	Increase in paw volumes [PV] (mL)				
		1h	2h	3h	4h	5 h
I	Normal Saline 10ml/kg+0.1 ml of 1% solution of carrageenan	1.33±0.06*	1.86±0.04*	2.3±0.09*	2.86±0.07*	3.43±0.09*
II	Cane Sugar +0.1 ml of 1% solution of carrageenan	1.16±0.03*	1.61±0.04*	2.06±0.10*	2.71±0.07*	3.16±0.06*
III	Indomethacin 20 mg/Kg b.w + 0.1 ml of 1% solution of carrageenan	0.51±0.06*	0.9±0.07*	1.03±0.04*	0.68±0.07*	0.36±0.05*
IV	Natural *Pooraparpam* 1.15mg/kg + 0.1 ml of 1% solution of carrageenan	0.83±0.08*	1.46±0.04*	1.78±0.06*	1.58±0.08*	1.26±0.03*
V	Natural *Pooraparpam* 2.30 mg/kg +0.1 ml of 1% solution of carrageenan	0.61±0.05*	1.5±0.07*	1.8±0.04*	1.61±0.05*	0.56±0.02*
VI	Synthetic *Pooraparpam* 1.15mg/kg + 0.1 ml of 1% solution of carrageenan	0.9±0.03*	1.383±0.06*	1.767±0.08*	1.5±0.03*	1.15±0.05*
VII	Synthetic *Pooraparpam* 2.30 mg/kg + 0.1 ml of 1% solution of carrageenan	0.78±0.04*	1.21±0.03*	1.5±0.05*	1.43±0.04*	0.93±0.09*

- *Values are expressed as mean ± SEM (n = 6) and units are in ml.*
- *Symbols represent statistical significance: *p<0.05, **p<0.01, ***p<0.001.*
- *One way ANOVA followed by Dunnett's test*

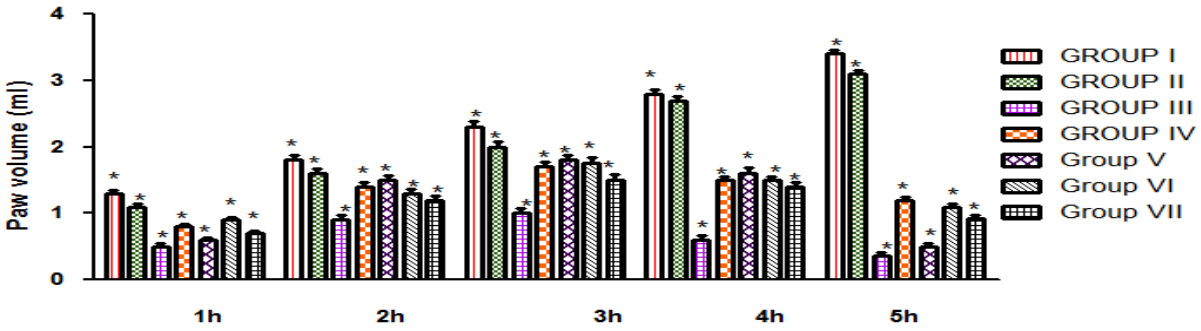

Figure 05: Effect of Natural and Synthetic *Pooraparpam* on carrageenan induced paw edema

Percentage inhibition of Paw edema by Natural and Synthetic *Pooraparpam*

The result obtained from the study shows that indomethacin treated group exerted maximum 89.31 % inhibition in carrangeenan induced paw edema at the dose of 20 mg/kg, whereas oral treatment of rat with Natural *Pooraparpam* exhibit 63.09 and 83.49 % inhibition at the dose of 1.15 and 2.30mg / kg respectively. Similarly rats treated with Synthetic *Pooraparpam* exhibit 66.5 and 72.81 % inhibition at the dose of 1.15 and 2.30mg / kg respectively. The results are tabulated in Table 6 and illustrated in Figure 06.

