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Evaluation of a herbal formulation *Kurosani Omam Choornam* for its anti-inflammatory, analgesic and antispasmodic activity in animal models

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Abstract

Background: Siddha system of medicine is a distinct therapeutic science with many single drugs and compound formulations used for treating a broad spectrum of ailments. The Kurosani omam choornam (KOC) is a mono-herbal siddha formulation used in the treatment of ceganavatham (cervical spondylosis). **Objective:** The objective is to assess the therapeutic potential of KOC for its anti-inflammatory, analgesic and antispasmodic activities. **Materials and Methods:** Anti-inflammatory activity was studied using carrageenan induced paw oedema with dose of 100mg/kg and 200mg/kg in rats where indomethacin were used as a standard. 200 & 400 mg/kg was given for the models to study the analgesic activity through eddy's hot plate method and aspirin(100 mg/kg, p.o.) were used as a standard drug. Antispasmodic activity was evaluated through acetylcholine induced contractions in rat ileum. **Results:** The percentage reduction of paw volume against Carrageenan induced paw oedema was more than the control group and the analgesic activity was also in a significant levels. **Conclusion:** The present findings revealed that KOC possesses analgesic, anti-inflammatory and antispasmodic activity, which could be beneficial for the management of Ceganavatham(cervical spondylosis).

Keywords: *Kurosani omam Choornam, Anti-inflammatory, Analgesic, Antispasmodic, Ceganavatham*

Introduction

Medicinal plants are considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. According to the World Health Organisation survey report approximately 80% of the people in developing countries depend on traditional herbal related formulations to cure diseases. Today herbal medicines are in great demand in the developed world for primary health care due their efficacy, safety and lesser side effects. Siddha Medical system is a Dravidian based medicine with its roots in Tamil language speaking regions. The sources of medicines are utilized from herbal, herbo-mineral, metal and animal Kingdom. Of these, the medicines of plant origin are given prime importance and given first hand to treat diseases. Single herbal treatment is one of the specification of siddha system of medicine, there are many single herbal formulation mentioned in siddha text books. Kurosani omam chooranam,

which is a single herbal medicine used in treatment of vatha disease ceganavatham (cervical spondylosis) according to standard siddha literature. The present investigation was undertaken to demonstrate the pharmacological potential of Kurosani omam chooranam by using various animal models and thus to explore the drug for their potent antispasmodic, Anti-inflammatory and Analgesic effect.

Materials and Methods

Collection, authentication and purification

The raw drug was purchased from well reputed drug shop, Thakkalay, Kanyakumari. The raw drug was identified and authenticated by the medicinal botanist, Government Siddha Medical College, Palayamkottai. The trial drug is purified and shade dried. The seeds were powdered and sieved by using a fine cloth and then bottled up.

Table 1. Ingredients of Kurosani omam Choornam.

| S.No. | Tamil Name | Scientific name | Parts used | Quantity |
|-------|---------------|------------------------------|------------|----------|
| 1. | Kurosani omam | <i>Hyoscyamus niger.Linn</i> | Seeds | Q.S |

Experimental animals

Young wistar rats of either sex aged 4-5 weeks, average weight 20-25 gm were used for Anti-inflammatory and analgesic studies. The rats were purchased from the animal house, Nagarkoil. They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDR B formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983). Antispasmodic studies were carried out in rabbits. They were obtained from CAP laps animal house. Rabbits were maintained under standard laboratory conditions of temperature (25± 2°C) and 12/12 hour light/dark

cycle and for anticholinergic study the Rabbits were sacrificed by blow to the head and exsanguinated as per CPCSEA recommended guidelines.

Anti-inflammatory activity

Carrageenan induced rat paw edema

Evaluation of Acute Anti-inflammatory activity Carrageenan induced rat paw oedema the rats were divided into four groups containing six rats in each group. 0.1ml of 1.0% carrageenan in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The trial drug KOC was administered to the rats 1 h before carrageenan injection. Different groups were treated as follows:

Group I : Carrageenan (0.1 ml of 1.0% carrageenan/rat to the sub plantar region).

