ANTINOCICEPTIVE AND ANTIPYRETIC EFFECT IN ANIMAL MODELS TREATED WITH A POLYHERBAL UNANI MEDICINE - HABB-E-GUL-E-AAKH

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ABSTRACT
Unani medicine is an ancient system that relies on drugs of natural origin for treatment of different medical conditions. It is practiced throughout Asia and especially in India. Rich asset of natural drugs of Unani medicine encompasses single and compound drugs both. In the present study, Habb-e-Gul-e-Aakh (HGA)-a compound Unani preparation, was screened for its activity against pain and fever. This formulation is in the form of a pill containing four ingredients viz. flowers of Calotropis procera, R.Br. (Gul-e-Aakh), rhizome of Zingiber officinale, Roscoe. (Zanjabil,) fruits of Piper nigrum, Linn. (Filfilsiyah) and leaves of Bambusa arundinacea, Retz. (Barg-e-Bans) in equal proportion.¹² Antinociceptive activity was evaluated by the method of Davies (1946)³ with the help of analgesiometer. Three doses i.e. 400, 750 and 1000mg/kg of test drug were given to albino rats for this study. Method of Dhawan et al., (1962)⁴, modified by Amin et al., (1990)⁵ was used to see the antipyretic activity. For this study, test drug in doses of 250 mg/kg, 400 mg/kg and 570 mg/kg was given to albino rabbits. In analgesiometer test, the onset of analgesia and peak effect were observed at 15 min and 60 min. interval respectively but the magnitude of effect and level of significance was found to increase in dose dependant manner. Similarly the magnitude and duration of antipyretic effect increased correspondingly to the increase in dose. Peak effect was attained at 90 min. for all the three doses. Thus, HGA was seen to exhibit significant antinociceptive and antipyretic activity in a dose dependent manner. KEYWORDS: Unani system of medicine, Habb-e-Gul-e-Aakh, Antinociceptive activity, Antipyretic activity.

INTRODUCTION
Unani System of Medicine is a complementary and alternative medicine that has a glorious past. ‘Unani’ literally means “Greek.” The name Unani (Ionian) is reflected the Greek origin of the system.⁶ This system is based on the idea of balance in bodily systems by balancing the bodily humours and uses various regimens (Tadbeer), diet (Ghiza) and drugs (Dawa) of natural origin i.e. herbs, animals, metals and minerals to treat diseases.⁷ Pain is a most common manifestation of any illness or injury. Though pain is not unworthy as it gives the information about damage or possible damage to any part of the body,⁸ yet agony caused by pain drives to put a check on it. Similar is the case of fever which is immune response...
of body to infections. Pharmacotherapy is the customary way to relieve the pain and reduce fever. Drugs, commonly used for pain and fever are well known for their adverse effects. Herbs have the potential to relieve pain and reduce fever in a better and safer way. Traditional systems like Unani Medicine offer a number of drugs which can take the charge.

*Unani* System of Medicine claims to possess a number of effective and safe drugs useful in the treatment of various diseases. Habb-e-Gul-e-Aakh (HGA) is such a drug, which have been described in unani material medica and is widely used by the practitioners of Unani Medicine for arthritis. It is a compound formulation from the invaluable resources of Unani System of Medicine. It is a pill which contains four *flowers of Calotropis procera*, R.Br. *(Gul-e-Aakh)*, rhizome of *Zingiber officinale*, Roscoe. *(Zanjabil)*, *fruits of Piper nigrum*, Linn. *(Filfilsiyah)* and leaves of *Bambusa arundinacea*, Retz. *(Barg-e-Bans)* in equal proportion.* It has already been studied scientifically for its anti-arthritic and anti-inflammatory effects. Since most of the anti-inflammatory drugs also possess analgesic and antipyretic activity, it is relevant to study the drug for these activities. In addition, most of its ingredients individually have already been reported to possess these activities, therefore; present study was done to explore analgesic and antipyretic effect of this well-known unani formulation, Habb-e-Gul-e-Aakh (HGA).

**MATERIALS AND METHODS**

For the compound formulation under study,*flowers of Calotropis procera*, R.Br. *(Gul-e-Aakh)*, rhizome of *Zingiber officinale*, Roscoe. *(Zanjabil)*, *fruits of Piper nigrum*, Linn. *(Filfilsiyah)* have been purchased from market and leaves of *Bambusa arundinacea*, Retz. *(Barg-e-Bans)* were procured from F.R.I., Dehradun. All the four plant materials were identified from the Botany Dept. of A.M.U., Aligarh. The experiments were conducted in Dept of IlmulAdvia, Ajmal Khan Tibbiya College, Aligarh Muslim University and approved by the Institutional Animal Ethics Committee.

