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Original Research Article

Comparative Analgesic and Anti-inflammatory Activities of Two Polyherbal Tablet Formulations (Aujaie and Surangeen) in Rats

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Abstract

Purpose: *To investigate the analgesic and anti-inflammatory activities of two herbal polymedicines - Aujaie and Surangeen to ascertain their therapeutic claims.*
Methods: *A total of 96 rats were divided into two equal groups; one for determination of anti-inflammatory activity and the other for analgesic activity. Anti-inflammatory and analgesic activities were evaluated by carrageenan-induced paw edema and formalin-induced paw licking test, respectively. For both studies, group I (untreated control) received 1 ml/kg, (po) of gum suspension 1 h before carrageenan injection. Aspirin (100 mg/kg, po) was given to group II (treated control) before injection. Groups III, IV and V were administered orally aujaie (3, 4 and 5 mg/kg, po, respectively), while surangeen tablets (10, 20 and 40 mg/kg, po) were given to groups VI, VII and VIII, respectively. Pain was experimentally induced by injecting 0.1 ml of 2.5 % formalin (40 % formaldehyde in distilled water) via the subplantar region of the left hind paw.*
Results: *Significant ($p < 0.05$) anti-inflammatory activity for aspirin (group II as well as for groups III - VIII with paw edema inhibition (PDI) ranging from 24.6 - 90.2 %, There was significant (($p < 0.05$) analgesic activity in group II, VI and VII while in groups III - V and VIII the activity was insignificant ($p < 0.05$).*
Conclusion: *Aujaie and surangeen tablets exhibited pronounced analgesic and anti-inflammatory activities in rats depending on the dose employed.*

Keywords: *Aujaie, Surangeen, Anti-inflammatory, Analgesic.*

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INTRODUCTION

In Pakistan, Hakims (herbal practitioners) use various local herbal polypharmaceutical preparations for the treatment of different types of rheumatic diseases. Although the use of these products has a sound tradition and rational background according to herbal system of medicine, it is essential to investigate the validity by scientific methods [1-5]. Such studies can help to determine their therapeutic usefulness. Aujaie and Surangeen tablets tablets have been used in traditional (Unani) system of medicine for the treatment of inflammation and pain associated with rheumatoid arthritis and osteo-arthritis [6]. Surangeen tablet is a herbal polypharmaceutical formulation containing extracts of 6 medicinal plants. Each tablet contains *Withania somnifera* 31.79 mg, *Colchicum luteum* 31.79 mg, *Styrax Benzoin*

Dryand 31.79 mg, Ammonium chloride 31.79 mg, *Terminalia chebula* 31.79 mg and *Smilax china* 31.79 mg [6]. Aujaie tablet is another herbal polypharmaceutical formulation containing extracts of 9 medicinal plants. Each tablet contains *Calchicum luteum* 25 mg, *Withania somnifera* 20 mg, *Zingeber officinalis* 20 mg, *Aloe indica* 10 mg, *Curculigo orchioides* 10 mg, *Ptychotis ajowan* (Ajwain) 10 mg, *Pimpinella anisum* 10 mg, *Balsamodendron mukul* 5 mg and *Pistacia lentiscus* 5 mg [5-7].

Therefore, the objective of this study was to evaluate and compare the anti-inflammatory and analgesic activities of the aforementioned two herbal poly-pharmaceutical preparations in rats.

EXPERIMENTAL

Materials

Aspirin (Reckitt Benckiser, Pakistan) and formalin (37 % formaldehyde solution, Merck, Germany) were purchased from local pharmacy (Servier Pharmacy, Sargodha). Carrageenan (Sigma, USA) and gum tragacanth (Merck, Germany) were procured through local sources (Ahmad Dealers, Lahore). The tested herbal poly-pharmaceutical tablet preparations aujaie (Hamdard Laboratories, Karachi, Pakistan) and surangeen (Qarshi Laboratories, Lahore, Pakistan) were purchased from local pharmacy (Servier Pharmacy, Sargodha).

Animals and drug administration

Wister albino rats of either sex weighing 200-250 g were used. 96 animals were used, 48 for each experiment, i.e., analgesic and anti-inflammatory studies. These 48 rabbits were sub-grouped as shown in Table 1. The animals were housed in standard polypropylene cages and were kept under controlled room temperature (25 ± 10 °C, relative humidity 60 – 70 %) and fed with standard laboratory diet with water *ad libitum*. Two sets of eight groups of six animals each have been used for the experiments (Table 1). The doses of tablets administered to the animals were selected based on human doses (Table 1) as indicated in Unani literature [3-5]. The animal studies were approved (approval ref no. 33-BP/2009/SU) by the Departmental Ethical Committee and were conducted according to international guidelines [8] as well as the guidelines of the Institutional Animal Ethical Committee [7].