Group II: Carrageenan (0.1 ml of 1.0% carrageenan/rat to the sub plantar region).+ Indomethacin (10 mg/kg b. w., p. o.)

Group III and IV: Carrageenan (0.1 ml of 1.0% carrageenan/rat to the sub plantar region).+ KOC (200 mg/kg and 400 mg/kg b. w., p. o. respectively).

The paw volume was measured initially and at 1, 2, 3 and 4 h after carrageenan injection, using Plethysmograph, inflammation was calculated.

Analgesic activity

Hot plate method

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II, group-III and group-IV consisting of five Rats in each group for control, positive control and test sample group respectively. Each group received a particular treatment i.e. control (1% Tween-80 solution in water, 10ml/kg, p.o.), positive control (Asprin 100 mg/kg, p.o.) and the test sample (drug of 200 mg/kg, p.o. & 400 mg/kg, p.o. respectively). The animals were positioned on

Eddy's hot plate kept at a temperature of 55 ± 0.5 °C. A cut off period of 15 s (Franzotti *et al.*, 2000) was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples (Eddy *et al.*, 1953; Kulkarni,1999; Toma *et al.*, 2003).

Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean \pm SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons

Antispasmodic Activity

Animals sacrificed and a segment from ileum (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode solution (10 ml) between two stainless steel hooks under 0.5 to 1 g initial tension. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonic transducer. It was continuously aerated and maintained at 37 ± 0.5 °C. The equilibrium period was 60 minutes and the bath solution was refreshed every 15 minutes. After equilibrium period, a dose response curve for histamine in variant molar concentration, by maintaining 45 minutes time cycle was taken separately .

Statistical analysis

Ileum contractions induced by agonist were assumed as 100% and reductions induced by test drug calculated. Percentage of ileum contraction was expressed as mean \pm SEM. Results were analysed using one-way analysis of variance (ANOVA). Probability values less than 0.05 were considered as significant.

(ANOVA) followed by Dunnet "t" test. Differences were considered significant when $P < 0.05$ and very significant when $P < 0.01$.

Results

Anti-inflammatory activity

Table 2. Results of Anti-inflammatory activity of KOC

| | Treatment | 1hr | 2hr | 3hr | 4hr | % of inhibition |
|---------|---|-----------|-----------|-----------|-----------|-----------------|
| group 1 | carrageenan(1% w/v) | 0.92±0.52 | 2.57±0.17 | 2.92±0.52 | 2.94±0.54 | - |
| group 2 | carrageenan(1% w/v) +indomethacin(10mg/kg) | 0.41±0.01 | 1.75±0.35 | 1.94±0.54 | 1.45±0.05 | 50.68 |
| group 3 | carrageenan(1% w/v)+ KOC low dose(200mg/kg) | 0.51±0.11 | 1.78±0.38 | 1.67±0.27 | 1.56±0.16 | 46.93 |
| group 4 | carrageenan(1% w/v)+ KOC high dose(400mg/kg) | 0.52±0.11 | 1.78±0.38 | 1.74±0.34 | 1.52±0.12 | 48.29 |

Values are mean ± SEM (n = 6) (Dunnett' test). *** p < 0.001 when compared to control

The results of the effect of *KOC* on carrageenan-induced edema in hind Paw of rats is given in Table 2. The edema volume increased over time, this increase was greater in the group treated by the saline and lesser in *KOC* 200, *KOC* 400 and indomethacin. These results were significant

compared to the control group (P < 0.001). The percentage of inhibition was 46.93% and 48.36% for *KOC* 200 and *KOC* 400 respectively. In the same condition indomethacin 10mg /kg has a percentage of inhibition of 50.68%.

