Research Article

Antiarthritic Activity of Kanthaga Parpam (KP) (Official Siddha Drug) in Complete Freund's Adjuvant (CFA) Induced Arthritic rats

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ABSTRACT

Periarthritis of the shoulder (frozen shoulder) is the most common problem among aged individuals. It is a well-defined condition with its phases of severe pain, increasing stiffness, and the gradual recovery of full movement of the shoulder. The present study evaluates the safety and anti-arthritic efficacy of herbo-mineral formulation kanthagaparpam (kp) (official siddha drug) in animal models. The anti-arthritic activity of kp (in doses of 25 mg/kg and 50 mg/kg of body wt.) Was evaluated using the complete freund's adjuvant (cfa) induced arthritis models. Diclofenac sodium (45 mg/kg body wt.) Was used as the standard drug in all the models. This study includes examination of the paws, haematological parameters, body weight changes, organ weight changes and paw withdrawal latency. The lesions were measured again on the 7th, 14th, and 21st days after injection of the adjuvant. The marked reduction of the arthritis score by kanthagaparpam as observed in our study indicates a possible immune suppressant effect .a significant reduction in the levels of the biomarkers of inflammation and autoimmune stimulation in the treated rats. Significant ($p<0.05$) decrease in mean paw edema level of treated group compare to control. Also significant ($p<0.05$) decrease in body weight of treated group compare to control. Significantly inhibit the progression of the arthritis in animal models. The present study reveals that the kanthaga parpam (kp) can be more beneficial in the treatment of periarthritis.

Keywords: Kanthaga parpam, adjuvant induced arthritis, anti-arthritis, paw edema.

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INTRODUCTION

Kumbavatham in Siddha is congruent with the Periarthritis of shoulder. Arthritis is a generic term that describes inflammation of a joint. Inflammation is the general response of tissue to injury of any type [1]. Over time, the gliding drugs are taken by more than 30 million people worldwide; of these, 40% of consumers are older than 60. Population studies have shown that 10–20% of all people who are 65 years or older either are currently receiving or have recently received a prescription for nonsteroidal anti-rheumatic drugs. During the next 20 years the number of people over 65 is expected to increase from 380 million to 600 million surface wears out. Every day anti-arthritic [2]. Over the past 140 years chemical substances have been introduced for therapy, collectively termed non-steroidal anti-inflammatory drugs [3]. Though conventional treatment options for this condition have improved in terms of effectiveness, the use of non-steroidal anti-inflammatory drugs (NSAIDs) like etoricoxib, disease modifying anti-rheumatic drugs (DMARDs) like methotrexate, sulphasalazine, leflunomide, hydroxychloroquine, and corticosteroids like prednisolone, methylprednisolone have all been associated with adverse effects.

Because of this reason, patients suffering from chronic musculoskeletal disorders are likely to seek alternative methods for symptomatic relief and are amongst the highest users of complementary and alternative medicine [4]. This revival of herbal and other complementary therapies in the management of chronic diseases (RA and other inflammatory disorders) is well documented [5]. The present study was conducted to evaluate the possibility of using as a therapeutic agent to treat arthritis in the animal models. The herbo-mineral drug Kanthaga Parpam has been quoted by AnubogaVaithyanavaneetham, whose efficacy is not proved [6]. The drug holding the property against 80 vathadisease. The drug is chosen for the treatment of Kumbavatham (Peri arthritis) which is quoted by Yugi Muni [7].

MATERIALS AND METHODS

Aim

Aim of the study is to evaluate the safety and efficacy of the siddha drug 'Kanthaga Parpam'

Preparation of Kanthaga Parpam:

35gm of kanthagam (sulphur) (**Figure 1**) is taken and after purified (**Figure 2**), it kept it in lemon juice for 60 nazhigai (24 hr.) and dried, and maruthambattai ash (84 gm) is taken and calcidation process is done.

