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EVALUATION OF ANTINOCICEPTIVE AND ANTI - INFLAMMATORY ACTIVITY OF AMIRTHA KANTHI KUKIL VALLATHY-A POLYHERBAL SIDHA DRUG.

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ABSTRACT

Amirtha Kanthi Kukill Vallathy (AKKV), a Siddha herbal medicine is traditionally used for Osteoarthritis to manage pain and inflammation. So the study was carried out to test the analgesic and anti inflammatory effects of AKKV drug in animal models. Analgesic activity was evaluated by Tail flick method, acute and chronic anti-inflammatory activity was evaluated by carragneenan induced paw oedema and cotton pellet granuloma in Wistar albino rats. Diclofenac sodium and indomethacin were employed as reference drugs for analgesic and anti-inflammatory studies. In the tail flick method, AKKV dosage of 500mg orally increased the tail withdrawal time significantly (P<0.001) when compared to the control group. In carrageenan induced paw oedema model, AKKV drug showed significant (P<0.001) inhibition at 1st hr, 2nd hr, 3rd hr and 4th hr. At the 3rd hr, the test drug showed maximum percentage of (66.2%) inhibition than the standard drug. The results of cotton pellet granuloma method indicated that AKKV drug significantly (P<0.001) reduced the wet (30.57%) and dry weight (48.4%) of the cotton pellet granuloma. From the result it can be concluded that the trial drug AKKV has potent analgesic and anti inflammatory properties which confirmed the traditional use of this drug for clinical conditions.

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INTRODUCTION

Among the ancient traditional systems of India, Siddha is a very famous system of medicine and practiced mostly in its southern part (Tamilnadu) for treating various diseases, even chronic conditions [1].The history of Siddha medicine is as old as the history of the Tamil culture and civilization [2]. On the other hand is the allopathic medicine that emerges as the primary western medical model. Drugs used in modern medicine (Allopathic) for pain and anti-inflammation are analgesics or NSAIDs. NSAIDs in particular, can cause serious side effects including peptic ulcer and hepatic or renal failure. But for the same conditions, our traditional system has more potent and safe herbal preparations without any harmful or side effects to the body. One of the analgesic and anti-inflammatory drug preparations in Siddha system of medicine used in the treatment of pain management and preventing the inflammatory process is *Amirtha Kanthi Kukill Vallathy* (AKKV).The Siddha classical literature, '*Agasthiyarvallathy* 600*'* mentioned the above said properties [3] and used for the conditions like osteoarthritis. sOsteoarthritis (OA) is the second most common rheumatologic problem and is the most frequent joint disease with prevalence of 22% to 39% in India [4-6]. It is also the cause of locomotor disability conditions in the elderly people [7]. Greatest burden of OA is due to degenerative and inflammation of joints, more likely involve knee joints [8].Primary aim in OA is to give relief from pain, inflammation and to improve the function of inflamed area. So the study was designed to test the analgesic and anti inflammatory action of AKKV on animal model and compare it with the standard allopathic drug.

MATERIALS AND METHODS

Preparation of AKKV:

The standard operating procedures for the preparation of the drug *AmirthaKandhi Kukkil Vallathy* (AKKV) were followed as per the text *Agasthiyarvallathy* 600 which is an authoritative book listed in the drug and cosmetics act 1940. The raw drugs were collected from a country shop and authenticated by Botanist of Government Siddha Medical College, Palayamkottai, Tamilnadu.

INGREDIENTS:

