Evaluation of antiinflammatory activity of a Unani formulation

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The study presents the acute antiinflammatory effect of a non-pharmacopoeal Unani formulation. Both the aqueous and alcoholic extracts were tested for acute antiinflammatory activity by carrageenin oedema test. Since, most of the mainstream antiinflammatory drugs have significant gastric toxicity; therefore test combination was subjected to the test for ulcerogenic activity. The standard drug Piroxicam was used for comparison of antiinflammatory and ulcerogenic activity. The LD50 was also performed to point out the safety margin of test drug. The test drug was found to possess significant antiinflammatory activity against acute inflammation. The test drug also exhibited gastro-friendly effect and the safety margin was very large. The alcoholic extract was more effective than that of aqueous extract.

Key words: Antiinflammatory activity, Ulcerogenic activity, Unani formulation

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Tibb-e-Unani possesses many antiinflammatory drugs that are highly effective and quite safe. However, such drugs have not been scientifically evaluated, therefore, there is an element of doubt regarding their efficacy; secondly their role in inflammation is not described in necessary details. For instance their differential activity in acute & chronic phase is not described. There is a general belief that Unani drugs are relatively benign. However, it has not been objectively tested for the likely adverse reaction such as gastric irritation, ulceration, etc. Unani physician frequently prescribe a non-pharmacopoeal preparation (Table 1) for arthritis. From the Unani point of view, the combination is perfectly rational as all ingredients are considered to be resolvent. Alpinia galanga, Withania somnifera, Calotropis procera (flower), Lactuca scariola and Zingiber officinale have been unambiguously shown to possess significant antiinflammatory activity in modern scientific research1-8. However, the combination has not been scientifically studied for antiinflammatory activity. Potentially active principles could be extracted exclusively in water & alcohol; therefore, the aqueous and alcoholic extracts of test combination were used. The animal dose of extract of test combination was determined by multiplying the Unani clinical dose with standard conversion factors9. The extracts were studied for antiinflammatory activity by carrageenin rat paw oedema test. Since, most antiinflammatory mainstream medicine have significant gastric toxicity, therefore, the combination was also subjected for ulcerogenic activity. The LD50 of test drug was determined in mice to obtain an index of safety of the combination.

Methodology

The crude drugs were procured from Dawakhana Tibbia College, AMU, Aligarh and identified. All the ingredients were shade dried, powdered and extracted in distilled water and 70% alcohol, respectively for 6 hrs. After filtration, the aqueous & alcoholic extracts were evaporated on a hot plate and water bath, respectively. The extracts were freshly dissolved in distilled water before each experiment and were administered orally in all the tests. The dose of extracts was calculated in the usual manner by multiplying Unani clinical dose by 7 far rat9. The dose of aqueous & alcoholic extracts thus determined was found to be 80 mg/100 gm and 52 mg / 100 gm, respectively. Albino rats of either sex, weighing 150-200 gm were divided into 4 groups of 6 animals each. The animals in group I and II were served as plain and standard control and administered with distilled water.
and piroxicam 0.25 mg/100 gm while the animals of group III and IV were administered with the aqueous and alcoholic extract in the dose of 80 mg/100 mg and 52 mg/100 gm, respectively. The volume of right hand paw was measured by displacement of volume in ml, with the help of plethysometer. This was followed by the injection of 0.05 ml freund’s adjuvant aseptically under the planter aponeurosis of right hind paw. The test and standard drugs were administered orally once a day for 13 days. On 14th day, the volume of the right hind paw was again measured. The per cent increase in volume of foot in comparison to the control animals was determined in each group10. The observation for the severity of secondary lesion i.e. swelling of the left hind paw, forepaw, ear and tail was recorded to the scale of mild, moderate, moderately severe and severe. The per cent inhibition of increase in volume of the foot was calculated by the following formula:

\[ I = 100\left(1 - \frac{a-x}{b-y}\right) \]

Where:
- \( I \) = the percentage inhibition
- \( a \) = mean volume of injected food of treated rats on 14th day of injection
- \( x \) = mean volume of injected foot of treated rats before injection
- \( b \) = mean volume of injected foot of control rats on 14th day of injection
- \( y \) = mean volume of injected foot of control rats before injection

The mean volume of the right hind paw on the 14th day was compared and statistically analyzed by student’s “t” test to determine the significance of the difference. Albino rats of either sex, weighing 150–200 gm, were divided into 4 groups of 6 animals each. The animals in group I and II were served as plain and standard control and administered with the distilled water (3ml orally) and piroxicam (0.25 mg/100gm), respectively, by oral route. The animals in group III & IV (test groups) were administered with aqueous and alcoholic extracts in the dose of 80 mg/100gm and 52 mg/100 gm, respectively by oral route. Animals were kept on fasting for another period of 2 hrs, following which diet was allowed. The procedure was repeated for 7 days. On the last day, animals were sacrificed (under ether anesthesia) 2 hrs after drug administration. The stomach was removed, cut along the lesser curvature, gently washed with normal saline and fixed in 10% formalin. The gastric mucosa was examined11,12. The degree of single ulceration (DSU) for each stomach was determined and graded according to the following scale13:

