Evaluation of antiinflammatory activity of Habbe-Gule-Aakh in albino rat

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Abstract

In the present study, Habbe-Gule-Aakh (HGA)-a Unani pharmacopoeial preparation, was studied for anti-inflammatory effect. Carrageenan-induced rat paw edema test and Cotton pellet implant test were used to determine the effect against acute and sub-acute inflammation, respectively in different groups of albino rats treated with HGA in the dose of 400, 750 and 1000 mg/kg. Diclofenac sodium was used as standard drug. In Carrageenin-induced rat paw edema, the maximum effect was observed at three hours when HGA produced 26.32%, 47.3% and 50% inhibition of inflammation, respectively. In Cotton pellet implant test, it was found to induce inhibition of 30.94%, 47.78% and 49.80% of granuloma formation. HGA demonstrated significant anti-inflammatory activity in both acute and sub-acute inflammatory condition. The effect in sub-acute inflammation was found more pronounced

Keywords: Habbe-Gule-Aakh, Anti-inflammatory activity, Carrageenin, Granuloma

Introduction

The arthritis is among the most prevalent conditions and one of the largest health care problems, as about one third of all adults over the world suffer from one or other form of arthritis (Denqueker, 1999). Since it is a disease of varied etiology and diverse pathological and symptomatic manifestation that transform soon into chronic stage therefore its management is complex and frequently ends up in a poor prognosis. Despite tremendous development in understanding the complexities of the disease and the rationale of its treatment, in allopathic medicine, successful treatment and cure are still elusive (Gaitonde *et al*., 2000). The partial success to the extent of symptomatic relief claimed, appears to be achieved at very high cost, considering the adverse effect of the medications used in the treatment of various forms of arthritis (Chopra *et al*., 1994). That is why even the practitioners of modern medicine at times prescribe drugs of alternative medicine without necessary understanding of their role (Gaitonde *et al.*, 2000) and about 68% of the patients of chronic arthritis have been estimated to approach non-allopathic physicians for the treatment (Shekhran *et al.*, 2000). Inflammation is the hallmark symptom of arthritis and many other joint diseases and most of the therapeutic regimens evolve antiinflammatory and analgesic attributes of a drug intended to be used in joint disorders. Unani Medicine claims to possess a number of effective

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and safe drugs useful in the treatment of arthritis. These drugs are in use since centuries in various forms of arthritis with a good recuperation rate and without any major side effect. One such pharmacopoeal preparation is Habbe Gule Aakh (HGA) which has been described to possess analgesic and antiinflammatory effect; useful in arthritis and other inflammatory conditions of joints (Lubhaya, 1979; Khan, ynm). It contains four ingredients viz. Gule Aakh (flowers of *Calotropis procera* R. Br.) Zanjabil (*Zingiber officinale* Rosc.), Filfil Siyah (*Piper nigrum* Linn) and Barge Bans (leaves of *Bambusa arundinaceae*, Retz) in equal proportion. Anti-inflammatory and analgesic activity of all the ingredients have been reported in certain recent studies (Jana *et al*., 1993; Mascolo *et al*., 1988; Muniappan *et al*., 2003; Lee *et al.*, 1984). Further, *P. nigrum* is a known bioavailability enhancer (Shoba *et al.*, 1997), while *Z. officinale* has anti-ulcerogenic (Kawai *et al.*, 1992) and anti emetic activity (Kawai *et al*., 1993), thus the combination is having added value of improving some of the side effects frequently associated with anti-inflammatory agents. In view of the above, HGA was studied for its anti-inflammatory effect in acute and sub acute and experimental models.

Materials and Methods

Drug material

'Zanjabil', 'Filfil siyah' and 'Gule aakh' were procured from Dawakhana Tibbiya College, AMU, Aligarh, while 'Barge-Bans' was procured from Forest Research Institute (F.R.I.), Dehradun (Uttrakhand) (now Indian Council of Forestry Research ICFRI). All four plant materials were identified by Dr. Athar, Associate Professor, Department of Botany, Aligarh Muslim University, Aligarh.