PurifiedSulphur (kandhagam)	:	280 gm
PurifiedShorea robusta (kukkil)	:	140 gm
PurifiedSemecarpus anacardium (serankottai)	:	140 gm
Tinospora cordifolia (Seenthil sarkarai)	:	140 gm
PurifiedSesamum indicum (ell)	:	140 gm
PurifiedWithania somnifera (amukkura)	:	105 gm
Purified Smilax chinensis (parangipattai)	:	105 gm
PurifiedPlumbago zeylanicum (kodiveli verpattai)	:	70 gm
Celastrus paniculatus (Vazzhiluvai arisi)	:	35 gm
Costus species (Kottam)	:	35 gm
Piper cubeba (Valmilagu)	:	35 gm
Phoenix species (Paereechu)	:	35 gm
Nardostachys jatamansi (Sadamanjil)	:	35 gm
Indigofera asphalanthoides (Sivanarvembu)	:	35 gm
Vettiveria zizhinoides (Vetti ver)	:	35 gm
Enicostemma axillare (Vellarugu)	:	35 gm
Clerodendron inerme (Sanganguppi)	:	35 gm
Terminalia chebula (Kadukkai thol)	:	35 gm
Terminalia bellerica (Thandrikkai)	:	35 gm
Emblica officinalis (Nellikai)	:	35 gm
Mesua ferra (Sirunagapoo)	:	35 gm
Illicium verum (Thakkolam)	:	35 gm
Cuminum cyminum (Seerakam)	:	35 gm
Psoralea corylifolia (Karboki)	:	35 gm
Nigella sativa (Karunjeerakam)	:	35 gm
Taxus buccatum (Thalisapathri)	:	35 gm
Hemidesmus indica (Nannari)	:	35 gm
Tribulus terrestris (Nerunji mul)	:	35 gm
Curculigo orchioides (Nilappanai)	:	35 gm
Asparagus racemosus (Thanneervittan kilangu)	:	35 gm
Coriandrum sativum (Kothumalli)	:	35 gm
Asphaltum (Kalmatham)	:	35 gm
Asbestos (Kalnar)	:	35 gm
Elettaria cardamomum (Elam)	:	35 gm
Zingiber officinale (Chuku)	:	35 gm
Piper nigrum (Milagu)	:	35 gm
Piper longum (Thippili)	:	35 gm
Cinnamomum verum (Karuvappattai)	:	35 gm
Alpinia galanga (Chittrathai)	:	35 gm
Palm jaggerry(Panai vellam)	:	1540gm
Honey (Then)	:	280 gm

PURIFICATION OF RAW DRUGS:

1. PurificationofSulphur:

By using cow's urine it was steamed well by placing it underground. Then the same process was repeated one by one with the following juices i.e. Onion juice, solanumnigram juice, Amaranthus tricolor juice and copper sulphate mixed with curd.

2. Purification of Kukkil:

By using neem bark decoction it was fermented for about three days and washed with cold water. The same fermentation process was repeated with a mixture of butter milk, vinegar and lime juice. The final product was boiled with milk and fried by using ghee.

3. Purification of Semecarpusanacardium:

It was molded with lime stone and then was fried with vinegar.

4. Purification of smilax chinensis:

The barks were steamed with milk and then dried.

5. Purification of Withaniasomnifera:

The rhizomes were steamed by using milk and then dried before using.

6. Purification of other drugs:

All the other drugs were purified generally by cleaning and by frying slightly.

METHOD:

All the above ingredients except honey and palm jaggery were powdered well to fine consistency and the resultant powder was grounded well with palm jaggery and honey for about 12 hours and then taken.

Maintenance of animals:

Wister albino rats weighing approximately 150-200 mg of either sex were used in this study. The animals were acclimatized for 7 days prior to experiment. They were housed in standard laboratory conditions of temperature 25±1 ^{o}C under a 12hr dark -light cycle and allowed free access of water (ad libitum) and standard pellet diet. All animals kept overnight fasting before conducting the experiment. Institutional animal Ethical committee (34/IAEC/GSMC/2011-2012) approved the experimental protocol.

Chemicals:

Carrageenan, diclofenac, indomethacin and all other the chemicals used in the study were of analytical grade and were purchased from sigma.

Acute toxicity studies:

Acute oral toxicity studies were subjected in rats as per OECD guidelines 425 [9]. Animals were kept overnight fasting prior to drug administration. Each animal was separately observed for first 30 minutes after dosage, then periodically during first 24 hours but Special attention given at 4th hour and daily thereafter for up to 14 days. Body weight and behavioral changes were noted [10]. Rats were observed individually and systematic changes were recorded.

Antinociceptive activity:

Tail flick method

Withdrawal of tail for radiant heat was used to determine the central analgesic activity in animal model. Radiant heat was passing through nichrome wire at constant strength of 5 Amps. The base of the tail of the rats was placed on nichrome wire. Tail-flick latency was assessed by the analgesiometer. Normally rat's withdrawal of their tails within 3-5 secs and cut off time of 10-12 secs was fixed to prevent damage of the tail tissues [11].Any animal failing to flick their tail within 3-5 secs was rejected from the study. Animals were divided into three groups and each group had 6 animals .Group I received distilled water, served as a control. Group II received diclofenac sodium 20mg/100mg p.o (Per. orally). Group III received tested drug of AKKV at 500mg/p .o. All the animals received the drugs 1 hr before conducting the experiments. Reaction times of the animals were taken at intervals of 30, 60 and 120 mts. A reaction time (withdrawal time) increment of 2-5 secs more than the control animals was considered for the analgesic activity of the drug.