Analgesic activity

Table 3. Results of Analgesic activity of KOC

| GROUP | DOSE | Mean latency before and after drug administration | | | | % inhibition | | |
|-----------|---------|---|--------------|---------------|----------------|--------------|-------|-------|
| | | 0 min | 30 min | 60 min | 90 min | 30min | 60min | 90min |
| Group I | Vehicle | 3.76 ±0.119 | 3.95±0.986 | 3.66±0.258 | 3.108±0.687 | - | - | - |
| Group II | 100 | 2.84± 0.076* | 6.102±0.295* | 8.127±0.365** | 12.117±1.002** | 35.26 | 54.96 | 74.35 |
| GROUP III | 200 | 3.71± 0.017 | 4.77±0.387* | 6.69±0.376** | 6.97±0.377** | 17.19 | 45.29 | 55.40 |
| Group IV | 400 | 3.67± 0.025 | 4.107±0.672* | 7.84±0.874** | 6.133±0.245** | 11.12 | 53.31 | 49.32 |

Values expressed in mean ±SEM and units in seconds, Significant *p<0.05, **P<0.01 (n=6)

Results of hotplate test are presented in Table for drugs respectively. The drug were found to exhibit a dose dependent increase in latency time when compared with control. At 90 minutes, the percent inhibition of two different doses (200 and 400 mg/kg body weight) was 55.40% & 49.32% respectively.

The results of the effect of koc on pain induced by hot plate method are given in Table 3. As Asprin, KOC 200 and KOC 400 significantly increased percentage of reaction time with dose dependent response.

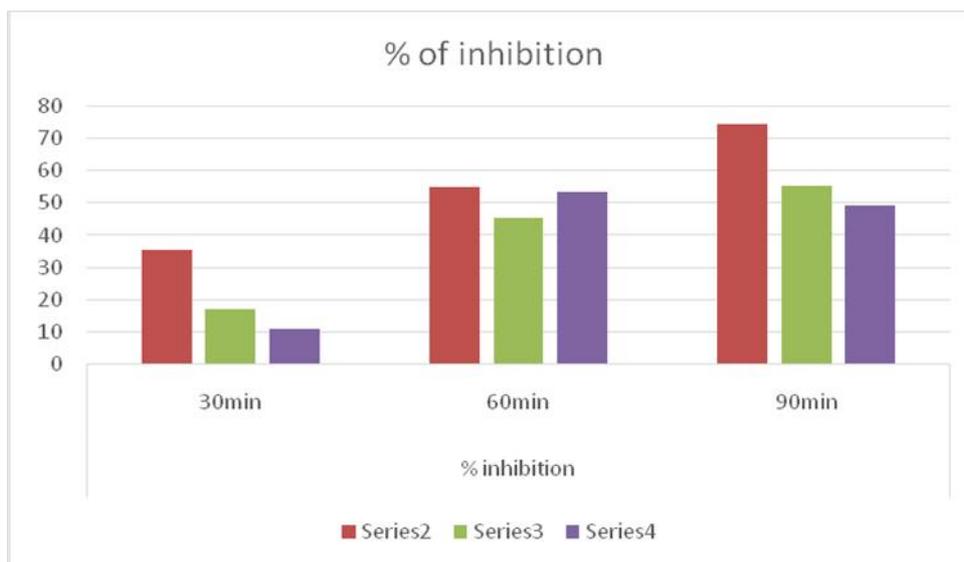


Figure 1. Effect of KOC on Eddy's hot plate method.

Antispasmodic activity

The compounds presence in KOC showed significant inhibition in ACH induced albino rat ileum contraction. KOC reduce the contraction of

albino rat ileum preparation induced by ACH, the real mechanism may be by calcium ions. As the contraction of the KOC was decreased when compared to standard, a more pronounced relaxant effect was observed in Table.