Ingredients of Kanthaga parpam:

Fig. 1: Kanthagam

Fig. 2: Purified Kanthagam

Drugs, Chemicals and Preparation of stock Solution

Diclofenac sodium and Carboxymethyl Cellulose were obtained from Sigma Aldrich, USA. Incomplete Freund's adjuvant and Mycobacterium tuberculosis H37RA was obtained from DIFFCO Laboratories. CFA emulsified solution was prepared by triturating 1 mg of non-viable, desiccated Mycobacterium tuberculosis in 1 ml of Freund's adjuvant [8]. Diclofenac was prepared by dissolving in water for injection. Kanthaga Parpam was dissolved in 2% of CMC and made into suspension form.

Animals

Wistar rats of either sex (100–150 g weight) were used in this study. The animals were maintained in plastic cages at 22 ± 2°C with free access to pellet food and water. The experimental protocols were approved by the Institutional Animal Ethical Committee (Approval No. XIII/VELS/PCOL/07/2000/ CPCSEA/IAEC/08.08.2012) constituted as per the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

CFA-Induced Arthritis in Rats

Each treatment group contained six wistar rats. The rats were randomly divided into four groups: CFA control, Kanthaga Parpam ($25\ mg\ kg^{-1}\ day^{-1}$, p.o.), Kanthaga Parpam ($50\ mg\ kg^{-1}\ day^{-1}$, p.o.), and Diclofenac sodium ($45\ mg\ kg^{-1}\ day^{-1}$, p.o.). On day 0, arthritis was induced by injection of 100 μL CFA, containing heat-killed and dried Mycobacterium tuberculosis into the paw of the right hind limb of each rat. The Severity of Arthritis was evaluated with the consideration of the primary and secondary lesions, that is, paw volumes of injected and non-injected paws, were measured using a plethysmometer, after which adjuvant was administered [9]. The lesions were measured again on the 7^{th}, 14^{th}, and 21^{st} days after injection of the adjuvant [10,11].

During the experimental period, the body weight was measured using a digital weighing balance every 3rd day after adjuvant injection. The severity of arthritis was recorded by a blinded observer using the visual arthritis scoring systems [12,13]. The arthritis score ranged from 0 to 4; where 0 indicates the least but definite

swelling and 4 represents the maximum swelling. This scoring system involves observations of all four paws and giving a separate score for each limb. Scores were assigned for evaluation of the pain associated with the arthritis.

Apart from this, the hematological parameters were evaluated using routine laboratory methods. The level of serum CRP and RF was determined using commercial kits. Similarly, on day 21, animals were anesthetized with anesthetic ether. The severity of the swelling of the soft tissue around the joints of the hind paws, periarticular bone resorption, periarticular bone erosion and narrowing of the joint space were evaluated [14,15] .

Statistical Analysis

The results are expressed as the mean ± SEM. The significance of the difference was evaluated by one-way ANOVA followed by Dunnet's multiple comparisons test. Data were considered statistically significant if $P<0.05$.

RESULTS

Observations such as the paw volumes, body weight, hematological and biochemical parameters were recorded on the 7th, 14th, and 21st days after adjuvant injection. The CFA-induced arthritis control group showed signs of arthritis development, as seen by the increase in the paw volumes in both CFA-injected and CFA-non-injected paws, which indicates primary and secondary arthritic lesions (barto). Other indications, such as a decreased body weight and alterations in the arthritis scores, also showed induction of arthritis in the CFA-treated control group rats.

The assessment made on the 21st day showed that the standard drug Diclofenac sodium and Kanthaga Parpam treatments had significantly reduced the adjuvant-induced primary and secondary lesions in the respective treatment groups as compared with the CFA control group **(Table 1) (Figure 3)**. It is noteworthy that the reduction in the secondary lesions was comparable in the Diclofenac sodium-treated and Kanthaga Parpam 50 mg/kg treated groups. The average gain in the body weight on day 21 was compared with the initial body weight in each treatment group. The rats in the CFA control group gained less body weight and statistically not significant as compared with the Kanthaga Parpam and Diclofenac sodium-treated groups. This effect on the body weight was clearly evident even at the lowest tested dose of 25 mg/kg of Kanthaga Parpam **(Table 2)**.

