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Original Research Article

Anti-inflammatory and analgesic activity of *Habbe Gule Aakh*, A polyherbal Unani formulation in animal models

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ABSTRACT

Background: *Habbe Gule Aakh* is extensively used in Unani medicine for anti-inflammatory and analgesic activity.
Objective: To evaluate anti-inflammatory and analgesic effects of *Habbe Gule Aakh* on Wistar rats and Swiss mice of either sex.
Materials and methods: The study was carried out in Wistar rats for anti-inflammatory activity while Swiss mice were used for analgesic activity. In both the tests animals were divided into five groups of six animals each which served as control, standard and test groups A, B and C. For anti-inflammatory activity, method reported by Amman was followed. For analgesic activity, Koster's protocol was adapted.
Results: Significant ($P < 0.01$) reduction in the paw volume was noted in all the test groups but less than the standard drug. Mean writhes of group B and C reduced significantly ($P < 0.01$) demonstrating analgesic effect.
Conclusion: The study validated the claim of Unani medicine of use of *Habbe Gule Aakh* in inflammation and pain. Further, phytochemical studies are needed to know the exact mechanism of action of this formulation.

1. Introduction

Inflammation and pain are commonest manifestations of many diseases [1]. Inflammation is a nonspecific response that has a beneficial effect on the host [2]. Pain is physical discomfort caused due to illness or injury [1]. Conventional management of inflammation and pain includes varied groups of drugs; of them Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used anti-inflammatory and analgesic drugs. These drugs block prostaglandin synthesis and inhibit lipoxygenase and superoxide radical production, affect neutrophil aggregation and adhesion, cytokine production and cartilage metabolism etc. [3]. Anti-inflammatory, analgesic and antipyretic actions are due to a common mechanism, that is, inhibition of COX-2 and COX-1, however, simultaneous inhibition of COX-1 result is unwanted side effects [4].

In Unani medicine, inflammation has not been described as has been in conventional medicine. However, a term *waram* (swelling) mentioned in Unani medicine referring to any abnormal swelling, be it due to accumulation of blood, pus, water and flatus, may be considered as a term nearest to inflammation to some extent especially when it is *har* (acute). But, *waram,* a general term including all types of abnormal swellings, should not be exactly correlated with modern concept of inflammation; instead, inflammation may be considered as a type of *waram* [5]. In Unani medicine, management of *waram* varies according to the nature and presence of matter e.g. hot, cold, hard etc. It also depends on organs in which swelling has occurred. Unani Medicine claims many single and compound drugs for reducing inflammation and pain. Many single as well as compound drugs are in use as *Mohlil-e-waram* (anti-inflammatory) as well as *musakkin* (analgesic).

Habb (pill) is a dosage form of Unani medicine. It is round, small, uniformly shaped, of different sizes e.g. pea, gram, black pepper etc. *Habbe Gule Aakh* (HGA) is extensively used in Unani medicine in the management of inflammation and pain [6]. Some of its ingredients (Table 1) are said to be anti-inflammatory and are used in arthitis and other painful conditions [6,7]. The ingredients of this

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Table 1
Ingredients of *HabbeGuleAakh*.

Unani name	botanical names	weight (g)	parts used
Zanjabeel	*Zingiber officinalis* Roscoe.	100	rhizome
FilfilSiyah	*Pipernigrum* Linn.	100	Fruit
GuleMadar	*Calotropis gigantea* Linn.	100	flower
Barge Bans	*Bambusa arundinacea* Willd.	100	leaf

formulation have been investigated individually for anti-inflammatory and analgesic effects [8–11], but the compound has not been evaluated for anti-inflammatory and analgesic actions.

2. Materials and methods

2.1. Experimental animals

Wistar rats weighing 150–200 g and Swiss mice weighing 25–30 g of either sex were procured from a registered breeder. The animals were kept under standard laboratory condition throughout the experimentation and provided food and water *ad libitum*. They were housed in clean polypropylene cages at room temperature 25 ± 2 °C, humidity 45–55% with 12 h light and dark cycle. The experimental protocol followed Committee for the purpose of control and supervision of experiment on animals (CPCSEA) guidelines and was approved by the Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine (NIUM) vide Reg. No. IAEC/11/02/IA.

2.2. Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade. Acetic acid (Nice chemicals Pvt. Ltd), Histamine (Sigma) and Diclofenac sodium (Cipla) were purchased from local market of Bengaluru.

