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Research Article

ANTI-INFLAMMATORY ACTIVITY OF AN AYURVEDIC HERBO-MINARAL FORMULATION: CHANDRAPRABHA VATI

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ABSTRACT

The study investigated the anti-inflammatory activity of an Ayurvedicherbo-mineral formulation, Chandra prabhavati (CV) which consist of 37 ingredients, in order to justify its claimed anti-inflammatory action, and also its underlying main anti-inflammatory mechanisms. Acute anti-inflammatory activity of CV was assessed using rat carrageenan- induced paw oedema test (doses tested: 1000, 2000 and 4000mg/kg, day following oral administration) and chronic anti-inflammatory activity with rat formaldyhyde-induced paw oedema assay (doses tested: 1000, 2000 and 4000mg/kg/day for 7 consecutive days). The results revealed that CV possesses marked and significant ($P<0.05$) acute (interms of simultaneous suppression of initial, (by 43.7%) maintance (by 47.6%) and late (36-56%) phases of carrageenan- induced paw oedema test) and chronic (interms of inhibition of oedema in formaldyhyde test) anti-inflammatory activity. These anti-inflammatory activities were dose-dependent and comparable to reference drug, indomethecin. In addition, CV showed marked and significant ($P<0.05$) anti-histamine activity (as determined by wheel test) and prostaglandin inhibitory activity (when tested in mouseenteropool assay). Together, these findings overly justify, for the first time, the recommendation of CV for inflammatory conditions in Ayurvedic medicine.

INTRODUCTION

Chandraprabhavati (CV) is a very popular Ayurvedicherbo-mineral preparation consisting of 37 ingredients which is often recommended to have a young looking wrinkle free glowing skin and to treat several diseases (Shastri, 2002). These include diseases of skin, urinary system, respiratorysystem, gastrointestinal system, eye, teeth. Besides, it is also claimed to possess pain relieving and anti-inflammatory properties (Shastri, 2002). However, as yet, this claimed anti-inflammatory activity of CV has not been scientifically tested and validated. Accordingly, this study was undertaken to investigate the anti-inflammatory potential of CV and to justify its use as an anti-inflammatory Ayurvedicherbo-mineral formulation, using well established rodent models.

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MATERIALS AND METHODS

Preparation of *ChandraprabhaVati*

Rhizomes of *Acoruscalamus, Zingiberofficinale* and *Curcuma longa,* tubers of *Cyperusrotandus*, whole plant of *Berberisaristatam*, *Andrographisp aniculata* and *Tinosporacordifolia,* heart wood of *Cedrusdeodara*, roots of *Ipomeaturpethum, Aconite heterophyllium, Plumbagozeylanica* and *Baliosperummontanum* fruits anddried spikes of *Piper longum*, fruits of *Coriandrumsativum, Terminaliabelarica, Terminaliachebula, Emblicaofficinale, Emblicaribe, Scindasusofficinalis, Piper nigrum, Piper cheba* and *Elettariacardomomum,* outer covers of *Cinnamomumzeylanicum* and stemandsugar were purchased from a registered Ayurvedic drug sales outlets in Colombo, Sri Lanka. These were identified and authenticated by Head of the Department of MateriaMedica, Institute of Indigenous Medicine, University of Colombo, Sri Lanka and shade dried

for 3 days. Leaves of *Cinnamomumtamala* and 5 salts (Rock salt, Black salt, Ammonium chloride, Sodium chloride and Potassium carbonate), metal ashes (Ferrum and Copper), Shilajatu and Guggulu were purchased from the Indian Medical practitioners co-operative pharmacy and stores, LT, Chennai, India. These materials were also identified and authenticated by the Head of the Department of MateriaMedica. Voucher specimens were deposited at the Institute of Indigenous Medicine University of Colombo, Sri Lanka. Using appropriate weights of each of these ingredients of CV was made according to the details description given in the Ayurveda Pharmacy text (Nagodavithana, 2001) at pharmacy of theInstitute of Indigenous Medicine, University of Colombo, Sri Lanka, under the supervision of Head of the Department of Materia Medica.