*Preparation of test drug*

All the four ingredients were dried under shade and powder was made from each drug separately. Then the equiproportional mixture of the four powders was extracted in 50% ethanol with the help of soxhlet’s apparatus for the ease of administration to animals. 50% ethanolic extract was used for the study because most of the potential constituents whether polar or non-polar are extracted out in water and alcohol. 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%. However, at the time of administration to animals, fresh solution of extract...
was prepared by dissolving it in distilled water and administered orally with the help of gastric cannula.

**Dose**

The dose for animals was calculated by extrapolating the human dose of HGA by conversion factor of 7 and 4 for rat and rabbit respectively.\(^{(17)}\) The dose of extract, thus calculated was found to be 400 mg/kg for rat and 250 mg/kg for rabbits. Two doses higher than human were also used for the study.

**Test for Antinociceptive Activity**

Test for Antinociceptive activity was carried out by the method of Davies (1946)\(^{(3)}\). Effect of test drugs was obtained in terms of tail flick latency period (the time required for flicking of tail, i.e., reaction time), using an analgesiometer at 0, 15, 30, 60, and 120 minutes. Radiant heat was directed to the proximal third of the tail through a hot wire of the analgesiometer and the reaction time was noted, when the mouse tried to pull the tail away. Albino rats of either sex, weighing 100-150gm, were divided into 3 groups of 6 animals each. The initial reaction time of each rat was determined by outing the tail on nichrome wire of an analgesiometer. The variac was adjusted at a point where the reaction time was found to be 3-6 seconds and the corresponding variac reading was noted. The variac was set at the same point for subsequent testing of particular animal. All the three groups were fed with 50% ethanolic extract in the doses of 400 mg/kg, 750 mg/kg and 1000 mg/kg by oral route, respectively. The reaction of each animal was recorded at intervals of 15 minutes for 120 minutes. The reaction time at each post treatment interval within a group was compared with the initial reaction time by Student’s t-test. P-value<0.05 is considered as significant.

**Test for Antipyretic Activity**

The antipyretic activity of the HGA was evaluated by the method of Dhawan et al. (1962)\(^{(4)}\), modified by Amin et al., (1990)\(^{(5)}\). Dhawan et al. have used T.A.B. vaccine as pyretic agent, while Amin et al., have used D.P.T. vaccine for this purpose, which produced wide and quite significant pyrexia fairly reproducible in both the degree and duration over a series of pilot studies carried out in untreated animals. The vaccine on intravenous administration in rabbits, produced the maximum rise in temperature of 4°F, 90 minutes after the injection and the maximum pyrexia persisted for 3 hours.

Albino rabbits of either sex, weighing 1.0 to 1.5 kg were divided into 4 groups of 3 animals each. The initial temperature of each animal was recorded by a clinical thermometer, inserted ½ inch into the rectum. The alcoholic extract, in the doses of 250 mg/kg, 400 mg/kg and
570 mg/kg was administered to group I, II and III respectively, by oral route. The animals in Group IV were administered with distilled water in usual manner. D.P.T. vaccine was injected intravenously in the ear vein in the dose of 0.5 ml/kg. The rectal temperature of test and control group was recorded 30 minutes after vaccine injection and at 30 minutes interval consequently for a period of 3 hours. The mean rectal temperature of test groups was compared with that of control group at corresponding time and analysed statistically by Student’s t-test. P-value<0.05 is considered as significant.

RESULTS

Antinociceptive effect

In analgesiometer test, the initial reaction time measured before the drug administration was found to be 4.3 ± 0.21 seconds, 5.3 ± 0.33 seconds and 5.6 ± 0.21 seconds in Group I, II and III respectively.

The reaction time for Group I was found to be 4.6 ± 0.21 seconds (N.S.), 4.8 ± 0.30 seconds (N.S.), 5.1 ± 0.16 seconds (N.S.), 5.3 ± 0.21 seconds (P<0.05), 4.8 ± 0.16 seconds (N.S.), 4.7 ± 0.33 seconds (N.S.), 4.7 ± 0.30 seconds (N.S.) and 4.5 ± 0.22 seconds (N.S.) at 15, 30, 45, 60, 75, 90, 105 and 120 minutes after the drug administration.