Table 4. Dose Response Relationship Observations of Acetylcholine

| S.No | Concentration/dose | Acetylcholine |
|------|--------------------|---------------|
| | | Response (cm) |
| 1 | 0.1 ml | 2.8 cm |
| 2 | 0.2 ml | 3.1 cm |
| 3 | 0.4 ml | 3.5 cm |
| 4 | 0.8 ml | 3.8 cm |
| 5 | 1.6 ml | 4.1 cm |

Table 5. Dose Response Relationship Observations of Atropine

| S.No | Concentration/dose | Atropine |
|------|--------------------|---------------|
| | | Response (cm) |
| 1 | 0.1 ml | - |
| 2 | 0.2 ml | - |
| 3 | 0.4 ml | - |
| 4 | 0.8 ml | - |
| 5 | 1.6 ml | - |

Table 6. Dose Response Relationship Observations of Acetylcholine and KOC

| S.No | Concentration/dose | Acetylcholine + KOC |
|------|--------------------|---------------------|
| | | Response (cm) |
| 1 | 0.1 ml +0.1 ml | 2.0 cm |
| 2 | 0.2 ml +0.2 ml | 2.6 cm |
| 3 | 0.4 ml +0.4 ml | 2.8 cm |
| 4 | 0.8 ml +0.8 ml | 3.2cm |
| 5 | 1.6 ml + 1.6 ml | 3.6 cm |

Table 7. Comparative Dose Response of Acetylcholine followed by KOC

| S.No. | Treatment | Dose(ml) | Response | % of response |
|-------|--------------------|---------------|----------|---------------|
| 1 | Acetylcholine | 0.1 ml | 2.8 cm | 68.29 |
| 2 | | 0.2 ml | 3.1 cm | 75.60 |
| 3 | | 0.4 ml | 3.5 cm | 85.36 |
| 4 | | 0.8 ml | 3.8 cm | 92.98 |
| 5 | | 1.6 ml | 4.1 cm | 100 |
| 6 | Acetylcholine +KOC | 0.1 ml+0.1 ml | 2.0 cm | 55.55 |
| 7 | | 0.2 ml+0.2 ml | 2.6 cm | 72.22 |
| 8 | | 0.4 ml+0.4 ml | 2.8 cm | 77.77 |
| 9 | | 0.8 ml+0.8 ml | 3.2cm | 88.88 |
| 10 | | 1.6 ml+1.6 ml | 3.6 cm | 100 |

Discussion

The carrageenan-induced hind paw oedema model in rats is known to be the acute inflammatory model sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAID), which primarily inhibit the cyclooxygenase involved in prostaglandin (PG) synthesis. In case of the time course of oedema development in carrageenan induced paw edema model in rats is generally two phases are found. The first phase, which occurs between 0 to 2.5 h of injection of the phlogistic agent, has been attributed to the release of histamine or serotonin. The edema volume reaches to its maximum approximately 3 h post treatment and then begin to decline. The second phase of inflammatory reaction which is measured at 3h is caused by the release of bradykinin, protease, prostaglandin and lysosome. Therefore, it can be inferred that the

inhibitory effect of the extract on the carrageenan induced inflammation could be due to the inhibition of enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Thus, the results of the present study demonstrate that the KOC exhibited acute anti-inflammatory activity in the tested models which was found to be the most effective at higher concentrations employed. The hot plate test is commonly used to test central-mediated anti-nociceptive effects and analgesic effect. The cyclooxygenase pathway promotes inflammatory pain via conversion of AA to PGE2 by COX-2, an important inflammatory mediator. In this study, koc at the dose of 200 and 400 mg/kg significantly increased latency time (during about 60 min in hot plate test) The drug of both the doses showed significant analgesic action compared to the reference drug Aspirin 100 mg/kg was found to exhibit higher analgesic activity.

Conclusion

In conclusion, the KOC showed dose dependent anti-inflammatory activity in carrageenan induced method and has significant analgesic properties in Eddy's hot plate method. The results demonstrate that the test drug was found to more effective in antagonism against ACH at 100µg/ml when compared with the standard antagonistic drug Atropine. From the present findings, it is manifest that the had shown marked anti cholinergic activity in isolated albino rat ileum. And also it is suggested that significantly protected the albino rat against ACH. This study results confirmed the validity of traditional indications of KOC in pain, spasmodic and inflammatory disease conditions.

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