- 0.0 : Absence of any detectable lesion
- 0.5 : Small hemorrhagic effusion
- 1.0 : Hemorrhagic effusion
- 1.5 : Mucosal ulceration of limited diffusion involving not more than 2/3 of whole surface
- 2.5 : Mucosal ulceration of generalized diffusion
- 3.0 = deep ulceration of limited diffusion
- 3.5 = deep ulceration of generalized diffusion
- 4.0 = perforated ulcer

The average degree of ulceration (ADU) for each group was determined by adding DSU of each animal divided by number of animals in a group. The ulcerative Index (UI) was calculated by means of the following formula: UI = ADU (% RU/100); where: % RU = percentage of rats with ulceration in a group.

A pilot study with 2 mice to each dose of test drug administered orally was carried out to find out the approximate dose producing 50% mortality. Thus, 50% mortality produced with the aqueous and alcoholic extracts was found to be 2,400 mg / 100 gm and 2,000 mg / 100gm, respectively. This dose and two lower and two higher doses were selected for main study. Mice of either sex, weighing 20–25 gm were divided into 10 groups of 6 animals each. The animals in group I, II, III, IV, V were administered with the aqueous extract in the dose of 2,300 mg, 2,200 mg, 2,400 mg, 2,500 mg, 2,600 mg/100 gm, respectively, by oral route. Animals in group VI, VII, VIII, IX and X were administered with the alcoholic extract in the dose of 1,900, 1,800, 2,000, 2,100, 2,200 mg/100gm, respectively, also by oral route. Animals kept in separate cage were observed continuously for 2, 6 and 24 hrs for mortality. The number of animals dying within 24 hrs in each group was recorded and the LD50 was determined by Arithmetical method14.

**Results**

In the control group, the mean increase in volume of right hind paw was found to be 0.05 ± 0.01 ml, while in the standard group, it was found to be 0.05 ± 0.018 ml (P<0.001) and amounted to 200% inhibition of inflammation. In the test group treated with the aqueous and alcoholic extracts, the mean increase in
Table 1—Non-pharmacopoeial Unani preparation

<table>
<thead>
<tr>
<th>Unani name</th>
<th>Plant name</th>
</tr>
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<tbody>
<tr>
<td>Bozidan</td>
<td>(<em>Tanaecetum umbelliferum</em> Boiss)</td>
</tr>
<tr>
<td>Khulanjan</td>
<td>(<em>Alpinia galanga</em>, Willd.)</td>
</tr>
<tr>
<td>Asgand</td>
<td>(<em>Withania Somnifera</em>, Dunal)</td>
</tr>
<tr>
<td>Gul-e-Madar</td>
<td>(<em>Calotropis Procera</em> R.Br.)</td>
</tr>
<tr>
<td>Tukhm-e-Kahu</td>
<td>(<em>Lactuca scaiola</em>, Linn.)</td>
</tr>
<tr>
<td>Zanjabil</td>
<td>(<em>Zingiber officinale</em> Rosc.)</td>
</tr>
</tbody>
</table>

Fig. 1—Antiinflammatory activity of test drugs

Table 2—Antiinflammatory effect of aqueous and alcoholic extract with Piroxicam as standard drug

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thickness of rat paw in ml means ± SE</th>
<th>Increased thickness of paw in ml</th>
<th>% Inhibition inflammation</th>
<th>‘P’ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before car Injection 3 hrs after car Injection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC (DW)</td>
<td>0.53±0.02</td>
<td>0.78±0.01</td>
<td>0.25±0.03</td>
<td>—</td>
</tr>
<tr>
<td>SC (Pirox 0.25 mg/100gm)</td>
<td>0.47±0.03</td>
<td>0.55±0.01</td>
<td>0.08±0.02</td>
<td>77.14%</td>
</tr>
<tr>
<td>Aq. extract (80 mg/100gm)</td>
<td>0.43±0.02</td>
<td>0.62±0.03</td>
<td>0.15±0.04</td>
<td>45.71%</td>
</tr>
<tr>
<td>Alc extract (52 mg/100gm)</td>
<td>0.48±0.03</td>
<td>0.61±0.02</td>
<td>0.13±0.02</td>
<td>62.86%</td>
</tr>
</tbody>
</table>

n = 6; PC = Plain control; DW = Distilled water; SC = Standard control
Aq. Ext. = Aqueous extract; Alc. Ext. = Alcoholic extract

Fig. 2—LD50 of the alcoholic extract

Fig. 3—LD50 of the aqueous extract

The percentage inhibition of inflammation was found to be 120% and 80%, respectively. The percentage inhibition was lesser than the inhibition of inflammation produced by the standard drug Piroxicam. A few secondary lesions were present in the control group, but were not found either in test or standard group (Table 2 & Fig. 1).