2.3. Plant materials

The ingredients of *Habbe Gule Aakh* (HGA) *viz*., *Zingiber officinalis* Roscoe., *Pipernigrum* Linn., *Calotropis gigantea* Linn. and *Bambusa arundinacea* Willd. were procured from local market of Bengaluru and were authenticated by Dr. S. Noorunnisa Begum, Senior Assistant Professor, Centre for Repository of Medicinal Resources (C-RMR), Bengaluru (No. FRLHT 3469,3471,3472, and 3470). The voucher specimens of the same have also been submitted in the drug Museum of NIUM. The ingredients were cleaned from impurities and coarse powder was made with the help of electrical grinder, 100 g of the powder was then used for extraction in (50% distilled water and 50% ethanol) solvent about 6 h by using Soxhlet apparatus at 80 °C. The extract was cooled and filtered by filter paper (Whatman no. 44) and then evaporated on water bath (80 °C) till it dried [12,13]. The yield % of hydro alcoholic extract was found to be 40% *w/w*.

2.4. Dosage and administration

The dose of HGA was calculated by the method described by Frierch et al., (1966) [14] and was found to be 400 mg/kg [6]. Since, the test drug was used in extract form; the final doses were taken based on yield % of extract. So, the dose selected was 160 mg/kg. To find out dose related effect, two more doses were taken i.e. 220 mg/kg and 480 mg/kg body weight of rats respectively.

For mice the first dose was extrapolated from the rat dose which was found to be 800 mg/kg. Since, the test drug was used in extract form; the final doses were taken based on yield % of extract and were found to be 320 mg/kg body weight. Second and third doses were found to be 640 mg and 960 mg/kg for a mice of 25 g. The drugs were used orally. Each dose was prepared freshly before administration.

2.5. Experimental design for anti-inflammatory activity

The anti-inflammatory activity was carried out by the method of Amann et al. (1995) [15]. Wistar rats of either sex, weighing 150–200 g were divided into five groups of six animals each. Initial right hind paw volume of each rat was noted at '0' h by Plethysmometer. Control group was administered 0.1 ml of 1% aqueous suspension of Histamine. Standard group was given Diclofenac Sodium in the dose of 6 mg/kg. Test groups A, B, and C were treated with test drug in the dose of 160 mg, 220 mg and 480 mg/kg, respectively. One hour after the administration of the drug, animals were injected 0.1 ml of 1% suspension of Histamine under the plantar aponeurosis of right hind paw, SC. The % inhibition of inflammatory edema in animals of test and standard groups was calculated by Newbould's (1963) formula [16].

$$[I = 100\{1-(a-x)/b-y)\}]$$

I = % inhibition.
a = mean right hind paw volume of test/standard animals after Histamine injection.
b = mean right paw volume of control animals after Histamine injection.
x = mean right hind paw volume of test/standard animals before Histamine injection.
y = mean right hind paw volume of control animals before Histamine injection.

2.6. Experimental design for analgesic activity

The test was carried out by the method of Koster et al. [17], Swiss mice of either sex; weighing 20–30 g were divided into five groups of six animals each. Control group was administered Acetic acid solution (0.6%) I.P, Standard group received Diclofenac sodium in the dose of 10 mg/kg. Test groups A, B, and C were treated with test drug in the dose of 320 mg, 640 mg, and 960 mg, respectively. After 1 h of administration of test and standard drug, all the animals were administered Acetic acid solution (0.6%) I.P. immediately after administering Acetic acid, the animals was placed individually in a one liter glass beaker and number of writhes was counted for 20 min. A reduction in the number of writhes as compared to control group was considered as evidence as analgesic effect which is expressed as percent inhibition of writhing as:

$$\%\text{ age inhibition} = 100 \times \frac{\text{mean no. of writhes in control group} - \text{Mean no. of writhes in each group}}{\text{Mean no. of writhes in control group}}$$

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2.7. Statistical analysis

Data were expressed as mean ± SEM and the values were compared by using one way analysis of variance by Tukey kramer multiple comparison test. The significance level was considered ($p < 0.05$).