The CFA-induced hematological perturbations, such as an increase in the WBC count, a decreased RBC count, a decreased hemoglobin count and an increased erythrocyte sedimentation rate were also observed in Kanthaga Parpam treatment group of animals. The serum CRP and RF are markers of systemic inflammation and antibody production against the injected adjuvant. High levels of serum CRP (8.24 mg/dL) and serum RF (76 IU/mL) were observed in the CFA control group rats. The Kanthaga Parpam and Diclofenac sodium treatments reduced the increase in the levels of both CRP and RF in the serum. The effects of Kanthaga Parpam were dose-dependent, and the 50 mg/kg dose of Kanthaga Parpam and 45 mg/kg dose of Diclofenac sodium had almost equipotent effects in decreasing the serum RF levels **(Table-3) (Figure-4) (Figure 5)**. Kanthaga Parpam treatment favorably affected the pain scores, indicating a significant decrease in the pain associated with the adjuvant-induced arthritis. All the estimated pain scores, including the flexion pain test score, mobility score and stance score were significantly altered in Diclofenac sodium treated and Kanthaga Parpam (50 mg/kg)-treated rats. The reduction in the mobility score was greater in the Kanthaga Parpam (50 mg/kg)-treated group as compared with the standard drug treated group. It is clearly observed in the lab investigation and histopathology of joint that the soft tissue swelling around the joints, periarticular bone resorption, periarticular bony erosions and joint space narrowing in the rats treated with Kanthaga Parpam have been protected from the CFA-induced arthritis-related joint changes.

Table 1: Effect of Kanthaga Parpam on CFA-Induced Chronic Arthritis in Albino Rats

Treatment	Dose	Mean paw edema (ml)± S.E.M			
		Day 1	Day 7	Day 14	Day 21
Control	3ml/kg p.o.	0.20± 0.04	0.28±0.05	0.35±0.06	0.25±0.06
KanthagaParpam	25 mg kg^{-1} day^{-1}, p.o.	0.16±0.04	0.26±0.04	0.19±0.03	0.16±0.03
KanthagaParpam	50 mg kg^{-1} day^{-1}, p.o.),	0.11±0.02	0.18±0.03	0.11±0.03	0.09±0.02
Diclofenac sodium	(45 mg kg^{-1} day^{-1}, p.o.).	0.08±0.02	0.15±0.03	0.12±0.04	0.10±0.03

Results are expressed as mean ± S.E.M. (n=6). The significance of results was considered statistically significant at *P < 0.05

Table 2: Effect of Kanthaga Parpam on Body Weight Changes in CFA-Induced Arthritic Albino Rats

Treatment	Dose	Changes in Body Weight (g)			
		Day 1	Day 7	Day 14	Day 21
Normal control	3ml/kg p.o.	118.46 ± ± 3.69	121.02 ± 2.52	124.60 ± 4.40	126.44 ± 3.64
Arthritic Control (2% CMC)	3ml/kg p.o.	120.15 ± 2.54	122.35 ± 2.47	125.43 ± 5.00	120.63 ± 5.29
KanthagaParpam	25 mg kg^{-1} day^{-1}, p.o.	124.24 ± 3.00	126.62 ± 2.50	117.21 ± 5.58	115.43 ± 3.15
KanthagaParpam	50 mg kg^{-1} day^{-1}, p.o.),	126.02 ± 3.58	126.10 ± 2.58	110.54 ± 3.41*	103.89 ± 4.30**
Diclofenac sodium	(45 mg kg^{-1} day^{-1}, p.o.).	123.36 ± 2.96	122.41 ± 3.46	124.23 ± 5.74	130.26 ± 5.95

Values are mean ± SEM of 6 animals. One-way ANOVA followed by Dunnet test. *p<0.05, ** p<0.01 Vsarthritic control

