Exploration of Mechanism of Anti-inflammatory Action of Habbe-Gule-Aakh

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Abstract

Habbe-Gul-e-Aakh (HGA) is an important pharmacopoeal drug of Unani medicine commonly used in a number of inflammatory diseases such as arthritis and gout etc. It contains four ingredients of plant origin drugs. All the ingredients have been evaluated individually and shown to possess analgesic, anti-inflammatory and related effects. HGA as such has also been studied on experimental models of acute, sub-acute and chronic inflammation and reported to be safe and effective anti-inflammatory agent. However, its mechanism of anti-inflammatory effect has not been studied so far. Therefore, in the present study HGA was evaluated experimentally to detect its possible mechanism of action.

The study was carried out by inoculating the potent chemical mediators like Serotonin (5-HT), Bradykinin, Histamine and PGE_1, directly in albino rats by subplantar injection to cause edema. The animals were treated with 1000 mg/kg of 50% hydroalcoholic extract of HGA one hour before injecting the edemagen. HGA produced 28.0% ($p<0.02$) inhibition against 5-HT and 41.01% ($P=0.001$) against PGE1. Although HGA inhibit the Histamine and Bradykinin induced inflammation also but the inhibition was found non-significant (16.30% and 3.30% respectively). It may be concluded therefore that HGA mainly acts by its anti-5HT and anti-PGE_1 activity.

Keywords: Unani system of medicine, Habb-e-Gul-e-Aakh, Anti-inflammatory, Mechanism of action

Introduction

Habbe-Gule-Aakh (HGA) is an anti-arthritic polyherbal formulation of Unani medicine. It is commonly used in the treatment of inflammatory diseases, mainly in arthritis and other diseases of joints (Lubhaya, 1979; Khan, ynm).

It is a solid dosage preparation available in a pill form of a gram size containing four ingredients wiz. Gule-Aakh *(Calotropis procera*), Zanjabil (*Zingiber officinale*), Filfil siyah *(Piper nigrum*) and Barg-e-Bans (*Bambusa arundinacea* Retz.) in equal proportion (Lubhaya, 1979, Khan, ynm). All the four ingredients of HGA are described to possess anti inflammatory activity, in Unani literature (Attar, 1988; Ibn Baitar, 1999; Ibn Sina, 1887). Their anti inflammatory activity has also been proved in various scientific studies (Mascolo *et al*., 1988; Vendruscolo *et al*., 2006; Bang *et al*., 2009; Muniappan *et al*., 2003).

Inflammation is a series of pathological changes associated with local vascular reaction and cellular response of the living tissue to a sub-lethal injury. All

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inflammatory reactions are constructed from the same few elements but the mediators that affect the elements are different. A number of chemicals play major roles to carry out the process of inflammation (Harsh Mohan, 2000). As chemical mediators influence inflammatory response, in the very same manner drugs play roles to influence different mediators in order to show their effects on inflammation. This influence of drug on chemical mediators is referred as the mechanism of action of that particular drug. HGA has been described in Unani literature vividly and is widely prescribed by the physicians in the management of chronic inflammatory diseases such as arthritis, gout and lumbago etc. Further it has also been studied scientifically for anti-inflammatory and antiarthritic effect (Nafees *et al*., 2012) and shown to possess significant ameliorating property but its mechanism has still not been elucidated. Therefore present study was undertaken to explore its mechanism of antiinflammatory action in experimental animals. The efficacy of HGA was determined against four important inflammatory mediators viz. Serotonin (5-HT), Bradykinin, Histamine and PGE_1. Since these mediators intervene at different stages of inflammation therefore the exploration of mechanism will also indicate appropriate therapeutic application of HGA in different acute, sub acute and chronic forms of inflammation.

Materials and Methods

Barge-Bans (*Bambusa arundinacea* Retz.) was supplied by Forest Research Institute (FRI), Dehradun while the remaining three drugs were purchased from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh, which has valid drug license issued by Govt. of India. Botanists at department of Ilmul Advvia and department of Botany, AMU, Aligarh identified the drug samples. A voucher specimen has been deposited in the department of Ilmul Advia, AMU, Aligarh, for future reference.