Group II reacted at 5.7 ± 0.21 seconds (N.S.), 6.2 ± 0.31 seconds (P<0.05), 6.5 ± 0.34 seconds (P<0.02), 6.7 ± 0.21 seconds (P=0.01), 6.5 ± 0.22 seconds (P<0.05), 6.3 ± 0.21 seconds (P<0.05), 6.1 ± 0.31 seconds (P<0.05) and 5.8 ± 0.31 seconds (N.S.) at 15, 30, 45, 60, 75, 90, 105 and 120 minutes after the drug administration.

The reaction time estimated for Group III was 6.2 ± 0.17 seconds (N.S.), 7.0 ± 0.45 seconds (P<0.03), 7.3 ± 0.49 seconds (P<0.03), 7.5 ± 0.34 seconds (P<0.01), 7.3 ± 0.42 seconds (P=0.02), 6.7 ± 0.33 seconds (P<0.05), 6.5 ± 0.34 seconds (P<0.05) and 6.4 ± 0.26 seconds (P<0.05) at the same above mentioned time intervals.

The response was dose dependent but increase in reaction time of all the groups started from 15 minutes and reached at peak at 60 minutes.
Table 1 ANALGESIC EFFECT OF HGA

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reaction time in seconds Mean ± SE</th>
<th>Initial</th>
<th>After Drug Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>HGA (400mg/kg)</td>
<td>4.3±0.21</td>
<td>4.6±0.21</td>
<td>4.8±0.30</td>
</tr>
<tr>
<td>HGA (750mg/kg)</td>
<td>5.3±0.33</td>
<td>5.7±0.21</td>
<td>6.2*±0.31</td>
</tr>
<tr>
<td>HGA (1000mg/kg)</td>
<td>5.6±0.21</td>
<td>6.2±0.17</td>
<td>7.0*±0.45</td>
</tr>
</tbody>
</table>

* significant

Chart 1

ANTALGESIC EFFECT OF HGA

Antipyretic effect

In the control group, mean initial rectal temperature of animal was 102.1±0.24°F and the rise in the temperature after intravenous administration of D.P.T. vaccine (0.5 ml/kg) was found to be 102.9±0.52°F, 103.4±0.54°F, 105.4±0.58°F, 105.2±0.33°F, 105.48±0.52°F and 104.84±0.35°F after 30, 60, 90, 120, 150 and 180 minutes, respectively.

The various test groups showed reduction in temperature depending upon the dose. The initial temperature of Group I, II and III was found to be 102.4±0.20°F, 101.8±0.23°F and 101.9±0.13°F, respectively. The temperature at different time intervals after drug administration was recorded as follows,

At 30 minutes: Group I – 103.0±0.37°F, Group II - 102.0±0.26°F and Group III – 102.0±0.18°F
At 60 minutes: Group I – 103.2±0.22°F, Group II – 102.3±0.21°F and Group III – 102.2±0.23°F

At 90 minutes: Group I – 104.1±0.46°F Group II – 103.1±0.18°F and Group III – 102.7±0.36°F

At 120 minutes: Group I – 104.9±0.28°F Group II – 103.9±0.48°F and Group III – 103.4±0.40°F

At 150 minutes: Group I – 105.3±0.36°F Group II – 104.0±0.54°F and Group III – 103.6±0.47°F

At 180 minutes: Group I – 104.6±0.57°F Group II – 103.8±0.39°F and Group III – 130.1±0.50°F

Table 2 ANTI-PYRETIC EFFECT OF HGA

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean rectal Temperature ± SE°F</th>
<th>Controls</th>
<th>30 Min.</th>
<th>60 Min.</th>
<th>90 Min.</th>
<th>120 Min.</th>
<th>150 Min.</th>
<th>180 Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>102.1 ±0.24</td>
<td>102.9 ±0.52</td>
<td>103.2 ±0.54</td>
<td>104.1 ±0.58</td>
<td>105.2 ±0.33</td>
<td>105.1 ±0.32</td>
<td>104.8 ±0.35</td>
</tr>
<tr>
<td>HGA (250mg/kg)</td>
<td></td>
<td>102.4 ±0.20</td>
<td>103.0 ±0.37</td>
<td>103.2 ±0.22</td>
<td>104.1* ±0.46</td>
<td>104.9* ±0.28</td>
<td>105.3 ±0.36</td>
<td>104.6 ±0.57</td>
</tr>
<tr>
<td>HGA (400mg/kg)</td>
<td></td>
<td>101.8 ±0.23</td>
<td>102.0 ±0.26</td>
<td>102.3* ±0.21</td>
<td>103.1* ±0.18</td>
<td>103.9* ±0.48</td>
<td>104.0* ±0.54</td>
<td>103.4 ±0.39</td>
</tr>
<tr>
<td>HGA (570mg/kg)</td>
<td></td>
<td>101.9 ±0.13</td>
<td>102.0 ±0.18</td>
<td>102.2* ±0.23</td>
<td>102.7* ±0.36</td>
<td>103.4* ±0.40</td>
<td>103.6* ±0.47</td>
<td>103.1* ±0.50</td>
</tr>
</tbody>
</table>