Anti-inflammatory activity:

Carrageenan-induced rat paw oedema

Male albino rats were divided into 3 groups (n=6 in each groups).Group I received water (0.9% Nacl, 5ml/kg p.o.) served as a control. Group II received indomethacin 10mg/kg/p.o. Group III received AKKV 500mg/ p.o. In tibio-tarsal junction on the both hind paw a mark was made on all the rats [12]. The administration of drug was 30 min prior to injection of 0.1ml of 1% freshly prepared suspension of carragneenan in normal saline in the right hind paw sub-plantar region of the rats. Paw volume was measured by plethysmometer initially and then after 1, 2, 3 and 4hrs. The anti-inflammatory effects of drugs measured by using the following formula:-

Anti-inflammatory activity (%) =1-D/C×100 where D is percentage difference in paw volume after administration of drugs in treatment groups and C is percentage difference in paw volume in control groups[13].

Cotton pellet-induced granuloma:

Chronic anti-inflammatory effects in rats assessed by cotton pellet induced granuloma method [14].Animals were divided into 3 groups of six each. The rats were anesthetized and sterile cotton pellet weighing 20±0.5mg was implanted subcutaneously into both sides of the groin region of each rat. Group I was kept as a control and received water only (0.9% Nacl, 5ml/kg).Group II received diclofenac sodium 20mg/kg.p.o. once a day and served as a standard. Group III received the test drug AKKV 500mg.p.o twice a day for seven consecutive days from the day of Cotton pellet implantation. On the eighth day rats were sacrificed and cotton pellet with granuloma was dissected out and made free from extraneous tissues. The wet weight of granuloma was weighed and then dried in an oven at 60°C for 24hrs to constant weight, after that dried granuloma was weighed again. Increment in the dry weight of the pellet granuloma was taken as a measure of granuloma formation.

Statistical analysis:

All data were analyzed in R free software. The values were expressed as Mean ± SD and compared with the corresponding control values. *P* values were calculated by using one – way ANOVA followed by Tukey HSD post hoc test.

RESULT

Acute toxicity test:

In table -1, acute toxicity of Amirthakandhi kukkil valathi did not occur at 5000mg (as per the OECD-425) on rats after 48 hours of oral drug treatment. Hence one-tenth of the dose which was 500mg was selected as therapeutic dose from maximum limit dose for further pharmacological study.

Table: 1 .Acute toxic activity of AKKV.

S.NO	DOSE Mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	500	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	1000	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3	2000	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
4	5000	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.Alertness, 2.Aggressiveness, 3.Pile erection, 4.Grooming, 5.Gripping, 6.Touch Response, 7.Decreased Motor Activity, 8.Tremors, 9.Convulsions, 10.Muscle Spasm, 11.Catatonia, 12.Muscle relaxant, 13.Hypnosis, 14.Analgesia, 15.Lacrimation, 16.Exophthalmos, 17.Diarrhoea, 18.Writhing, 19.Respiration, 20. Mortality.

Analgesic activity:

The test drug AKKV at the dose of 500mg/kg/p.o showed significantly (*P*<0.001) increased response time at 60mins and 120mins in tail flick test when compared to the control. (Table-2).

TABLE: 2 Analgesic effects of AKKV by tail-flick method.

Paw licking response (sec)					
Groups	Drug(dose),Route	0min	30min	60min	120min
Control	0.9%Nacl(5ml/kg),p.o	2.25±0.30	2.31±0.28	2.33±0.21	2.16±0.21
Standard	diclofenac sodium (20mg/kg),p.o.	2.28±0.29	2.75±0.30	4.71±0.34***	6.76±0.41***
Test drug	AKKV(500mg/kg),p.o	2.23±0.32	1.75±0.30**	3.15±0.42***	5.38±0.46**

N=6.Values are expressed as Mean±SD.* P<0.05, ** P<0.01, *** P<0.001 as compare to control.

Acute inflammatory activity:

Intable-3, carrageenan induced paw edema model the administration of AKKV at the dose of 500mg/kg/p.o showed significant (*P*<0.001) reduction in paw volume at 2nd hr, 3rd hr and 4th hr. The maximum percent inhibition in paw edema found at 3hr was 66.20 % in case of test drug AKKV.

TABLE: 3 Protective effects of AKKV on paw edema induced by carragneenan in rat.