Preparation of test drug

All the ingredients were dried under shade and the powder was made from each drug separately. Then the equiproportional mixture of the four powders was extracted in 50% alcohol with the help of Soxhlet's apparatus for a period of 6 hours. The extract was filtered and evaporated on a hot plate till it dried. The yield percentage of the extract was calculated with reference to the crude drug and it was found to be 40.23%. The dried extract however was reconstituted at the time of administration to animals in to a fresh suspension by adding distilled water to it. The suspension was administered orally with the help of a gastric cannula.

Test for mechanism of anti-inflammatory activity

The method described by Parmar *et al*. (1978) was employed to detect the possible mechanism of antiinflammatory effect of HGA. Albino rats of either sex, weighing 100-150 gm were divided into 8 groups of 6 animals each. Four groups served as control and remaining four were used as test groups for each edemagen. Right hind paw edema was induced by subplantar injection of 0.1 ml. of chemical mediators viz. 5-HT (1gm/ml) to Group I and II, Histamine (1mg/ml) to Group III and IV, Bradykinin (20µg/ml) to Group V and VI, PGE_1 (1µg/ml) to Group VII and VIII one hour after the HGA treatment. The volume of the hind paw was measured by plethysmometer (UGO Basile7041) at 0 hour and at predetermined intervals of 30 minutes after administration of 5-HT, Bradykinin and PGE_1 and 1 hour after histamine injection. Each group received 1000 mg/kg of 50% ethanolic extract of HGA while the animals in Control Group received the vehicle 1 hour before the edemagen administration. Results were statistically analysed using Student's t-test. A P-value less than 0.05 was considered significant.

Results and Observation

The mean increase in the paw volume produced by 5-HT was found to be 0.308 ml in control group and 0.218 ml. in test group. PGE_1 produced edema of 0.206 ml. in control group and 0.11 ml. in test group. Histamine produced a mean increase of 0.235 ml. in control group and 0.195 ml. in test group while Bradykinin produced edema of 0.305 ml. and 0.300 ml. in the control and test group, respectively (Table 1, Fig 1).

Table 1: Effect of HGA on inflammation induced by different endemagen

Groups		Paw volume (ml.) mean ± SE		Mean increase in Paw volume (ml.)	Percentage of inhibition	'P' value
		After	treat-ment			
5-HT	Control	1.52±0.15	1.83±0.17	0.308	28.0	<0.02
	Treated	1.86±0.13	2.08±0.13	0.218		
PGE_1	Control	1.89±0.11	2.10±0.10	0.207	41.01	0.001
	Treated	1.62±0.13	1.74±0.12	0.110		
Histamine	Control	1.74±0.12	1.97±0.03	0.235	16.30	N.S.
	Treated	2.00±0.04	2.15±0.02	0.195		
Bradykinin	Control	1.84±0.22	2.14±0.24	0.305	3.30	N.S.
	Treated	1.51±0.06	1.81±0.12	0.300		