Table 6: Percentage inhibition of Paw edema by Natural and Synthetic *Pooraparpam* on Carrageenan induced paw edema

Group	Dose	% inhibition of Paw edema
I	Normal Saline 10ml/kg+0.1 ml of 1% solution of carrageenan	-
II	Cane Sugar +0.1 ml of 1% solution of carrageenan	7.74
III	Indomethacin 20 mg/Kg b.w + 0.1 ml of 1% solution of carrageenan	89.31
IV	Natural *Pooraparpam* 1.15mg/kg + 0.1 ml of 1% solution of carrageenan	63.09
V	Natural *Pooraparpam* 2.530 mg/kg +0.1 ml of 1% solution of carrageenan	83.49
VI	Synthetic *Pooraparpam* 1.15mg/kg + 0.1 ml of 1% solution of carrageenan	66.5
VII	Synthetic *Pooraparpam* 2.30 mg/kg +0.1 ml of 1% solution of carrageenan	72.81

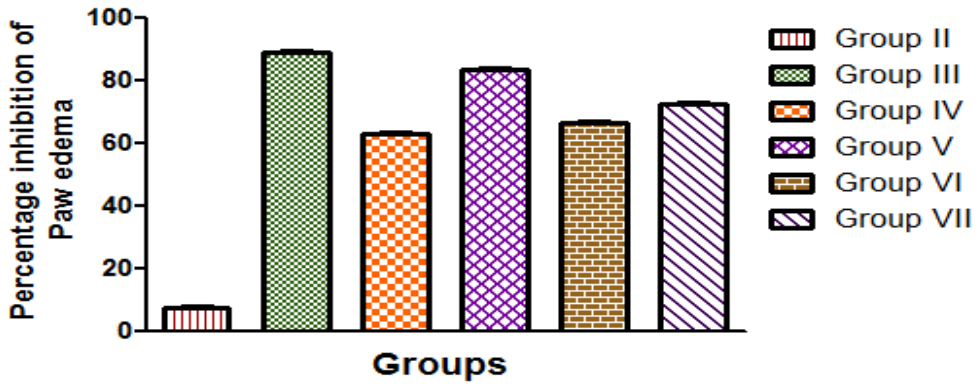

Figure 06: Percentage inhibition of Paw edema by Natural and Synthetic *Pooraparpam* on Carrageenan induced paw edema

Effect of Natural and Synthetic *Pooraparpam* on Cotton pellet induced granuloma in rats

Anti-inflammatory activity of Natural and Synthetic *Pooraparpam*were evaluated using chronic inflammatory model such as cotton pellet induced granuloma in rats. The results obtained from the study revealed that there was a significant increase in the weight of the granuloma and high level of granular formation on surgical incision of cotton pellet in sub plantar region of rats.

Treatment with Natural and Synthetic *Pooraparpam* exhibited dose dependent inhibition of granular formation at the dose of 1.15 and 2.30 mg / kg respectively. Whereas rats treated with standard drug Indomethacin (20 mg / kg) exerts highest level of reduction in the weight of cotton pellet when compared to positive control group. The results are tabulated in Table 7 and illustrated in Figure 07.

Table 7: Effect of Natural and Synthetic *Pooraparpam* on Cotton pellet induced granuloma

Group	Treatment and Dose	Weight of the Granuloma in mg	
		Wet Weight	Dry Weight
I	Normal Saline 10ml/kg + Surgical Incision of Cotton Pellet	165.7±1.43*	87.17±1.66*
II	Cane Sugar + Surgical Incision of Cotton Pellet	158.3±1.16	87.67±4.5
III	Indomethacin 20 mg/Kg b.w+ Surgical Incision of Cotton Pellet	85.33±1.18**	50.83±1.74**
IV	Natural *Pooraparpam* 1.15mg/kg + Surgical Incision of Cotton Pellet	123.7±1.28*	61.33±1.82*
V	Natural *Pooraparpam* 2.30 mg/kg + Surgical Incision of Cotton Pellet	108±1.80*	55.17±1.30*
VI	Synthetic *Pooraparpam* 1.15mg/kg+ Surgical Incision of Cotton Pellet	131.3±1.83*	64.83±2.42*
VII	Synthetic *Pooraparpam* 2.30 mg/kg+ Surgical Incision of Cotton Pellet	115.3±1.33*	58±3.08*

- *Values are expressed as mean ±SEM (n= 6) and units are in mg.*
- *Symbols represent statistical significance: *p<0.05, **p<0.01, ***p<0.001.*
- *One way ANOVA followed by Dunnett's test*