Table 1: Grouping of experimental animals, and doses of control and test drugs (n = 6)

Group	Treatment
Group I (Normal ontrol)	Gum tragacanth solution (1 ml/kg/p.o)[a]
Group II (Standard control)	Aspirin (100 mg/kg, p.o.)[a,b,c]
Group III (Aujaie)	3 mg/kg, p.o.
Group IV (Aujaie)	4 mg/kg, p.o.
Group V (Aujaie)	5 mg/kg, p.o.
GroupVI (Surangeen)	10 mg/kg, p.o.
GroupVII (Surangeen)	20 mg/kg, p.o.
GroupVIII (Surangeen)	40 mg/kg, p.o.

Note: *[a] [7], [b][8], [c][9].*

Preparation of solutions

Freshly prepared suspensions of all test tablets were prepared by suspending tablet powder in 0.5% suspension of gum tragacanth. 0.5 % (w/v) suspension of gum tragacanth was prepared by dissolving 0.05g of gum tragacanth in 10 ml of distilled water. 1 % (w/v) carrageenan suspension is prepared by dissolving 1g of carrageenan in 100 ml of normal saline. 2.5 % formalin solution was prepared by dissolving 6.8 ml of 37 % formalin in 100 ml of distilled water.

Evaluation of anti-inflammatory activity

The animals were fasted for 24 h with free access to water prior to experiments. Approximately 100 µl of 1 % carrageenan suspension (prepared 1 h before each experiment) was injected into the plantar surface of the right hind paw of rat [9,10] and the site of injection was marked.

Rats of group I (control group) received only gum tragacanth solution 1 h before carrageenan injection. Similarly, aspirin was given to the group II (standard group). Three different doses of each of the two herbal preparations were given orally to groups III, IV, V, VI, VII and VIII, respectively by gastric lavage. The anterio-posterior diameter of the rat paw was measured at 0, 1, 2 and 3 h intervals after carrageenan injection using vernier calipers (AM13, Emmay, Pakistan) at the marked site. The difference between the basal value of paw diameter and that measured at different time intervals was noted in millimeters and the difference was regarded as degree of edema (inflammation) developed after carrageenan injection [11-16]. Paw edema inhibition (PI) at different doses of test and standard drugs was calculated by comparing with untreated control rats, as in Eq 1 [17].

$$PI\ (\%) = 100\{(Vt - Vo)_C - (Vt - Vo)_T\}/(Vt - Vo)_C \quad \ldots \quad (1)$$

where, V_t is rat paw volume at time t, V_o is initial rat paw volume (basal value), $(V_t - V_o)_C$ is edema produced in the control group and $(V_t - V_o)_T$ is edema produced in the treatment group.

Evaluation of analgesic activity

Three different doses of each of the herbal preparations were given orally by gastric lavage to animals of groups III, IV, V, VI, VII and VIII, respectively. Group I and II animals received gum tragacanth and aspirin suspension, respectively. After 1 h, analgesic activity was determined using formalin-induced paw licking test. 100 µl of 2.5 % formalin was injected into dorsal surface left hind paw. After injecting formalin, the rats were observed for 30 min and the number of lickings observed [19,20]. Analgesic activity was expressed as "none", "mild" and "good" if reduction in number of lickings was < 20, ≥ 20 but < 40 and ≥ 40 % of control, respectively [20].

Behavioral pattern studies

For preliminarily screening of toxic effects of these herbal poly-pharmaceutical preparations, all the treated rats were kept under close observation for one week following the dosing. The symptoms including awareness, mood, motor activity, CNS excitation, posture, motor inco-ordination, muscle tone and reflexes were recorded for 7 days [4]. Any mortality occurring during next two weeks were also recorded.

Statistical analysis

Anti-inflammatory and analgesic activities were analyzed using Chi-square test with the aid of SPSS, version 13.0 software (IBM, USA), and $p < 0.05$ was considered statistically significant.

RESULTS

Anti-inflammatory activity

As shown in Table 2, there was significant ($p < 0.05$) anti-inflammatory activity in the aspirin group (group II) at the doses administered. For groups III – VIII, anti-inflammatory activity after 1 h (paw edema inhibition, PDI) after 1 h was in the range of 78.58 - 90.23) and 3 h and in the range of 24.59 - 59.0 %) after 3 h. However after 2 h, anti-inflammatory activity was significant only in groups IV and V with PDI of 44.62 and 63.39 %, respectively, but was insignificant ($p < 0.05$) in groups III, VI-VIII with PDI of 12.05 - 42.60 %.

Analgesic activity

There was significant ($p < 0.05$) analgesic activity (number of paw lickings) in group II but insignificant in groups III - V and VIII ($p < 0.05$) On the other hand, groups VI and VII showed significant ($p < 0.05$) analgesic activity (Table 2).