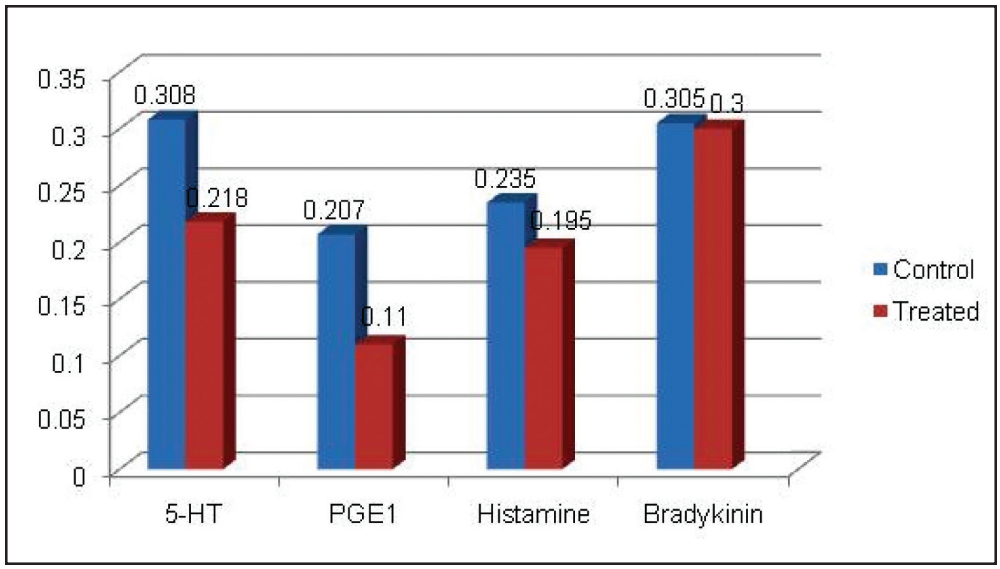

Fig. 1: Effect of HGA on inflammation induced by different endemogen

Thus, HGA produced 28.0% ($p<0.02$) inhibition against 5-HT and 41.01% ($P=0.001$) against PGE_1. Although HGA inhibit the Histamine and Bradykinin induced inflammation also but the inhibition (16.30% and 3.30%, respectively) was non-significant.

Discussion

HGA produced significant effect against 5-HT and PGE_1 induced edema suggesting that its action is mediated through the inhibition of these two edemagens. As far as the remaining two chemical are concerned HGA produced little response that did not amount to be significant statistically. The inflammatory process is mediated by many chemicals. Vasodilatation is caused by Histamine, Kinins and particularly by Prostaglandin E and Prostacyclin. Many factors that are able to increase vascular permeability have also been identified. There are two or more phases of increased permeability governed by different mediators. An initial phase begins within the few minutes of injury and lasts for half an hour. It is usually caused by vasoactive amines like Histamine and Serotonin (Rang et al, 2003, Ritchie, 1990). The delayed phase which reaches a maximum in few hours and persists as long as the inflammation lasts is mediated by prostaglandin E. In the present study HGA was observed to produce significant antiinflammatory effect against PGE_1 and 5-HT induced inflammation. The maximum effect however was found against PGE_1 induced inflammation while the effect against 5-HT induced inflammation was although significant statistically but was comparatively of lesser degree. Therefore, it can be inferred that HGA is less effective in early phase of inflammation. This is so, because 5-HT, Bradykinin and Histamine are

the chemicals which mediate the inflammatory reactions in early phase and the test drug found to be less effective against these mediators. On the other hand HGA was able to reduce the inflammation mediated through PGE_1 enormously therefore it may be deduced that the test drug is more effective in delayed phase of inflammation and may be therapeutically useful in chronic inflammatory conditions. This finding is in conformity with our previous report where it has been shown that HGA has very striking antiarthritic effect (Nafees *et al*., 2012). A drug specially that having antiinflammatory activity such as HGA if used over a long period of time, is liable to induce side effects particularly the gastritis and gastric ulcer etc. However, one of the ingredients of HGA viz. Zanjabil (*Zingiber officinale*) possesses comparatively weak anti-inflammatory effect but has been reported to protect the gastric mucosa significantly (Nanjundaiah *et al*., 2011). Inclusion of Zanjabil in the compound is probably meant to minimize the side effect of HGA which is likely to be used for long period of time in chronic inflammatory condition. It indicates that HGA is effective and safe PGE_1 inhibitor.

Conclusion

It can be concluded therefore that HGA produces anti-inflammatory activity mainly through inhibiting PGE_1. Since this mediator is responsible for delayed phase of increased permeability and inflammatory response therefore it is likely that the test drug will be more useful in the management of chronic inflammatory conditions.

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