Analgesic effect of the aqueous extract of Artimisia herba alba Arial part

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Abstract

The present study was aimed to investigate the analgesic effects of the aqueous extract of Artimisia herba alba Arial part in rats and mice (AEAHA). The AEAHA (400-700 mg/kg; p.o.) was evaluated for its analgesic activity by employing acetic acid-induced writhing test, hot plate test and tail immersion tests i.e. in hot and cold water. AEAHA (400-700 mg/kg; p.o.) showed significant (P<0.01) reduction in the number of writhing induced by acetic acid, increased reaction time in hot plate test and elevated pain threshold in hot and cold water tests. AEAHA exhibited the dose-dependent analgesic effects.

KEYWORDS- Artimisia herba alba, Analgesic activity, Writhing, Hot plate, Tail immersion tests

Introduction

The use of plants products is increasing in many segments of the population [1]. At the present, thousands of plant metabolites are being successfully used in the treatment of variety of diseases. According to an estimate 80% worlds population relied upon plants for their medication. The use of medicinal plants is increasing in many countries where 35% of drugs contain natural products [2]. Medicinal herbs are an indispensable part of the traditional medicine practiced all over the world due to the low costs, easy access, ancestral experience [3]. Plants are considered as natural chemical factory because synthetic process in biological systems particularly in plants is going on by the nature ordinary temperature and pressure. The laboratory synthesis of anti-malarial drug Quinine requires an intensive work extending over half a century but Cinchona plant can do it everyday without difficulty. The presence of diversified chemical compounds as steroids, terpenoids, flavinoids, chalcones, alkaloids, and glycosides in plants has already been reported [2]. Artimisia herba alba which is commonly known as "the desert worm wood" is locally known by Arabic name "Shih", the plant is a perennial dwarf gray woolly shrublet growing in Iraq, and it belongs to the family Compositae [4]. Several workers reported that Artimisia herba alba (AHA) possesses antithelmentic activities, antibacterial effect, and used in folk medicine in the treatment of diabetes mellitus in Iraq [5]. The photochemical screening on the plant has shown the presence of flavinoids, steroids triterpene and sesiquiterpene, volatile oil, coumarins, anthraquinones, tannins and volatile base [6]. Different species of Artimisia have been reported to show an anti-nociceptive and anti-inflammatory activities [7,8]. Meanwhile, methanolic extract of Artimisia absinthium showed significant analgesic effects [9]. An attempt was, therefore, made to evaluate the analgesic activity of the aqueous extract of the aerial parts of Artimisia herba alba in different models of pain in albino mice and rats.

Materials and Methods

Extraction of the plant material

The plant AHA was collected from the area "Tikreet –Iraq", and were authenticated by the authority of Abu-Ghraib Botany Directorate, then the plant were shed dried at 25 C and grounded by an electrical mill mesh no. 50and the powdered plant were kept in a nylon bags in a deep freeze until the time of use. A suspension of 100 g of the powder in 200 ml of distilled water was stirred magnetically overnight (16 h) at room temperature; this was repeated
three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a low temperature (<40°C) under reduced pressure in a rotary evaporator. The powdered extract was dissolved in normal saline whenever used in the experiment [5].

**Animals**
Albino Rats (Wistar strain) weighing between 200-250 g and Swiss albino mice weighing between 20-25g of either sex were used and were maintained at 25±3°C. They were kept in well ventilated animal house in large polypropylene cages and were fed standard rat chow and water ad libitum [3].

**Evaluation of analgesic activity**

**Acetic acid-induced writhing test.**
Acetic acid solution at the dose of 300 mg/kg body weight was injected (i.p.) and the number of writhes during the following 30 min period was observed [10]. A significant reduction in the number of writhes by drug treatment as compared to vehicle treated animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated. Aspirin (100 mg/kg, i.p.) was used as standard.

**Acetic acid induced writhing test (Chemical stimulation)**
The aqueous extract was evaluated for its analgesic activity by acetic acid-induced writhing model described by Siegmund et al [10] and modified by Köster et al [11]. Swiss albino mice were divided into four groups. First group was used as negative control and received 5 % gum acacia (5 ml/kg), an hour before injection (i.p.) of 0.6 % v/v acetic acid (10 ml/kg). Second group served as positive control and received aspirin 300 mg/kg, while the third and fourth groups were administered orally with divided doses of the aqueous extract of Artimisia herba alba (AEAHA) i.e. 400, 700 mg/kg, respectively, an hour before acetic acid injection. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted for 15 minutes after administering acetic acid. Percent protection against writhing movement was taken as index of analgesia.

**Hot Plate method (Thermal stimulation)**
In the hot plate method [11] albino rats (200-250 g) were divided into 4 groups each consisting of six animals. First group served as negative control (received 5 ml/kg of 5 % Gum acacia). The second group served as positive control (received pentozocin 5mg/kg) while the third and fourth group received AEAHA (400-700 mg/kg; p.o.). The basal reaction time was noted before 1, 2, 3 hrs after the administration of the drugs at a temperature about 100 °C.

**Tail immersion methods**

**Hot tail flick test**
Swiss albino mice were screened by exposure to the thermal stimulus. The mice showing positive response were divided into four groups of six animals each. The animals of first and second groups were treated, respectively, with 5 % w/v Gum acacia, pentazocine (5mg/kg; p.o.). The animals of third and fourth groups were administered orally with divided doses of AEAHA i.e. 400 and 700 mg/kg, respectively. About 5 cm of the tail of mice was dipped in warm water, kept constant at 50±0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 60 sec. The latent period of the tail flick response was taken as the index on antinociception and was determined immediately after injection [12].