FIGURE 3

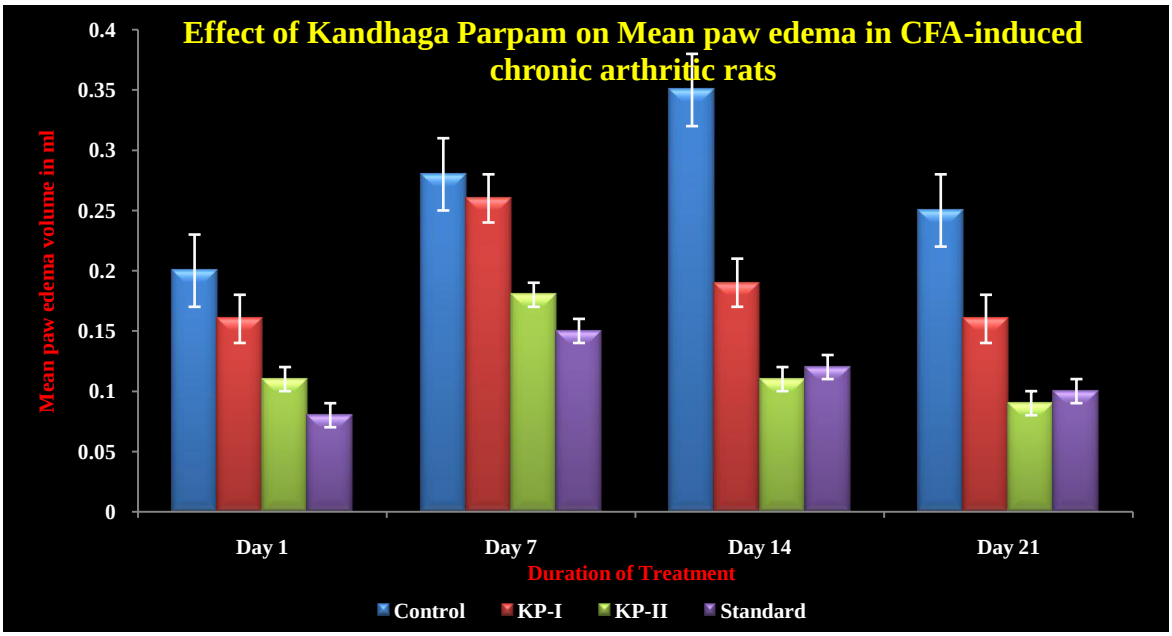


Table 3: Effect of Kanthaga Parpam on Hematological and Biochemical Parameters in CFA-Induced Arthritic Rats

Parameters	Normal control	Arthritic Control	KP (25mg/kg/ day)	KP (50mg/kg/ day)	Diclofenac (45mg/kg/ day)
Hb (g/dl)	18.66 ±1.42	19.00 ± 1.26	17.05 ± 0.92	14.98 ± 0.86	19.04 ± 1.05
PCV (%)	61.58 ± 1.86	59.99 ±1.72	60.77 ±1.28	61.18 ±1.60	61.23 ±1.44
RBC ($x10^6$/ml)	6.24 ± 0.12	6.24± 0.10	5.36 ±0.07**	5.20 ±0.09**	7.64 ± 0.12**
WBC(10^3/mm^3)	9224±206	9310±212	9410±217	9448±256	9324±244
ESR (mm/hr.)	2±00**	8.15±0.25	6.52±1.04	4.38±1.42*	3.24±1.00**
Creatinine (mg/dL)	0.44±0.03*	0.68±0.08	0.53±0.07	0.51±0.06	0.49±0.04
Total Protein (g/dL)	5.50±0.18	5.45±0.20	5.54±0.19	6.22±0.23*	5.72±0.21
SGOT (IU/L)	129.11±4.25	131.41±4.72	130.20±5.61	172.11±6.32**	180.02±5.98**
SGPT(IU/L)	44.95±1.72	45.79±2.00	47.02±1.88	46.33±1.51	46.12±1.42
ALP (IU/L)	51.22±4.45	49.64±3.88	50.26±3.95	51.08±4.20	52.00±4.12
CRP (mg/dL)	3.14±0.18**	8.24±0.73	6.20±0.36*	5.03±0.31**	4.64±0.52**
RF (IU/mL)	25.05±0.79**	76.58±2.62	44.16±0.88**	38.51±1.04**	33.25±1.44**