3. Results

3.1. Effect of HabbeGuleAakh on histamine induced paw edema

The paw volumes of each animal were observed at 0, 1, 3 and 5 h. In control group, paw volume at 0 h was noted to 0.215 ± 0.021 but after 1st and 3rd h, a remarkable increase ($p < 0.001$) in the volume was observed as 0.757 ± 0.06 and 0.51 ± 0.08, but after 5th h a gradual reduction ($p < 0.01$) was noted as 0.351 ± 0.04. When standard group and all the three test groups were compared with control at 0 h reduction in the paw volume was observed in all the groups i.e. 0.165 ± 0.0; 0.130 ± 0.01; 0.126 ± 0.009 and 0.115 ± 0.01 in respectively but statistically not significant. When these groups were compared with control at 1st h, standard drug showed more significant effect but test formulation in all three doses also produced significant reduction ($p < 0.001$) in paw volume. The percentage of reduction was noted as 80.6, 43.4, 65.8 and 75.8 in standard and test groups respectively. At 3rd h result was noted in similar way ($p < 0.001$) as the inhibitory percentage was observed 74.2, 28.2, 59.3 and 71.8 in standard and test groups respectively. While at 5th h the reduction was found to be slightly increased 86.1, 22.7, 60.3, and 74.2 in standard and test groups respectively (Table 2).

3.2. Effect of Habbe Gule Aakh on acetic acid induced writhing

The mean writhes of positive control were found to be 15.16 ± 1.4, standard 4.83 ± 1.3, test group A 11.8 ± 1.4, test group B 8.66 ± 1.3, and test group C 5 ± 1.0. When the mean of various groups was compared it was observed that mean writhes of Test group B showed significant reduction ($p < 0.05$) and there was very significant reduction ($p < 0.01$) in mean writhing in Test group C with respect to control group (Table 3).

4. Discussion

According to Unani medicine, *waram* is an abnormal swelling, whether solid or even caused due to accumulation of excessive water, flatus, blood, pus and certain other matters in normal or abnormal cavities of the body. Accordingly, for a *Muhallil-e-waram* drug (a drug that reduces the swelling), appropriate term will be resolving. Unani physicians have classified *waram* in many types; one of them is *warmehar* that can be correlated with acute inflammation. In this view point, *mohallil-e-waram* drugs may be considered as anti-inflammatory whenever correlation with a modern medical term sought.

Conventional anti-inflammatory and analgesic agents are very often misused and are responsible for many serious side effects like nephrotoxicity, hepatotoxicity and gastric ulceration. Unani medicine offers many anti-inflammatory and analgesic drugs for the management of various inflammatory conditions [6]. HGA is an important Unani pharmacopeial formulation used for the management of arthritis and other inflammatory conditions, but scientific data are lacking.

Edema caused due to injection of various inflammatory agents in animals, is usually taken as a parameter for testing anti-inflammatory activity of drugs. Edema formation in the paw of animals is the cumulative effect of various inflammatory mediators [18]. This model has been appropriate for screening anti-inflammatory activity and is used by many investigators [19]. In case of injury, release of Histamine starts acute inflammatory process [20] because it is a potent vasodilator and thereby one of the main inflammatory mediators [21]. In the present study we found statistically significant ($p < 0.001$) decrease in hind paw

Table 3
Analgesic effect of HGA in Acetic acid induced writhing.

Treatment	No. Of writhes observed	Percentage Inhibition
Control 0.1 ml of Acetic acid	15.166 ± 1.42	–
Standard group 10 mg/kg	4.833 ± 1.30**	68.2
Test group A 320 mg	11.83 ± 1.49	22.16
Test group B 640 mg	8.66 ± 1.30*	42.6
Test group C 960 mg	5 ± 1.06**	66.8

Values expressed as Mean ± SEM (n = 6). Test employed ANOVA one way with post hoc Tukey Kramer multiple comparison test.
* - $p < 0.05$ vs. Control.
**- $p < 0.01$ vs. Control.

Table 2
Anti inflammatory effect of HGA in Histamine induced oedema.