Experimental animals

Healthy adult cross bred male albino rats (200-225g) and CRI male mice (35-40g) from our own colony were used. They were kept under standard environmental conditions (temperature: 28-31^{0}C, photoperiod: approximately 12h natural light per day, relative humidity: 50-55%). The animals were fed withpelleted food (Ceylon Grain Elevators, Colombo, Sri Lanka) and clear drinking water *ad labidum*. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guidelines and rules of the Faculty of Science, University of Colombo, Sri Lanka. Further, ethical clearance (Registration No; ERC 12/07) was obtained from the Ethical Committee of the Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Anti-inflammatory activity

Carrageenan –induced paw oedema

Forty four male rats were selected and randomly divided into five equal groups (n=9/group). These rats were orally treated with 1000, 2000 and 4000mg/kg of CV, 5ml/kg water and 5mg/kg indomethacin (State Pharmaceutical Corporation, Colombo, Sri Lanka) respectively (Forestieri *et al.*, 1996). After 1 h, 0.05ml of 1% carrageenan (Sigma Chemical Company, St. Louis, MO, USA) suspension was injected subcutaneously into the planter surface of the left hindpaw as described by (Winter *et.al*. Forestieri *et al.,* 1996). The paw volume of these rats were measured using a Plethysmometer (Letica Scientific Instruments, Barcelona, Spain) at 1 h before, and 1,2,3,4 and 5h after the carrageenan injection and the paw oedema was calculated.

Formaldehyde – induced paw oedema

Different group of male rats (n=9/group) were orally treated with 1000 or 2000 or 4000mg/kg/day of CV or 5ml/kg/day of water for 7 consecutive days. After 1h, on days 1 and 3 of treatment, these rats were injected with 0.1 ml of 2% formaldehyde into the foot pad of left hind paw (Ratnasooriya, 2009). Paw oedema was measured 1 h before formaldehyde injection and at 4 h after the injection on day 1 and everyday at 1 h after the treatment for 7 consecutive days.

Mechanisms of anti-inflammatory activity

Antihistamin activity

Twenty seven male rats were randomly divided into three equal groups (n=9/group) and their fur on the posterior left side was shaved. Twenty fours later, rats in the three groups were orally treated in the following manner: group 1; 2000 mg/kg of CV; group2; 5 mg/kg of Chlorpheniramine (State Pharmaceutical Corporation,Colombo, Sri Lanka) (Selye, 1949) and group 3; 5ml/kg of distilled water. After 1 h, these rats were subcutaneously injected with 0.05 ml of 200µg/ml histamine dihydrochloride into the fur removed area of the skin (Spector, 1956) under mild ether anaesthesia and the area of the wheal formed after 1.5min was measured.

Effect on small intestinal secretion

Intestinal secretion was indirectly evaluated by the enteropooling assay described by (Vitali *et al*, 2005). Briefly, 18 mice were randomly assigned into three groups (n=6/group). Mice in group 1 were orally treated with 0.2 ml of Distilled water (DW), group 2 with 0.2 ml of castor oil with 0.2 ml of DW and group 3 with 2000 mg/kg of CV. Forty minutes later, mice in groups 2 and 3 were orally administered with 0.2 ml of castor oil. After 30 minutes, the mice were sacrificed with ether and their small intestines were removed and weighed. The weights were then expressed as mg/20g body weight. The difference in the intestinal weight between the normal control and castor oil treated control was considered as the castor oil-induced accumulation of intestinal fluid.

Qualitative analysis of phytoconstituents

CV was subjected to qualitative analysis for alkaloids, flavonoids, tannins, phenolics, anthroquinone glycosides, sterols, saponins and carbohydrates as described by (Fransworth, 1988).

Statistical analysis

Data are given as means±SEM. Statistical analysis wasmade one-way ANOVA followed by Tukey's Family Error Rate test, $P<0.05$ was considered as significant.Curvilinear regressions were made using Minitab 13 package.

RESULTS

Anti-inflammatory activity

Carrageenan-induced paw oedema test

Compared to the control, treatment with all three doses of CV significantly inhibited the paw oedema at all the time point measured (Table 1); 1st h (by 56%-73%), 2nd h (by 43%-62%), 3rd h (by 47%-61%), 4th h (by 43%-56%) and 5th h (by 36%-43%). Overall, the anti-inflammatory activity of CV was curvilinearly dose dependent ($r^2=1$, $P<0.05$). EC_{50} value of this anti paw oedema action was 2550mg/kg at 1 st hour. Indomethacine also induceda significant ($P<0.05$) impairment of oedema at all time points measured (by 36%-56%).

Formaldehyde -induced paw oedema test

The treatment with 2000 and 1000mg/kg/day of CV for 7 days, significantly (P<0.05) reduced the paw oedema from days 1-7 when compared with the control.In contrast, 4000mg/kg/day dose significantly (P<0.05) reduced the paw oedemaonly on 1st and 2nd days compared to the control (Table 2).The reference drug, indomethacin also significantly (P<0.05) impaired the paw oedema in all seven days.