Values are mean ± SEM of 6 animals. One-way ANOVA followed by Dunnet test. *p<0.05, ** p<0.01 Vsarthritic control

DISCUSSION

Frozen shoulder is medically called Periarthritisor adhesive capsulitis. It means a shoulder joint with significant loss of motion in all directions. The motion is limited when the patient attempts to move the arm (active motion) and when the physician or physical therapist moves the arm with the person relaxed (passive motion). The cause of a frozen shoulder probably is an underlying inflammatory process& arthritic process [16].

Chronic inflammation in the CFA model is manifested as a progressive increase in the volume of the injected paw. It is noteworthy that the inhibitory effect of Kanthaga Parpam (50 mg kg^{-1} day^{-1}) on the volume of the injected paw was comparable with that of Diclofenac sodium (45 mg kg^{-1} day^{-1}). CFA-induced polyarthritis is associated with an immune-mediated inflammatory reaction and the rat is unique in developing polyarthritis after CFA treatment. The initial reaction of edema and soft-tissue thickening at the depot site in this model is caused by the irritant effect of the adjuvant, whereas the late-phase arthritis and flare in the injected foot are presumed to be immunologic events. The appearance of secondary lesions, that is, non-injected paw swelling is a manifestation of cell-mediated immunity. The suppression of such secondary lesions by a drug shows its immunosuppressive activity [17,18].

Kanthaga Parpam effectively reduced the secondary lesions in arthritic rats. Moreover, this effect of Kanthaga Parpam was more potent than that of diclofenac. This reveals potent suppression by Kanthaga Parpam of cell-mediated immunity in arthritic rats. Similarly, it reduced the arthritic score and secondary paw swelling. A selective reduction in the arthritis score distinguishes the immunosuppressive effects of a drug from its anti-inflammatory effects. The reduction of the arthritis score by Kanthaga Parpam as observed in our study indicates a

possible immune suppressant effect. CFA-induced arthritis in rats is associated with an increase in the plasma levels of RF and CRP.

The treatment with Kanthaga Parpam significantly reduced the levels of these biomarkers of inflammation and autoimmune stimulation in the treated rats. This study includes examination of the paws, haematological parameters, body weight changes, organ weight changes and paw withdrawal latency. The visual observations of the rats show that the treatment with Kanthaga Parpam and Diclofenac inhibited the arthritis-associated joint changes. In the Kanthaga Parpam and Diclofenac treated groups there was restoration of the body weights of the rats. A report suggests that the decrease in the body weight during inflammation is due to deficient absorption of nutrients through the intestine and that treatment with anti-inflammatory drugs normalizes the process of absorption.

The evident restoration of the body weight of rats in the Kanthaga Parpam and Diclofenac-treated groups may involve improvement of intestinal absorption of the nutrients and a reduction in the distress caused by the severity of the arthritis. It has been reported that a moderate rise in the WBC count occurs in arthritic conditions due to an IL-1B-mediated rise in the respective colony-stimulating factors. The present study reveals that Kanthaga Parpam 25 mg/kg and Diclofenac treatments tend to normalize the WBC count. In addition to this, other characteristic hematological alterations such as the decreased Hb count and increased erythrocyte sedimentation rate were also altered by the Kanthaga Parpam and Diclofenac treatments. It is proposed that the reduction in the Hb count during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells.

