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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF AANAITHIPILI DHRAVAGAM, A SIDDHA HERBO-MINERAL FORMULATION, IN EXPERIMENTAL RAT MODELS

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ABSTRACT

Background: Pain and inflammation are the main symptoms of Thandagavadham (lumbar spondylosis). Aanaithipilidhravagam, a Siddha herbo-mineral distillate, has not been examined experimentally. We evaluated its analgesic and anti-inflammatory activity in rats. Materials and Methods: The dhravagam was prepared from Scindapsus officinalis fruit, purified potassium nitrate, purified potash alum and Trachyspermumammi by classical distillation. Analgesic activity (Eddy's hot plate) and anti-inflammatory activity (carrageenan paw oedema) were studied in rats (n = 6 per group) at 100 and 200 mg/kg orally, against pentazocine (30 mg/kg) and indomethacin (40 mg/kg). Data were analysed by one-way ANOVA with Dunnett's test. Results: Vehicle-treated rats had hot plate reaction times of 1.5–1.8 s. At 60 min, reaction time was 5.1 ± 0.6 s (100 mg/kg) and 7.4 ± 0.7 s (200 mg/kg) with the dhravagam, against 10.8 ± 0.9 s with pentazocine, and was longer with the higher dose at every time point. In carrageenan controls, paw measurement rose from 1.21 ± 0.17 to 2.62 ± 0.17 mm at 5 h; at 5 h it was 1.60 ± 0.34 mm (100 mg/kg), 1.20 ± 0.12 mm (200 mg/kg) and 1.15 ± 0.16 mm with indomethacin. Conclusion: Aanaithipilidhravagamshowed dose-related analgesic activity and reduced carrageenan-induced paw oedema in rats, supporting its use in painful inflammatory conditions such as Thandagavadham. The mechanism and the ingredients responsible were not investigated.

Keywords: Aanaithipilidhravagam, Siddha medicine, Thandagavadham, Analgesic, Anti-inflammatory, Hot plate, Carrageenan

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INTRODUCTION

Lumbar spondylosis is a degenerative condition of the lumbar spine in which pain, stiffness and restricted movement are the dominant complaints.[1] In Siddha medicine it corresponds to Thandagavadham, one of the vatha diseases, and its management combines internal medicines, external applications and varmam therapy.

Aanaithipilidhravagam is a herbo-mineral distillate described in YakoppuVaithiya Sinthamani.[2] It is prepared from the fruit of Scindapsus officinalis (Roxb.) Schott (Aanaithipili), purified potassium nitrate (vediuppu) and potash alum (seenam). The seeds of Trachyspermumammi (L.) Sprague (omam; synonym Carum copticum) are added during preparation of the distilled medium (pugaineer) that is used to process the fruit.

Experimental reports on the individual ingredients are limited. The fruit of S. officinalis has shown analgesic and anti-inflammatory activity in rodent models[3] and antioxidant activity in vitro,[4] and extracts of T. ammi (reported as C. copticum) have shown analgesic[5] and antispasmodic[6] effects. These reports concern crude extracts of single ingredients. To our knowledge, the pharmacological activity of the formulation made by the classical distillation method has not been reported.

Because pain and inflammation are the principal problems in Thandagavadham, we evaluated the analgesic activity (Eddy's hot plate method) and the anti-inflammatory activity (carrageenan-induced rat paw oedema) of Aanaithipilidhravagam at two dose levels. The work formed part of the pre-clinical evaluation of the drug before its clinical use, alone and with varmam therapy, in patients with lumbar spondylosis. (CTRI/2020/03/024166).[7]

MATERIALS AND METHODS

Preparation of Aanaithipilidhravagam

Source and authentication. The raw drugs were purchased from raw drug stores in Chennai and authenticated by the Department of Gunapadam, Government Siddha Medical College, Chennai. The drug was prepared in the Gunapadam laboratory of the same institution following the method in YakoppuVaithiya Sinthamani.[2]

Ingredients and purification. The ingredients were S. officinalis fruit (500 g), potassium nitrate (350 g), potash alum (525 g) and T. ammi seeds (quantity sufficient). The fruit and seeds were cleaned of dust and debris. Potassium nitrate was dissolved in four parts of water and boiled, four egg whites were added and the scum was removed, and the solution was filtered and left undisturbed for a day; the salt was then sun-dried, and the whole process was repeated seven times. Potash alum was dissolved in water, filtered and sun-dried.

Preparation. The purified potassium nitrate and potash alum were powdered and heated in a distillation apparatus. A small first fraction was discarded and the remaining distillate (pugaineer) was collected and measured. T. ammi seeds, equal to half the volume of the distillate, were soaked in it for three days, and the mixture was distilled again. The fruit of S. officinalis (500 g) was powdered, 2 L of the pugaineer was added, and the mixture was allowed to ferment and was then distilled in the dhravaga apparatus. The distillate (dhravagam) was stored in a clean, dry, airtight glass container.