*significant

Chart 2

ANTIPYRETIC EFFECT OF HGA
DISCUSSION

The Tail flick test is a commonly used method for evaluating the antinociceptive activity of medicinal substances. This is a rapid yet convenient method as it does not need the use of classy apparatus.\cite{18} In tail flick test HGA decreased the sensitivity to pain even at the lowest dose of 400 mg/kg though it was non-significant but at the dose of 750 mg/kg and 1000 mg/kg significant reduction in sensitivity was started at 30 min. after drug administration and reach to peak at 60 min. this significant nociceptive effect remain continued up to 105 min. and till the end of study for 750 mg/kg and 1000 mg/kg respectively. In analgesiometer test, the onset of analgesia and peak effect were observed at 15 min and 60 min. interval respectively but the magnitude of effect and level of significance was found to increase in dose dependant manner. As tail flick test is used to evaluatenot only centrally acting analgesics\cite{19} but peripherally acting analgesics like NSAIDs also which inhibit cyclooxygenase in peripheral tissues and make hindrance in transduction in primary afferent nociceptors,\cite{20} it is appear that HGA acts via the central and peripheral mechanisms of analgesia. This activity sure be contributed to those ingredients i.e. flowers of Calotropis procera, R.Br. (Gul-e-Aakh), rhizome of Zingiber officinale,Roscoe. (Zanjabil,) fruits of Piper nigrum, Linn. (Filfilsiyah) and leaves of Bambusa arundinacea, Retz. (Barg-e-Bans),which have already proved scientifically to possess antinociceptive activity in different studies. In the test for antipyretic activity, HGA, in the dose of 250mg/kg significantly reduced the temperature at 90 min. The recorded temperature in the Group I (250 mg/kg) was 104.1±0.46 \(F^0\) (P<0.03). The two test groups administered with 400 mg/kg and 570 mg /kg of drugs showed significant decrease in the temperature at 60 minutes as the recorded temperature was 102.3±0.21 \(F^0\) (P<0.05) and 102.2±0.23 \(F^0\) (P<0.05) respectively. The magnitude and duration of antipyretic effect increased correspondingly to the increase in dose however peak effect was attained at 90 minutes for all the three doses. Antipyretic action of flowers of Calotropis procera, R.Br. (Gul-e-Aakh), rhizome of Zingiber officinale,Roscoe. (Zanjabil,)and fruits of Piper nigrum, Linn. (Filfilsiyah) has been reported by many workers. Piperine, an alkaloid present in P. nigrumand [6]-shogaol in Z. officinale have been mainly attributed to possess antipyretic activity. All the findings of present study on antinociceptive and antipyretic activity of HGA also validate the similar reported actions of these individual drugs in Unani literature where, rhizome of Zingiber officinale,Roscoe. (Zanjabil), fruits of Piper nigrum, Linn. (Filfilsiyah) and leaves of
Bambusa arundinacea, Retz. (Barg-e-Bans) are described as analgesic and antipyretic while Calotropis procera, R.Br. (Gul-e-Aakh) is stated as analgesic.\textsuperscript{[21,22,23,24,25]}

**CONCLUSION**

In this study, inhibition of reaction to pain or increase in pain threshold was observed on administration of HGA. Test drug was also seen to exhibit significant reduction in temperature as compared to control, in a dose dependent manner. Thus, it can be concluded that HGA possesses significant antinociceptive and antipyretic activity. Results of this study strongly suggest using HGA in all those conditions associated with fever and pain other than its conventional use, arthritis.

However further experimental and clinical studies are required by using different protocols and gather extensive knowledge of drug to assured the use of HGA for its antinociceptive and antipyretic properties.

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