DISCUSSION

This study accessed the anti-inflammatory activity of a Ayurvedicherbo-minaral formulation, CV, (which consists of 37 ingredients) in rats using two widely used, sensitive, reliable and validated modeles: carrageenan- induced paw oedema test (Winter *et al.,*1962) and formaldyhyde –induced paw oedema test (Ratnasooriya, 2009).

Table 1. Effect of the oral treatment of Chandraprabhavati on the carrageenan-induced paw oedema in rats (mean±SEM, n=6/group)

Dose	Paw volume (ml)				
	1h	2h	3h	4h	5h
Control	0.34±0.01	0.42±0.03	0.57±0.04	0.53±0.03	0.55±0.03
4000mg/kg	0.12±0.05*	0.17±0.07*	0.22±0.02*	0.27±0.01*	0.31±0.02*
2000mg/kg	0.15±0.01*	0.2±0.04*	0.28±0.02*	0.30±0.01*	0.34±0.01*
1000mg/kg	0.09±0.02*	0.16±0.01*	0.23±0.02*	0.26±0.01*	0.31±0.02*
Indomethacin	0.15±0.01*	0.24±0.06*	0.3±0.02*	0.33±0.02*	0.35±0.01*

As compared with control: *P<0.05

Table 2. Effect of oral treatment of Chandraprabhavation the Formaldehyde -induced paw oedema on rats. (mean±SEM, n=6/group)

Dose	Paw volume (ml)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	0.25±0.04	0.86±0.03	0.21±0.03	0.36±0.02	0.32±0.01	0.28±0.02	0.24±0.02
4000mg/kg	0.16±0.02*	0.18±0.02*	0.17±0.01	0.39±0.04	0.36±0.03	0.35±0.02	0.31±0.02
2000mg/kg	0.18±0.01*	0.16±0.01*	0.12±0.01*	0.26±0.02*	0.18±0.02*	0.17±0.03*	0.12±0.01*
1000mg/kg	0.14±0.02*	0.10±0.01*	0.08±0.08*	0.23±0.03*	0.16±0.02*	0.12±0.02*	0.10±0.02*
Indomethacin	0.15±0.02*	0.13±0.02*	0.08±0.02*	0.15±0.01*	0.09±0.01*	0.06±0.01*	0.08±0.01*

As compared with control: *P<0.05

Table 3. Effect of orally administered Chandraprabhavation castor oil-induced enteropooling in mice (small intestinal secretion) (mean±SEM: 6/group)

Treatment	Small intestine weight (mg/20g)	Castor oil induced fluid accumulation
Normal control (water)	716±30.15	-
Castor oil control (0.2 ml castor oil +water)	1098±70.32[a]	382
(0.2 ml castor oil +2000mg/kg Chandraprabhavati)	912±50.21[ab]	196

[a]P< 0.05 compared to normal control, [ab]P< 0.05 compared to castor oil control

Mechanisms of anti-inflammatory activity

Antihistamine activity

In the histamine- induced vascular permeability test, the CV significantly (P<0.05) reduced the area of the wheal by 31.01% (control *vs.* treatment, 68.43±3.01 *vs.* 52.23±5.60mm^2). Also, chlopheniramine the reference drug, significantly (P<0.05) (by 26%) impaired the area of wheal (control *vs.* treatment, 68.43±3.01 *vs.* 50.70±2.74mm^2). As shown in Table 3, oral administration of castor oil significantly (P< 0.05) and profoundly increased (by 53%) the weight of small intestine compared to the normal control group. In contrast, the highest dose of Chandraprabhavatisignificantly (P <0.05) and markedly reduced (by 27%) the castor oil induced intestinal weight gain.

Phytoconstituent analysis

Phytoconstituent analysis of CV showed to presence of akoloides, flavonoids, tannins, phenolics, triterpenoides, sterols and carbohydrates but not saponins and anthroquinone glycosides.