Experimental animals and ethics

Adult Wistar rats weighing 200–250 g were used. Food was withdrawn the night before each experiment. The experiments were carried out under the approval of the Institutional Animal Ethics Committee (see Declarations). Details of the sex, source, housing conditions and acclimatisation period of the animals were not recorded in the available study records.***Analgesic activity***

Analgesic activity was assessed by Eddy's hot plate method.[8] Twenty-four rats were divided into four groups of six: vehicle control (0.5% carboxymethylcellulose, orally), pentazocine (30 mg/kg, intraperitoneal, standard), and Aanaithipilidhravagam at 100 mg/kg and 200 mg/kg, orally. Each animal was placed individually on Eddy's hot plate maintained at 55 °C, and the latency to a nociceptive response (paw licking or jumping off the plate) was recorded at 15, 30, 45 and 60 min after administration of the drug or vehicle.

Anti-inflammatory activity

Anti-inflammatory activity was assessed by carrageenan-induced paw oedema.[9] Thirty rats were divided into five groups of six: Group I, vehicle control (3% gum acacia, 10 mL/kg, orally); Group II, carrageenan control (0.1 mL of 1% w/v carrageenan suspension); Group III, indomethacin (40 mg/kg, orally); and Groups IV and V, Aanaithipilidhravagam at 100 mg/kg (low dose) and 200 mg/kg (high dose), orally. All drugs were given in a constant volume of 10 mL/kg, one hour before carrageenan (0.1 mL of 1% w/v suspension) was injected into the sub-plantar region of the left hind paw of the animals in Groups II–V. Paw size up to the tibiotarsal joint was measured at 0, 1, 2, 3, 4 and 5 h with a plethysmometer (Model 7150, UGO Basile, Italy).

Statistical analysis

Values are expressed as mean ± deviation as recorded in the original study (n = 6 per group). Differences among groups were analysed by one-way analysis of variance followed by Dunnett's test.

RESULTS

Characteristics of the formulation. Aanaithipilidhravagam was a pale yellow, strongly aromatic liquid with a pH of 4.8, a specific gravity of 1.021 g/cm^3 at 25 °C, a boiling point of 50 °C, a negligible essential oil content and a viscosity of 0.5833 mPa·s. Lead was not detected; arsenic, cadmium and mercury were within the limits of 3.0, 0.3 and 1.0 ppm respectively. No pesticide residue was found, and Escherichia coli, Salmonella, Staphylococcus and Pseudomonas were absent. The shelf life was more than 12 months.

Analgesic activity.

The reaction time of the vehicle control group stayed between 1.5 and 1.8 s throughout the observation period (Table 1). Pentazocine raised the reaction time from 5.6 ± 0.2 s at 15 min to 10.8 ± 0.9 s at 60 min. Aanaithipilidhravagam raised the reaction time in both dose groups, and the effect increased with time: from 2.8 ± 0.8 s to 5.1 ± 0.6 s at 100 mg/kg and from 4.2 ± 0.8 s to 7.4 ± 0.7 s at 200 mg/kg. At each time point the 200 mg/kg group had a longer reaction time than the 100 mg/kg group, and both were shorter than the pentazocine group. Statistical analysis was reported in the original study as one-way ANOVA followed by Dunnett's test, but the individual p-values were not available, so the differences described above are based on the mean values.

Effect of Aanaithipilidhravagam (ATD) on reaction time (s) in Eddy's hot plate test in rats.

Group	Dose (mg/kg)	15 min	30 min	45 min	60 min
Vehicle control	0.5% CMC	1.6±0.8	1.8±0.3	1.5±0.8	1.8±0.3
Pentazocine	30	5.6±0.2	8.2±0.3	8.9±0.8	10.8±0.9
ATD, low dose	100	2.8±0.8	3.23±0.8	4.62±0.6	5.1±0.6
ATD, high dose	200	4.2±0.8	4.9±0.3	5.8±0.3	7.4±0.7

n = 6 per group. Values are mean ± deviation as recorded in the original study. Analysis in the original study: one-way ANOVA followed by Dunnett's test; p-values not available.

Anti-inflammatory activity.