All the animals of control, standard and both test groups were sacrificed and their stomach was removed. The stomach was cut along with the lesser curvature and washed gently with normal saline. The organ was fixed in 10% formalin and examined to find out the ulceration and other lesions. In all the control and test groups, the ulcerogenic index (UI) was found to be 0.00. In animals treated with Piroxicam, the ulcerogenic index was 0.05. Both the aqueous and alcoholic groups were found to be devoid of any abnormal gastric lesion (Table 3).

A pilot study was conducted in group of 2 mice to each dose of the test drug (increasing order) in order to find out the approximate dose causing 50% mortality. This dose and two lower and two higher doses were selected for main study. In the group...
administered with the aqueous extract in the dose of 2,200 mg, 2,300 mg, 2,400 mg, 2,500 mg and 2,600 mg/100gm, the mortality within 24 hrs was observed as 2,3,3,3 and 4, respectively. In the group administered with the alcoholic extract in the dose of 1,800 mg, 1,900 mg, 2,000 mg, 2,100 mg and 2,200 mg / 100 gm, the mortality within 24 hrs was observed as 2,3,3,3 and 4, respectively. The results were analyzed and the LD50 of the aqueous and alcoholic extracts was found to be 2,466.04 mg /100 gm and 2,066.14 mg/100 gm, respectively (Tables 4,5; Figs. 2 & 3)

**Discussion**

The test drug, a non-pharmacopoeal formulation (Table 1) was investigated experimentally for acute antiinflammatory and ulcerogenic activities, employing two tests, viz. carrageenin rat paw oedema test and gastric irritation liability test. Arthritis is one of the major health problems all over the world. However the therapy available for these disorders is insufficient to cope with the varied and fulminating nature of these disorders. Antiinflammatory drugs have been extensively reported in Unani literature, which may serve as an important therapeutically potential group of drugs and may be used in combating various joint ailments. Since, antiinflammatory drugs are therapeutically of great importance, it is one of the area, which is given priority in scientific research in Tibb-e-Unani. Many Unani physicians frequently prescribe this preparation (Table 1) for arthritis. Most of its ingredients have been shown to possess antiinflammatory activity in modern scientific research. However, the combination has not been studied scientifically. Therefore, in the study it was evaluated for acute antiinflammatory and ulcerogenic activities. The LD50 was also performed to point out the safety margin of test drug.

In the Freund’s adjuvant Arthritis test, the mean increase in volume of the right hind paw was 0.05 ± 0.013 ml in the control group, while in the standard group, the mean increase in volume was 0.05 ± 0.018 ml (P < 0.01), which amounted to 200% inhibition of inflammation. In animals, treated with aqueous and alcoholic extracts, it was reduced to 0.01 ± 0.02 ml, (P < 0.5), 0.01 ± 0.014 ml (P < 0.5) and amounted to 120% and 80% inhibition of inflammation, respectively. A few secondary lesions were present is the control group, but were not found either in the test groups or in standard group (Table 2, Fig. 1). The aqueous and alcoholic extracts did not show any statistically significant antiarthritic activity. However, the percentage inhibition of inflammation by the aqueous and alcoholic extracts (120% and 80%, respectively) compares well with the per cent inhibition seen with the standard drug piroxicam (200%).

The test combination was also studied for the ulcerogenic activity by the gastric irritation liability test11,12. In the control group, the ulcerogenic Index (UI) was found to be 0.0. In the animals treated with
aqueous and alcoholic extracts and standard drug piroxicam, the UI was 0.0, 0.0 and 0.5, respectively (Table 3). In control and test groups, the UI was 0.0, which indicates the gastro-friendly effect of test combination. The study therefore shows that the test combination despite possessing good antiarthritic activity is devoid of ulcerogenic activity. As mentioned, very few Unani drugs have been subjected to toxicity testing, so in addition to carrying out ulcerogenic activity its LD$_{50}$ was also determined in mice. The LD$_{50}$ of aqueous and alcoholic extract were found to be 2,466.04 mg/100 gm and 2,066.14 mg/100 gm, respectively. The aqueous and alcoholic extracts were found to possess striking antiinflammatory activity at 80 mg/100 gm and 52 mg/100 gm, therefore the margin of safety of the extract is very wide.

**References**