The former test measures acute inflammation (excudation phase) (Vinegar *et al.,* 1969) and the latter chronic inflammation (proloferative phase) (Vinegar*et al.,* 1969). The results conclusively demonstrate, for the first time, that CV possess marked and dose-dependent oral acute and chronic anti-inflammatory activity. This is on important experimental finding which overly justifies, for the first time, the recommendation of CV in Ayurvedic medicine for inflammatory conditions. The carrageenan- induced paw oedema model is sensitive to most clinically effective anti-inflammatory drugs and consists of two phases with a maintenance phase in-between (Vinegar*et al.,* 1969). The initial phase (1-2h) is primarily mediated by release of histamine and serotonin (Antonio, 1998), but platelet activating factor and arachidonic acid metabolites including prostaglandins also play a role (Antonio, 1998). The late phase (3-5h) is linked to the infiltration of phagocytic cells, polymorphonuclear cells, monocytes, mocrophages, prostaglandins and other autocoidsproduced by tissue macrophages, prostaglandin of oxygen free radicals, nitric oxide, proteolytic enzymes and platelet activating factor (Boughton-Smith *et al*., 1993, Bouriche*et al*., 2003).

The maintance phase (1-2h) which is between initial and late phases is thought to be due to the release of kinin-like substances, especially bradykinin (Vinegar *et al.,* 1969). CV, in this study, significantly impaired all these three phases simultaneously. Curtailment of the initial phase of the carrageenan-induced paw oedema test by CV can be attributed, at least partly, to its antihistamine activity (histamine receptor blockage and/or inhibition of histamine synthesis or its release): CV exhibited marked antihistamine activity, comparable to the reference drug chlopheniromine when evaluated by the wheel test (Spector, 1956). This effect can be attributed to triterpinoids in CV: triterpenoids are known to impair histamine release from mast cells and exert anti-inflammatory activity (Janaki*et al*.1999). CV also reduced the small intestinal weight in the castor oil experiment (enteropooling assay). This suggests inhibition of prostaglandin synthesis and/or its receptor blockage induced by CV (Vitali*et al.,* 2005, Guuakkanru*et al*., 2005).

Obviously, this mechanisms is likely to play a substantial role in reducing the initial phase of the carrageenan- induced paw oedema evident in this study. Interestingly, it is now recognized that expression of COX 1 (type of cycloxyganase enzyme) is maximal in the initial phase (Seilber*et al*., 1994). Hence, the inhibition of the initial phase of oedema by CV may indicate its cox-1 inhibitory activity. CV contained flavonoids and tannins which are known to inhibit cyclooxygenease activity (Carlo *et al*., 1999). However, at present, it is unknown whether CV has serotonin inhibitory activity but it is not unreasonable to speculate that CV has such activity since it contains 37 ingredients of which 25are herbal. CV also suppressed themaintenance phase of the carrageenan-induced paw oedema assay. This suggests that CVhas kinin synthesis and/or release inhibitory activity.

CV prominently inhibited the late phase of the carrageenan induced paw oedema test. This can be mediated by several mechanisms. This phase is sensitive to antioxidants (Boughton-Smith *et al*., 1993). We have previously shown that CV has marked and dose-dependent anti-oxidant activity (Weerasekera*et al*., 2013). Obviously, this antioxidant action of CV can be linked to its anti-inflammatory action in the late phase. As mentioned earlier, prostaglandins are also involved in the late phase of the paw oedema test and its depression induced by CV could be attributed, at least partly, to inhibition of prostaglandin synthesis and/or receptor blockage: since, CV reduced small intestinal weight in enteropooling study. Further, it is possible that this CV induced inhibition of late phase is mediated via impairment of COX 2 expression. Since its expression is maximal during the late phase of carrageenan induced paw oedema assay (Seilber*et al*., 1994). In addition to these specific mechanisms, several other nonspecific mechanisms may account for the simultaneous and almost equal inhibition of all the three phases of the carrageenan induced paw oedema assay by CV. Diuresis is one such mechanism (Barrachina*et al.,* 1995) CV is shown to possess marked diuretic activity in rats (Ratnasooriya*et al*., 2014) and this mechanism is likely to be operative in this study. In this formaldyhyde induced paw oedema test, low and mid doses of CV impaired oedemaat all the seven days of treatment while the higher dose was effective only on first and second days of treatment. Nevertheless, overallresults show that CV has promising chronic anti-inflammatory activity.

It is likely that the mechanisms responsible for the acute anti-inflammatory activity CV isresponsible for the chronic anti-inflammatory activity as well. In addition, CV could induced release of glucocorticoids (Spectar, 1969) and/or have glucocorticoidemimetic activity (since it contained steroidal constituents) which could account for chronic anti-inflammatory action. Sub chronic oral treatment of CV has been shown to be well tolerated (Ratnasooriya*et al*., 2014). Considering all these facts it can be concluded that CV has considerable anti-inflammatory activity as is claimed in Ayurvedic medicine.

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