Diclofenac sodium (Dfc) was used as standard drug while distilled water was used as vehicle.

Preparation of test drug

All the ingredients were dried under shade and ground in an electrical grinder to get the powder. Powder of each drug was taken in equal proportion and was put together and mixed. The mixture was then extracted in hydroalcoholic solution (50% each) with the help of Soxhlet apparatus. The extract was filtered and evaporated on a hot plate till it dried. The yield percentage of the extract was calculated with reference to crude drug and it was found to be 40.23%. At the time of administration, fresh suspension of extract was prepared in distilled water and administered orally to the animals with the help of gastric cannula.

Dose

The dose for albino rats was calculated by extrapolating the human dose of HGA by conversion factor of 7 (Freidrich et al., 1966). The dose of extract thus calculated, was found to be 400 mg/kg. Two higher doses i.e., 750 mg/kg and 1000 mg/kg were also used in rats to study the dose dependant response.

Animals

The experiment was carried out on healthy albino rats of Wistar strain weighing 150-200 g of either sex divided in to 5 groups of 6 animals each. Prior to the experiment, the animals were allowed to get acclimatized for one week. They were maintained under standard laboratory conditions throughout the experiment period and were provided with standard diet and water *ad libitum*. They were housed in clean propylene cages and maintained at a temperature of 25 $\pm$ 2^0C and humidity of 45-55% with 12 h light and 12 h dark cycle.

Carrageenin-induced rat paw edema test **(***Winter et al., 1962)*

The thickness of right hind paw of albino rats was measured in ml with the help of a plethysmometer (UGO Basile 7041), by dipping the paw up to the indelible mark just below the knee joint. Liquid displaced by the paw gives the paw volume. Animal in group I, II and III were treated with test drug in the dose of 400 mg/kg, 750 mg/kg and 1000 mg/kg, respectively. The animals in group IV served as control and received vehicle, while the standard drug Diclofenac sodium was given to the animals in group V in the dose of 5 mg/kg, orally. One hour after the drug/vehicle treatment, animals were injected 0.1 ml suspension of lambda type carrageenin in normal saline under the planter aponeurosis of right hind paw. The thickness of paw was again measured at 1,2,3,4 and 5 hours after the injection of carrageenin. The percent inhibition in paw volume in test and standard control was calculated in comparison with plain control group by the formula described by Newbould (1963).

Cotton pellet implant test **(***Winter and Porter, 1957)*

Sterile cotton pellet (50 mg ± 1 mg) soaked in 0.2 ml of distilled water containing penicillin (0.1 mg) was implanted subcutaneously in axilla, bilaterally, under ether anesthesia in 5 groups of 6 animals each. Animal in group I, II and III were treated with test drug in the dose of 400 mg/kg, 750 mg/kg and 1000 mg/kg, respectively. Animals in group IV served as control and received distilled water, while the standard drug diclofenac sodium (5 mg/kg) was given to the group V, daily for 10 days. On the 11^{th} day the pellet were dissected out. After removal of fat and extraneous tissues, the pellets were

dried overnight at 60^0C and the weight of dried pellets was determined. Mean weight of pellets of different groups was calculated.

Data analysis

Findings were tabulated and analyzed statistically using Student's't' test. P-value less than 0.05 was considered as significant.

Observations and Results

HGA produced a dose dependant inhibition of carrageenin induced paw edema. Oral administration of 400 mg/kg, 750 mg/kg and 1000 mg/kg of HGA produced significant effect at 2 hours that continued till 4 hours but the maximum effect was observed at 3 hours when it produced 26.32% (NS), 47.37% ($p<0.01$) and 50.00% ($p<0.01$) inhibition, respectively as compared to plain control group. Diclofenac sodium (5 mg/kg) showed 82.89% (<0.001) inhibition after the same period (Table 1, Fig. 1). No significant effect was observed after 5 hours of the administration of test drug. Intergroup comparison showed no difference between the two higher doses but the two doses demonstrated significant effect as compared to the lower dose. The effect of the standard drug was also found significantly increased as compared to the all three dose of test drug.