Table 2: Anti-inflammatory (in carrageenan-induced paw edema) and analgesic activities (no. of paw lickings) of two herbal products in rats

Activity		Treatment						
		Standard drug	*Aujaie*			*Surangeen*		
		Group II	*Group III*	*Group IV*	*Group V*	*Group VI*	*Group VII*	*Group VIII*
Edema inhibition (%)	1 h	93.91	78.58	86.26	93.64	84.73	90.23	92.87
	2 h	65.45	12.53	44.62	63.39	12.05	41.95	42.60
	3 h	52.45	24.59	52.45	50.81	24.59	37.70	59.01
Number of lickings/0.5 h		114±04	270±32	204±30	203±43	318±42	303±19	258±29

Table 3: Number of rats treated orally with different doses of the herbal preparations with side effects

Toxic/side effect	Aujaie			Surangeen		
	3mg/kg	*4mg/kg*	*5mg/kg*	*10 mg/kg*	*20 mg/kg*	*40 mg/kg*
Dullness	0	1	1	0	1	1
Restlessness	0	0	0	0	0	0
Diarrhoea	0	1	1	1	0	1
Abdominal pain	0	1	0	0	0	1
Tremors/shivering	0	0	0	0	0	0
Excitement	0	0	0	0	0	0
Respiratory distress	1	0	1	0	1	1
Coma	0	0	0	0	0	0
Death	0	0	0	0	0	0

Side effects observed in rats treated orally with different doses of herbal poly-pharmaceutical preparation are presented in Table 3.

DISCUSSION

One of the most widely used primary tests for screening anti-inflammatory activity of drugs is the carrageenan-induced paw edema in rats [17], while formalin-induced paw licking test has been recommended for screening of analgesic activity. The results obtained in the present investigation indicate the potent anti-inflammatory and analgesic activities of both polyherbal, Aujaie and Surangeen.

For anti-inflammatory activity, both the test herbal drugs were administered orally in the recommended doses and prescribed manner. Anti-inflammatory activity was observed from the very first hour and continued to the end of the test in all animal groups. This activity may probably be due to the inhibition of different aspects and chemical mediators (such as kinin, histamine, and 5-HT) of inflammation as established for aspirin [7,9-14].

The results indicate dose-dependent anti-inflammatory activity for both drugs. None to mild anti-inflammatory action of surangeen probably indicates that it was not able to inhibit sufficiently the kinin-like substance responsible for the 2nd hour plateau phase of inflammation [18].
In 3rd hour, both aujaie and surangeen exhibited non-significant anti-inflammatory activity suggesting that they were not able to combat completely prostaglandin release which might be responsible for the last accelerating phase of inflammation as described previously [13]. Histamine and 5-HT are mainly responsible for vasodilatation and increased vascular permeability.

The anti-inflammatory activity of aujaie was not intense in the 2nd and 3rd hour but comparable to that of aspirin indicating that aujaie and aspirin inhibit histamine and serotonin mediated first phase of inflammation but aujaie is less effective in shortening the kinin-mediated plateau interval of 1st phase and the prostaglandin-mediated acceleration phase of inflammation [15]. When kinin release occurs, it activates β_1 and/or β_2 receptors, releasing other inflammatory mediators such as prostaglandins, leukotrienes, histamine, nitric oxide, platelet activating factor and cytokines, among others derived mainly from leucocytes, mast cells, macrophages and endothelial cells, causing either cell influx and plasma extravasations and ultimately prolonging the second phase of inflammation [5]. Therefore, any anti-inflammatory agent that cannot inhibit kinin plateau of 1st phase, will not be able to inhibit 2nd phase of inflammation. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents [18,20]. This was observed in the positive control whereby aspirin significantly reduced edema.

Pain is associated with the patho-physiology of various clinical conditions such as arthritis, muscular pain, cancer and vascular diseases. Formalin induced paw licking is a suitable method for assessing analgesic activity as it is sensitive for various classes of analgesic drugs, therefore, can be used to clarify possible mechanism of the anti-nociceptive effect of an analgesic. Surangeen showed mild analgesic activity in the same manner as anti-inflammatory activity. Like for anti-inflammatory activity, aujaie exhibited higher analgesic activity than surangeen but less than aspirin.

Formalin test is a biphasic phenomenon involving the direct stimulation of sensory nerve endings that ultimately releases inflammatory mediators such as histamine and serotonin in the late phase. Centrally acting drugs such as opioids inhibit both phases equally but peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [19]. The effect of aujaie on the first and second phase of formalin-induced paw licking suggests that its activity may be due to its central action.

CONCLUSION

Both surangeen and aujaie showed significant and consistent anti-inflammatory and analgesic activities in experimental rats probably due to the inhibition of release of histamine, serotonin (5-HT), kinin and prostaglandin. These findings, therefore, support the folkloric use of these polyherbal preparations for the treatment of rheumatism. However, further studies are required to elucidate the exact mechanism(s) of the anti-inflammatory and analgesic activities as well as establish their efficacy and safety for clinical use in rheumatism.

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