Change in Paw volume (ml) Mean ± SD						
Groups	Drug(dose),Route	0Hr	1Hr	2Hr	3Hr	4Hr
Control	0.9% Nacl(5ml/kg), p.o	0.55±0.01	1.33±0.10	1.29±0.06	1.45±0.03	1.90±0.08
Standard	Diclofenac sodium(20ml/kg), p.o	0.55±0.02	0.87±0.02*** (34.58%)	0.82±0.02*** (32.55%)	0.75±0.02*** (48.27%)	0.68±0.03*** (64.21%)
Testdrug	AKKV(500mg), p.o	0.54±0.02	0.95±0.02*** (28.57%)	1.00±0.02*** (22.48%)	0.96±0.02*** (66.20%)	0.94±0.02*** (50.52%)

Values are expressed as Mean±SD. Percentage inhibition are in brackets.* P<0.05, ** P<0.01, *** P<0.001 as compare to control.

Chronic inflammatory activity:

In the cotton pellet granuloma model (table-4) significant reduction in the weight of cotton pellets was observed with AKKV (500 mg/kg/p.o) compared to the control group. The percentage inhibition of test drug AKKV in wet granuloma was 30.57 % and dry granuloma 48.4%.However the degree of reduction was less than the effect caused by indomethacin.

Table: 4 Protective effect of AKKV on Cotton pellet induced granuloma model.

Groups	Drug(dose), route	Weight of cotton pellet (mg)	Granuloma wet weight (mg)	% Inhibition	Granuloma Dry weight (mg)	% Inhibition
Control	0.9%Nacl (5ml/kg),p.o	10.38±0.09	118.83±12.9		88.83±6.24	
Standard	Indomethacin (10mg/kg),p.o	10.50±0.15	54.83±1.72***	53.85	22.83±2.31***	74.29
Test drug	AKKV (500mg),p.o	10.33±0.19	82.50±5.82***	30.57	45.83±5.07***	48.4

Values are expressed as Mean±SD.* P<0.05, ** P<0.01, *** P<0.001 as compare to control.

DISCUSSION

Tail flick method has been found to be suitable for the evaluation of centrally acting analgesics. The increase in the latency in acute pain model may be due to the possible partial opioid agonistic effect in the drug AKKV [15]. Since prostaglandins are involved in the pain perception; flavonoids were reported to have analgesic activity by reducing the availability of prostaglandins. Hence, the phytochemical study of AKKV shows the presence of flavonoids that may also contribute to the analgesic effects.

The carrageenan test was selected because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation [16-17]. The development of carrageenan induced edema is bi-phasic phenomenon; the first phase (1-2 hrs) is mainly mediated by release of histamine, serotonin and kinins and sustained release of prostaglandins and bradykinins is the second phase[18-20].

We observed that AKKV at the given dose i.e. 500 mg. p.o. possess significant inhibition against carrageenan induced paw edema in rats. This response tendency of the drug in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the drug. This anti-inflammatory effect of AKKV may be due to the presence of flavonoids. It has been reported that a number of flavonoids possess anti-inflammatory [21] and analgesic activities [22]. Acute Inflammation is mainly produced by the enzyme prostaglandin synthetase, more specifically the endoproxidase. Flavonoids are known to inhibit the action of these enzymes in acute inflammation [23-24].

The cotton pellet-induced granuloma is a widely used method to assess the transudative and proliferative components of chronic inflammation. So to find out the chronic anti-inflammatory effect of AKKV, granuloma rat model was selected. It has three distinct phases: the first transudative phase in which the wet weight of the pellet increased during the first 3 hours, second exudative phase where the plasma leaking from the blood stream around the granuloma that occurs between 3 hours and 72hours after the cotton pellet implantation and the final proliferative phase in which the dry weight of the granuloma increased during the $3^{rd}$to 6^{th} day after the implantation [25].

The weight of the wet cotton pellets correlates with transudate material and the weight of dry pellet correlates with the amount of granulomatous tissue. Non-steroidal anti inflammatory drugs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration/ inflammation, preventing the generation of collagen fibers and suppressing mucopolysaccharides. The AKKV drug showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

CONCLUSION

Present study significantly shows the efficacy of the drug AKKV in analgesic, acute and chronic anti-inflammatory action on animal model. Thus the siddha drug proves to have equal potency as that of an allopathic drug comparatively with fewer side effects. The Pharmacological properties of the drug AKKV confirm the indications mentioned in the siddha system of medicine. In future, clinical researches can be carried out to validate the utilization of AKKV drug.

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Conflict of interest: None

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