The percentage of inhibition observed in granuloma formation in cotton pellet implant test was 30.94% ($p<0.02$), 47.78% ($p<0.001$) and 49.80% ($p<0.001$) at the dose of 400 mg/kg, 750 mg/kg and 1000 mg/kg, respectively. Diclofenac sodium (5 mg/kg) showed 59.78% ($p<0.001$) inhibition (Table 2, Fig. 2). Intergroup comparison showed no difference between the two higher doses of the test drug. There was significant difference between Diclofenac sodium and the two lower doses of test drug but no difference was found when it was compared with higher dose. Difference was also not found in between the two higher doses of test drug. The lower dose however induced significantly lesser response as compared to the higher dose.

Discussion

Results of the present study demonstrated dose dependant anti-inflammatory activity possessed by HGA. In Carrageenin-induced rat paw edema test the lower dose of the drug did not induced significant response but the two higher doses were found effective. The effect started from the first hour but was found statistically significant at 2, 3 and 4 hours of the study and the peak effect was observed at 3 hours. The effect of two doses was not found significantly different from each other suggesting that the dose of 750 mg is sufficient to

induce the antiinflammatory effect. Thus the finding demonstrated that the two higher doses HGA possess significant antiinflammatory activity in acute inflammatory condition. The effect of diclofenac was found significantly better than the test drug in acute phase of inflammation.

The test drug also produced significant effect against sub-acute inflammation in Cotton pellet implant test. All the three doses of test drug demonstrated a dose dependent decrease in the weight of cotton pellets ($p<0.02$, $p<0.01$ and $p<0.001$). The two higher doses i.e. 750 mg/kg and 1000 mg/kg produced greater response than the lower dose. Further, the effect produced by the two higher doses was comparable with diclofenac sodium suggesting that the test drug is equally effective as that of the standard drug in reducing the sub-acute inflammation. The study also suggests that overall effect of test drug is more pronounced in sub acute condition as compared to the acute phase of the inflammation and even the lower dose of the test drug is effective against sub acute inflammation.

All the 4 ingredients of the HGA are described in Unani literature to possess anti-inflammatory and analgesic (Attar, 1988; Ibne Baitar, 1999). The antiinflammatory activity of Gule-Aakh (Mascolo, 1988), Zanjabil (Vendruscolo, 2006; Penna, 2003) Filfil siyah (Bang, 2009; Kumar, 2007) and Barge-Bans (Muniappan, 2003) has also been reported in several studies. However, the test combination has not been formulated simply by mixing the four antiinflammatory agents but all the ingredients are meant to serve specific purpose in this combination. The dominance of phlegmatic humor or derangement of its quality is an important cause of arthritis (Kabeeruddin, 2009); this combination was formulated to provide symptomatic relief on one hand and remove the causative factors on the other. By virtue of their anti-phlegmatic effect, all the ingredients help to resolve and remove the phlegm from the body (Ibn Sina, 1887) and on account having antiinflammatory and analgesic effect they provide symptomatic relief to the inflamed joint. In addition, *P. nigrum* has been reported to increase the bioavailability of other drugs and *Z. officinale* possesses antiulcerogenic and anti-emetic effect, thus the former help increase the concentration of the drugs at the site of action while the later protect the gastric mucosa from the likely ulcerogenic effect of an anti-inflammatory agent.

Conclusion

In view of the above, it can be concluded that HGA possesses significant anti inflammatory effect in both acute and sub acute phase of inflammation. The effect is dose dependant and is more pronounced in sub acute inflammatory condition. This combination can be used in arthritis and other inflammatory diseases of joints as described in Unani Medicine.