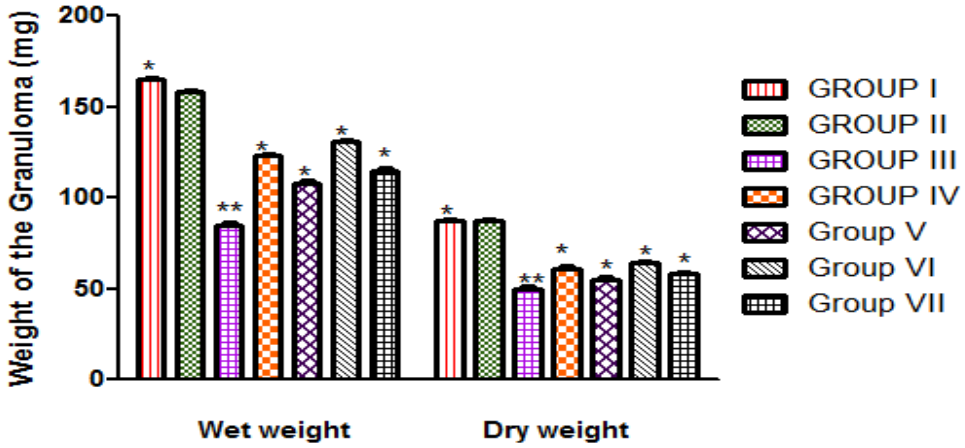

Figure 07: Effect of Natural and Synthetic *Pooraparpam* on Cotton pellet induced granuloma

Percentage inhibition of Granuloma by Natural and Synthetic *Pooraparpam*

Standard drug Indomethacin (20 mg / kg) exhibited the highest 56.06% inhibition on cotton pellet induced granuloma in rats. Natural*Pooraparpam* exhibited dose dependent percentage inhibition 20.57 and 32.72 % on granuloma formation at both the dose level of 1.15 and 2.30 mg / kg respectively. Similarly oral administration of rats with Synthetic *Pooraparpam* exerts percentage inhibition 15.35 and 27.03 % on granuloma at dose level of dose of 1.15 and 2.30 mg / kg respectively. The results are tabulated in Table 8 and illustrated in Figure 08.

Table 8: Percentage inhibition of Granuloma by Natural and Synthetic *Pooraparpam* on Cotton pellet induced granuloma

Group	Treatment and Dose	% inhibition of Granuloma
I	Normal Saline 10ml/kg +Surgical Incision of Cotton Pellet	-
II	Cane Sugar + Surgical Incision of Cotton Pellet	10.05
III	Indomethacin 20 mg/Kg b.w+Surgical Incision of Cotton Pellet	56.06
IV	Natural *Pooraparpam* 1.15mg/kg +Surgical Incision of Cotton Pellet	20.57
V	Natural *Pooraparpam* 2.30 mg/kg ++Surgical Incision of Cotton Pellet	32.72
VI	Synthetic *Pooraparpam* 1.15mg/kg++Surgical Incision of Cotton Pellet	15.35
VII	Synthetic *Pooraparpam* 2.30 mg/kg++Surgical Incision of Cotton Pellet	27.03

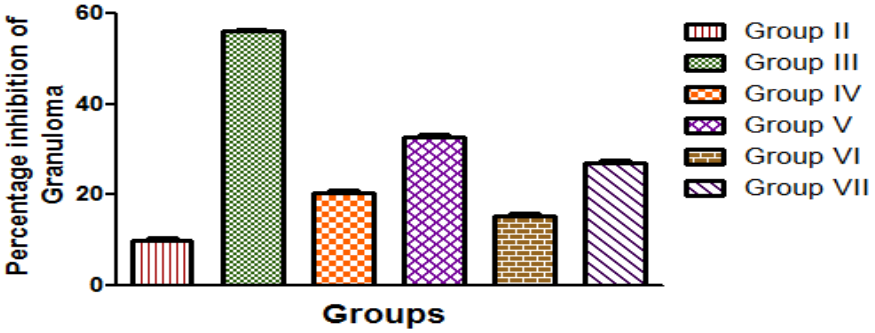

Figure 08: Percentage inhibition of Granuloma by Natural and Synthetic *Pooraparpam* on Cotton pellet induced granuloma.