In the carrageenan control group, paw measurement increased steadily from 1.21 ± 0.17 mm at baseline to 2.62 ± 0.17 mm at 5 h (Table 2). In the indomethacin group it was 2.10 ± 0.26 mm at 1 h and fell to 1.15 ± 0.16 mm at 5 h. With 100 mg/kg of the dhravagam it was 1.56 ± 0.12 mm at 1 h, reached 1.74 ± 0.24 mm at 3 h and was 1.60 ± 0.34 mm at 5 h. With 200 mg/kg it was 1.96 ± 0.32 mm at 1 h and fell at each later reading to 1.20 ± 0.12 mm at 5 h. At 5 h both dose groups were below the carrageenan control group, and the 200 mg/kg value was close to those of the indomethacin group (1.15 mm) and the vehicle control group (1.20
mm). Baseline paw measurements differed between groups, from 1.01 to 1.44 mm. As for the hot plate test, the p-values from the Dunnett's comparisons were not available in the original records, so the differences described above are based on the mean values.

Effect of Aanaithipilidhravagam (ATD) on paw measurement (mm) at different times after carrageenan in rats.

Group	0 h	1 h	2 h	3 h	4 h	5 h
I Vehicle control	1.20±0.14	1.20±0.14	1.20±0.14	1.20±0.14	1.20±0.14	1.20±0.14
II Carrageenan control	1.21±0.17	1.91±0.21	2.27±0.02	2.37±0.14	2.48±0.18	2.62±0.17
III Indomethacin 40 mg/kg	1.01±0.06	2.10±0.26	1.56±0.15	1.47±0.05	1.34±0.18	1.15±0.16
IV ATD 100 mg/kg	1.44±0.13	1.56±0.12	1.62±0.16	1.74±0.24	1.63±0.12	1.60±0.34
V ATD 200 mg/kg	1.24±0.42	1.96±0.32	1.84±0.34	1.64±0.44	1.61±0.18	1.20±0.12

n = 6 per group. Values are mean ± deviation as recorded in the original study. Analysis in the original study: one-way ANOVA followed by Dunnett's test; p-values not available.

DISCUSSION

Aanaithipilidhravagam raised the hot plate reaction time and lowered carrageenan-induced paw oedema in rats, and the hot plate response increased with dose. The hot plate test measures the response to thermal pain and is widely used to detect centrally acting analgesics, and pentazocine, the standard used here, is a centrally acting opioid analgesic.[8] The result therefore suggests that the dhravagam may act on pain processing, although the present experiments cannot show where or how.

Paw oedema after carrageenan develops in phases; the early phase is attributed mainly to histamine and serotonin and the later phase, from about the third hour, mainly to prostaglandins.[9] In this study the difference between the dhravagam groups and the carrageenan control group was clearest at the later readings, particularly with 200 mg/kg. That pattern is compatible with an effect on the later phase, but it is a hypothesis only, because no mediator or enzyme assay was done.

The results fit with earlier work on the ingredients. The fruit of S. officinalis has been reported to have analgesic and anti-inflammatory activity in rodents,[3] and T. ammi extracts have been reported to have analgesic[5] and antispasmodic[6] effects. However, these studies used crude extracts, whereas the dhravagam is a distillate made with potassium nitrate and potash alum, and it is not known which constituents pass into it. As the individual ingredients and the intermediate pugaineer were not tested alongside the final product, the contribution of each cannot be separated.

Pain and inflammation are the main symptoms that bring patients with Thandagavadham to treatment. The activity seen here gives a pharmacological basis for the clinical use of the drug, but results in rats do not by themselves show benefit in patients, and the doses used in rats cannot be converted directly to the human dose of 2 mL twice daily.

LIMITATIONS

This was a small pre-clinical study with six animals per group, two dose levels, one standard drug per model, and one thermal pain model and one inflammation model. Baseline paw measurements differed between groups, and the vehicle control group showed identical values at every time point. Randomisation, blinding and sample-size justification were not reported, no phytochemical or mechanism studies were done, and the ingredients were not tested separately. The work is reported retrospectively from the original records, so several details expected under the ARRIVE guidelines were unavailable: the sex, source, housing and acclimatisation of the animals, the hot plate cut-off time, the basis for expressing the dose in mg/kg, the unit of paw measurement, and the groups that received carrageenan. Whether the dispersion values were standard deviation or standard error, the reference group for the comparisons, and the exact p-values were also unavailable. The findings are therefore described as trends. They should be confirmed in a fully reported study with more doses, additional models, ingredient-wise controls and chemical profiling.

CONCLUSION

Aanaithipilidhravagam showed dose-related analgesic activity in the hot plate test and reduced carrageenan-induced paw oedema in rats. These findings support its traditional use in painful inflammatory conditions such as Thandagavadham, and they justify further work on its constituents and mechanism of action.

Declarations

Ethics approval: The animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of C. L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai (57th IAEC meeting; IAEC No. LVII/02/321/PO/Re/S/22/CPCSEA, dated 15 May 2020), for the project "Preclinical evaluation of anti-inflammatory and analgesic activities of Siddha herbo-mineral formulation Aanaithipilidhravagam: in vitro and in vivo studies".

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