Table 1: Carrageenean-induced rat paw edema test

Groups	1 h			2 h			3 h			4 h			5 h		
	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value
Control	0.62±0.04	-	-	0.70±0.04	-	-	0.76±0.02	-	-	0.70±0.20	-	-	0.65±0.06	-	-
Dfc 5mg/kg	0.23±0.05	62.90	< 0.01	0.15±0.05	78.50	< 0.001	0.13±0.02	82.89	<0.001[a]	0.15±0.04	78.57	< 0.001[a]	0.18±0.04	73.31	<0.001
HGA 400 mg/ kg	0.61±0.05	11.60	N.S	0.59±0.05	15.70	NS	0.55±0.11	26.32	N.S	0.62±0.04	11.43	NS	0.64±0.06	11.54	N.S
HGA 750 mg/ kg	0.50±0.07	17.74	N.S	0.49±0.05	30	<0.01[a,b]	0.40±0.08	47.37	<0.01[a] <0.05[b]	0.42±0.06	40.00	< 0.05[a,b]	0.55±0.06	15.38	N.S
HGA 1000 mg/ kg	0.50±0.05	19.35	N.S	0.42±0.09	40	< 0.01[a,b] < 0.05[c]	0.38±0.06	50.00	<0.01 <0.05[b]	0.40±0.03	42.86	< 0.05[a,b]	0.51±0.11	21.54	N.S

n=6

a= compared with plain control; b= compared with standard control; c= inter comparison between two higher doses

Table 2: Cotton pellet implant test

Groups	Weight of cotton pellet (Mean ± SE)	% inhibition	'P' value
Control	94.00± 5.6	-	-
Standard Dfc 5mg/kg	37.81± 3.4	59.78	<0.001[a]
HGA 400 mg/kg	64.91± 3.16	30.94	<0.02[a] <0.01[b]
HGA 750 mg/kg	49.09 ± 3.89	47.78	< 0.01[a] <0.05[b,c]
HGA 1000 mg/kg	47.19± 3.58	49.80	<0.01[a] <0.05[c]

n = 6
a= compared with plain control; b= compared with standard control; c= compared with the lowest dose

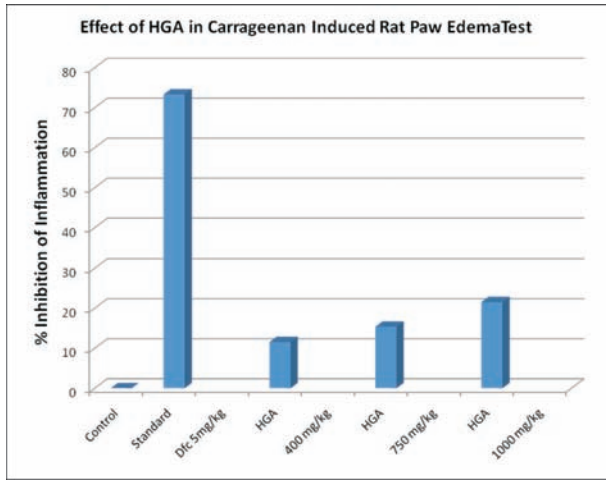

Fig. 1

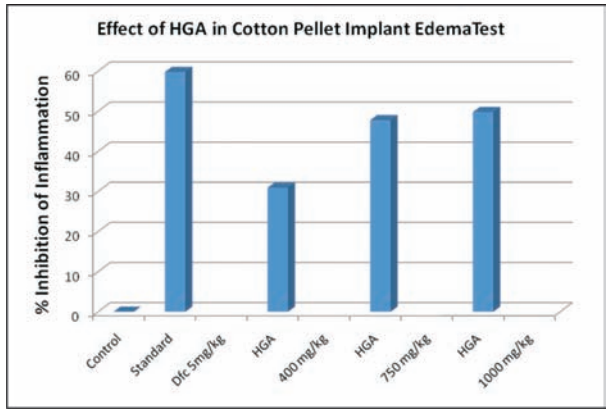

Fig. 2

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