DISCUSSION

Siddha medicines formulated based on the ancient vedic literature are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action. Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damage. Pain is produced by the excitation of nociceptors or their afferent free nerve endings. There are two types of pain, fast pain and slow pain, mediated through A-delta nerve fibers and C-nerve fibers nociception is the mechanism, whereby noxious peripheral stimuli are transmitted to the central nervous system. Nociceptive fibers terminate in the superficial layers of the dorsal horn, forming synaptic connections with transmission neurons running to the thalamus. Nociceptors release glutamate, substance *P* (SP) contributing to neurogenic inflammation [17]. Thermal nociception model was used to evaluate the central mechanism of analgesic activity which is known to elevate the pain threshold of mice towards heat [18].

The result obtained from Eddy's hot plate test in mice reveals that basal reaction time of Natural and Synthetic *Pooraparpam* treated groups were significantly increased when compared the control group. Similarly there was a significant increase in the basal reaction time of mice treated with standard drug Indomethacin 20 mg / kg b.w. Indomethacin treated group exerted maximum 63.37 % inhibition in Eddy's hot plate at the dose of 20 mg / kg, whereas mice treated with Natural *Pooraparpam* exhibit 46.37 and 50.12 % inhibition at the dose of 0.128 and 0.256 mg / kg respectively. Similarly mice treated with Synthetic *Pooraparpam* exhibit 34.06 and 36.04% inhibition at the dose of 0.128 and 0.256 mg / kg respectively. Synthetic *Pooraparpam* offers less percentage protection against thermal method of nociception in Eddy's hot plate test when compared to Natural *Pooraparpam*.

The brain and spinal cord play an important role in central pain mechanism. The dorsal part of the spinal cord is rich with substance *P*(SP), endogenous opioids, somatostatine and other inhibitory hormones which are the targets of pain and inflammation [19]. It is also established that tail clip, tail flick and tail immersion models are the well-established methods for measuring the central analgesic effects of drugs through opoid receptor [20].

The result obtained from mechanical tail clip test in mice revealed thatt here was a significant increase in reaction time of mice treated with Natural and Synthetic *Pooraparpam* at the dose of 0.128 and 0.256 mg / kg when compared to the control group animals. Similarly mice treated with standard drug Indomethacin has shown maximum reaction time at the dose of 20 mg / kg in clip removal time when compare to the control group. Natural *Pooraparpam* shown significantly increased reaction time in clip removal test when compared to Synthetic *Pooraparpam*.

Fever is a surrogate marker for disease activity in many infectious and inflammatory disorders. According to the classical view, the genesis of fever is induced by inflammatory mediators (i.e., cytokines, namely interleukin-1, interleukin-6, tumor necrosis factor and others) that are predominantly released by activated peripheral mononuclear phagocytes and other immune cells [21,22]. Due to the fact that direct access of the large hydrophilic cytokine proteins to the temperature-controlling brain structures within the pre-optic anterior hypothalamic (POAH) areas is prevented by the blood–brain barrier, the mechanisms described below have been suggested for producing pyrexia.

Fever is tightly regulated by the immune response. Inflammatory stimuli triggering the generation of pro-pyretic messages provoke the release of endogenous antipyretic substances [23]. Prostaglandin E2 (PGE2) is synthesized

from arachidonic acid, which is released from cell membrane lipid by phospholipase. Arachidonic acid is metabolized by two isoforms of the cyclooxygenase (COX) enzyme, COX-1 and COX-2. COX-1 usually is expressed constitutively and generates prostanoids important for housekeeping functions supporting homeostasis. COX-2, on the other hand, is inducible by inflammatory signals such as the pyrogenic cytokines, IL-1b, TNF and IL-6 as well as bacterial lipopolysaccharide. Many cells, including synoviocytes, macrophages, endothelial cells and chondrocytes have the capacity to rapidly up-regulate the expression of the COX-2 during inflammation [24]. The most likely cell type in the central nervous system responsible for producing PGE2 is the micro vascular endothelial cell, which expresses COX-2 exuberantly after stress.

The results obtained from the anti-pyretic study shows that there was significant increase in the body temperature of rats injected only with Brewer's yeast when compared to control group animal. Rats treated with standard drug Paracetamol (150 mg / kg) has shown maximum reduction in rectal temperature during fourth hour after injection of Brewer's yeast. Similar trend was observed in rats treated with Natural and Synthetic *Pooraparpam* at the dose of 1.15 and 2.30mg / kg when compared to the positive control group animals. Synthetic *Pooraparpam* has shown significant reduction in rectal temperature of rats in yeast induced pyrexia when compared to Natural *Pooraparpam*. An effective febrifuge like Natural and Synthetic *Pooraparpam* might interrupt pyrexogenesis at any step that connects peripheral inflammation with the central production of PGE2.