Treatment	0 h	After 1 h	After 3 h	After 5 h
Control 0.1 ml of Histamine	0.215 ± 0.021	0.757 ± 0.06[a,***]	0.51 ± 0.08[**,***]	0.351 ± 0.04[a,*]
Standard 6 mg/kg	0.165 ± 0.0[b]	0.27 ± 0.081[c,***] (80.6)	0.241 ± 0.01 [d,***] (74.2)	0.184 ± 0.009[e] (86.1)
Test group A 160 mg	0.130 ± 0.01[b,*]	0.437 ± 0.031[c,f,***] (43.4)	0.342 ± 0.02 [d] (28.2)	0.235 ± 0.01[e] (22.7)
Test group B 220 mg	0.126 ± 0.009[b]	0.311 ± 0.011[c,***] (65.8)	0.246 ± 0.006 [d,***] (59.3)	0.180 ± 0.007[e] (60.3)
Test group C480 mg	0.115 ± 0.01[b,*]	0.246 ± 0.016[c,f,***] (75.8)	0.198 ± 0.01[d] (71.8)	0.15 ± 0.01[e,*] (74.2)

Values are expressed as Mean ± SEM (n = 6/groups). Test employed ANOVA repeated for inter group analysis and ANOVA one way for intra group analysis. Inhibitory rate % was listed in the bracket.
*- $p < 0.05$, **- $p < 0.01$, ***- $p < 0.001$.
[a] Control 0 h vs 1 h, and 3 h: $P < 0.001$ and $p < 0.01$ respectively.
[b] Control 0 h vs standard, test A, B and C at 0 h: ns.
[c] Control 1 h vs standard, test A, B and C at 1 h: $p < 0.001$.
[d] Control 3 h vs standard: $p < 0.001$, Test B and C: $p < 0.001$.
[e] Control 5 h vs standard, test A and B: 5 h: ns, test C: $p < 0.05$.
[f] Test A 1 h vs test C 1 h: $p < 0.05$.

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edema of animals which was similar to inhibition percentage of the standard drug. The ability of test drug to inhibit Histamine induced paw edema suggests significant effect against acute inflammation in a dose related manner.

Acetic acid causes irritation in the peritoneum, which is responsible for vasodilatation, liberation of Histamine and Nitric acid, which are responsible for visceral pain [22]. Acetic acid induced writhing model is a reliable method for evaluation of peripheral analgesic action of drugs [23]. Drugs reducing writhing render analgesia by inhibiting prostaglandin synthesis [23,24]. Reduction in writhing is considered as a parameter for testing analgesic activity in Acetic acid induced writhing test [25], which is similar to the pain perceived by human's sensitive to NSAIDs [26]. In the present study the test drug showed statistically significant ($P < 0.01$) decrease in number of writhing at higher dose, which indicates dose dependent analgesic effect of HGA.

Drugs responding to chemical stimuli are said to have aspirin like effect. Pain reduction by the test drug indicates presence of aspirin like contents in it [27,28]. Aspirin like drugs inhibit PG biosynthesis [29]. Effect of herbal drugs due to phytochemicals has already been established. Some phytochemicals are reported to exhibit analgesic and anti-inflammatory properties [30]. Some ingredients of HGA have been investigated individually for anti-inflammatory and analgesic effects [7–10]. Flavonoids and gingerols may contribute to the analgesic activities. Several types of terpene compounds are known to prevent inflammatory and nociceptive activities [31]. Natural products responsible for its anti-inflammatory activity are sesquiterpenes [32]. It has been demonstrated that ginger contains gingerdiones and shogaols which have pharmacological properties like dual-acting NSAIDs. In a study it was found that gingerols inhibited both prostaglandins and leukotrienes in RBL-1 cells, and that the same with long alkyl side chains are more potent inhibitors of leukotrienes synthesis [33]. Bamboois an ingredient of HGA which is anti-inflammatory due to presence of α-amyrin and phenols [34]. Black paper is an important ingredient of the test drug. IL-6, TNF-α and IL1β the proinflammatory mediators were reduced by administration of the piperine isolated from Black paper [35]. Phenols and glycosides are reported to reduce pain though elevation of pain threshold or by inhibiting prostaglandin like chemical mediators [36]. Flavonoids act on prostaglandins in the late phase of acute inflammation and in pain perception [31]. Quercetin has an inhibitory effect on 5- lipoxygenase pathway [36]. Several types of terpene compounds are known to prevent anti-inflammatory and antinoceptive activities [31]. The anti-inflammatory and analgesic effect of HGA may be due to the presence of above phytoconstituents. Further studies are needed to know the exact mechanism of action of this polyherbal formulation.

5. Conclusion

From above discussion we can conclude that the extract of HGA has significant anti-inflammatory and analgesic actions in dose dependent manner. Mechanism of action of HGA can be inferred to be similar to NSAIDs. The study validated the claim of Unani Medicine about HGA used in inflammation and pain.

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Conflict of interest

We declare that we have no conflict of statement.

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