Similarly, an increase in the ESR is attributed to the accelerated formation of endogenous proteins such as fibrinogen and α/β globulin, and such a rise in the ESR indicates an active but obscure disease process [19]. Thus, the reduction in the ESR and increase in the Hb count brought about by Kanthaga Parpam treatment further support its anti-arthritic effect.

CONCLUSION

In the present study, based on the above results and reasons it can be concluded that the Kanthaga Parpam treatment at a dose of 25 and 50 mg/kg body wt. significantly inhibit the progression of the arthritis in animal models. So, this drug can be used clinically with minimum dose employed in this study.

FIGURE 4

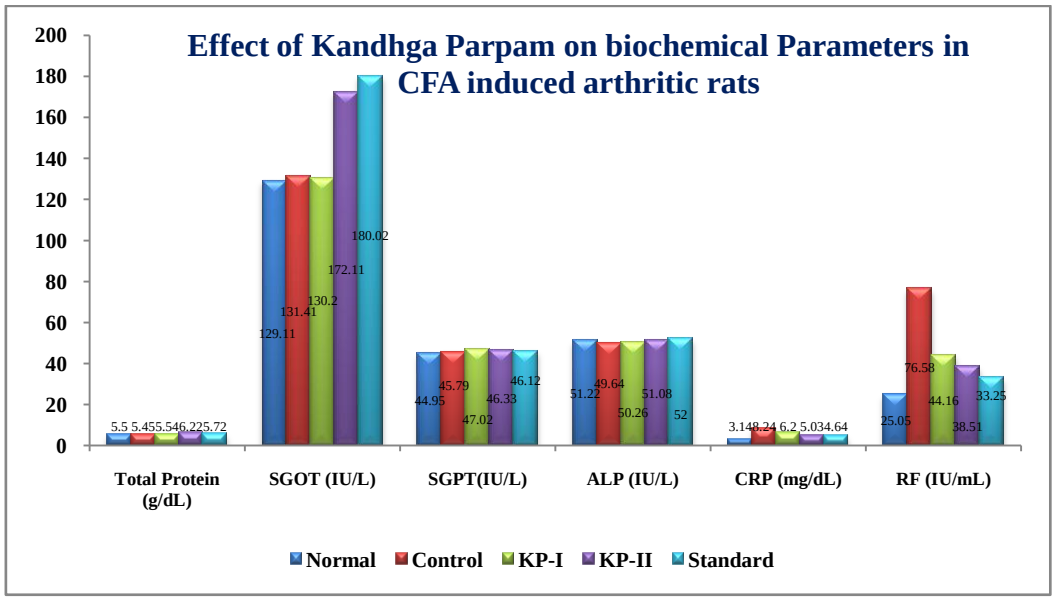
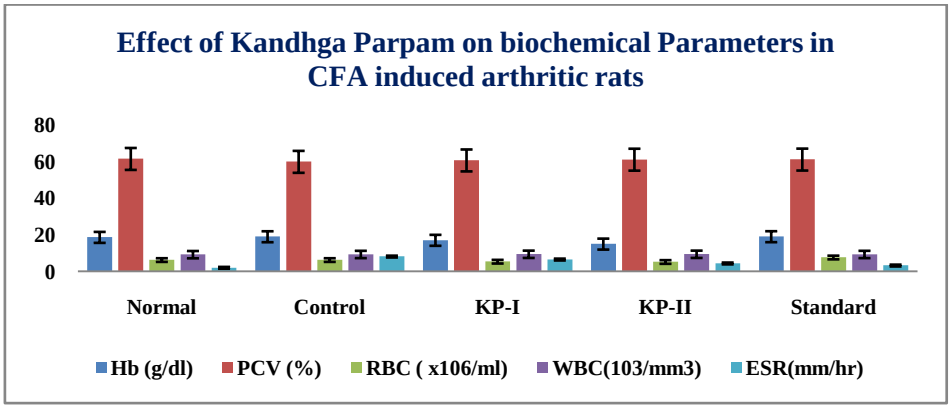


FIGURE 5



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