The carrageenan – induced paw edema is a prototype for the exudative phase of acute inflammatory effects. The development of edema in the rat paw after the injection of carrageenan has been described as a biphasic event [25]. The initial phase which starts immediately after injection and reduces within one hour, is attributed to the release of histamine and serotonin, while the second phase of swelling which begins at one and remains through three hours, is due to the release of prostaglandin – like substances [26].

The anti-inflammatory property of Natural and Synthetic *Pooraparpam* was studied using carrageenan induced paw edema method for acute inflammatory activity in rats. The results obtained from the study shows that there was a significant increase in the paw volume of rats injected with 0.1 ml of 1% solution of carrageenan. Rats treated with Natural and Synthetic *Pooraparpam*has shown a significant reduction in paw volume at the dose of 1.15 and 2.30 mg / kg which is same as that of reduction volume exhibited by standard drug Indomethacin (20 mg / kg). It was further observed that vehicle control group also exhibited very minimal level of reduction in paw volume at 4^{th} and 5^{th} hour of the experiment. Indomethacin treated group exerted maximum 89.31 % inhibition in carrangeenan induced paw edema at the dose of 20 mg / kg, whereas oral treatment of rat with Natural *Pooraparpam* exhibits 63.09 and 83.49 % inhibition at the dose of 1.15 and 2.30 mg / kg respectively. Similarly rats treated with Synthetic *Pooraparpam* exhibit 66.5 and 72.81 % inhibition at the dose of 1.15 and 2.30 mg / kg respectively. Natural *Pooraparpam* offers significantly higher level of percentage protection against carrangeenan induced paw edema when compared to Synthetic *Pooraparpam*.

The cotton pellet granuloma in rat is an excellent chronic inflammatory model that was selected to investigate chronic inflammation (the proliferative phase). Inflammatory response like extravasations, formation of granuloma and various biochemical exudates due to cotton pellet can be readily detected through this technique [27]. The results obtained from the study revealed that there was a significant increase in the weight of the granuloma and high level of granular formation on surgical incision of cotton pellet in sub plantar region of rats. Treatment with Natural and Synthetic *Pooraparpam* exhibited dose dependent inhibition of granular formation at the dose of 1.15 and 2.30 mg / kg. Whereas rats treated with standard drug Indomethacin (20 mg / Kg) exerts highest level of reduction in the weight of cotton pellet when compared to positive control group. Standard drug Indomethacin (20 mg / Kg) exhibited highest 56.06% inhibition on cotton pellet induced granuloma in rats. Natural *Pooraparpam* exhibited dose dependent percentage inhibition 20.57 and 32.72% on granuloma formation at both the dose level of dose of 1.15 and 2.30 mg / kg respectively. Similarly oral administration of rats with Synthetic *Pooraparpam* exerts percentage inhibition 15.35 and 27.03 % on granuloma at dose level of dose of 1.15 and 2.30 mg / kg respectively. Natural *Pooraparpam* offers significantly higher level of percentage protection against cotton pellet induced granuloma when compared to Synthetic *Pooraparpam*.

CONCLUSION

In the present research work traditional Siddha formulation Natural and Synthetic *Pooraparpam* were selected and investigated for its pharmacological activity against analgesic, anti-inflammatory and antipyretic activity in standard animal models.

From the result analysis of the present work it was concluded that the Siddha formulations Natural and Synthetic *Pooraparpam* has promising analgesic, anti-inflammatory and antipyretic activity in tested animals. While comparing the efficacy of the Natural and Synthetic *Pooraparpam*in pharmacological screening it was concluded that the drug *Pooraparpam* prepared from natural source has shown significantly higher level of activity when compared to Synthetic *Pooraparpam.*

Pooraparpam is a potent and very safe Siddha formulation, for many diseases like *Iduppu Soolai*(Lumbar spondilitis), *Suram* (Fever), *Mega noigal* (Venereal diseases), *Keel vatham* (Osteo arthritis) and *Sirangu* (Scabies). Clinical studies in sustained human participants can be done to establish the safety and efficacy further.

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