**Cold tail flick test**
Swiss albino mice were employed in this test and selected by exposure to cold stimulus. The mice showing positive response were divided into four groups. Before the experimentation, all the animals were fasted for 24 h (only water ad libitum was given). The animals of first and second groups were treated, respectively, with 5 % w/v Gum acacia, pentazocine (5 mg/kg; p.o.). Similarly, the animals of third and fourth groups were administered orally with divided doses of AEAHA i.e. 400 and 700
mg/kg, respectively. About 5 cm of the tail of mice was dipped in a cold 1:1 mixture of water and ethylene glycol kept constant at 10±0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 60 sec. The latent period of the tail flick response was taken as the index on antinociception and was determined immediately after injection [12, 13].

Results and Discussion

This study establishes the central and peripheral analgesic activity of aqueous extract of Artimisia herba alba aerial parts. Acetic acid-induced writhing and hot water test is used to study the action on peripheral nervous system. Cold water test and hot plate method was used to study the action on central nervous system [14]. The effect of AEAHA (400-700 mg/kg; p.o.) on acetic acid induced writhing is demonstrated in Table 1. The extract administered at the doses of 400 and 700 mg/kg orally showed the significant (P<0.01) reduction in the number of writhes induced by acetic acid in a dose dependent manner. Aspirin (300 mg/kg, p.o.) was used as a standard drug for comparison of results which exhibited significant (P<0.01) inhibitory effect on the writhing responses. Acetic acid causes analgesia by liberating endogenous substances including serotonin, bradykinin, histamine and prostaglandin which may stimulate pain nerve ending [15]. Therefore, AEAHA might inhibit the synthesis and/or release of these endogenous substances. The result of hot plate test indicated a significant increase (P<0.01) in reaction time at 1, 2 and 3 hours as comparable to the reference drug Pentazocin (5 mg/kg; p.o.) which is showed in Table 2. The results obtained from hot and cold tail flick experiments are shown in Table 3, in both the models, administration of AEAHA (490-700 mg/kg; p.o.) showed significant (P<0.01) protection against the pain induction. The result from hot and cold tail flick test also gave additional evidences for the analgesic activity of the root extract. The activity may be attributed due to the presence of flavonoids, alkaloids and other bioactive compounds. In conclusion, the present study demonstrated that AEAHA has intrinsic analgesic activity which needs to be investigated with more information on the bioactive principles responsible for the action. The results indicate that the AEAHA possesses significant analgesic activity.

Table 1. Effects of AEAHA and aspirin on writhing induced by acetic acid in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Number of writhes (per 30 min) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>30.31±2.98</td>
</tr>
<tr>
<td>- AEAHA 400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- AEAHA 700</td>
<td></td>
<td>13.53±1.18 *</td>
</tr>
<tr>
<td>- Aspirin 0.1</td>
<td></td>
<td>7.51 ± 1.49</td>
</tr>
<tr>
<td>- Aspirin +AEARA 400 + 0.1</td>
<td></td>
<td>3.22 ± 1.43 *</td>
</tr>
<tr>
<td>- Aspirin +AEARA 700 + 0.1</td>
<td></td>
<td>4.19 ± 0.62 *</td>
</tr>
</tbody>
</table>

The results given are mean ± S.E.M.; number of animal used (n=6) *P< 0.0005 Experimental groups were compared with control group (ANOVA followed by Dunnett T- test).
Table 2 Effect of AEAHA on thermal stimulus-induced pain (Hot plate test) in rats.

<table>
<thead>
<tr>
<th>Group(s)</th>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>I</td>
<td>Control 5% Gum acacia</td>
<td>5 mg/ kg</td>
<td>4.89 ± 0.18</td>
</tr>
<tr>
<td>II</td>
<td>Pentazocin</td>
<td>5</td>
<td>5.32 ± 0.12</td>
</tr>
<tr>
<td>III</td>
<td>AEAHA 400</td>
<td>5.18 ± 0.61</td>
<td>6.08 ± 0.36 *</td>
</tr>
<tr>
<td>IV</td>
<td>AEAHA 700</td>
<td>5.28 ± 0.14</td>
<td>7.81 ± 0.93 *</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n=6). * P<0.001 very significant with respect to control group (ANOVA followed by Dunnett t-test).

Table 3 Effect of AEAHA on pain threshold in hot and cold tail flick test.

<table>
<thead>
<tr>
<th>Group (s)</th>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Analgesic effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hot tail flick test</td>
</tr>
<tr>
<td>I</td>
<td>Control 5% Gum acacia</td>
<td>5 mg/ kg</td>
<td>8.43 ± 0.78</td>
</tr>
<tr>
<td>II</td>
<td>Pentazocin</td>
<td>5 mg/ kg</td>
<td>57.89 ± 0.13 *</td>
</tr>
<tr>
<td>III</td>
<td>AEAHA 400</td>
<td>400</td>
<td>16.01 ± 1.08 *</td>
</tr>
<tr>
<td>IV</td>
<td>AEAHA 700</td>
<td>700</td>
<td>21.18 ± 0.58 *</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n=6). * P<0.001 very significant with respect to control group (ANOVA followed by Dunnett t-test).
References

المفوع الموظف للمستخلص المائي لنبات الشيح / الجزء الهوائي

المؤلف: حسن قاسم خليل
كليّة التربية الأساسية / حليجة جامعة السليمانية
المعهد الطبي التقني / منصور هيئة التعليم التقني

الخلاصة

إن الهدف الأساسي من هذه الدراسة هو تحديد المفعول الموظف للمستخلص المائي لنبات الشيح / الجزء الهوائي Artemisia herba alba في الفئران والجرذان المختبرة. لقد تم تقديم النبات من خلال إعطاء جرعات مختلفة (400 -700 ملغم/كلغ) وزن إعطاء فموي باستخدام طريقة Acetic acid. لحصول على حالات من التوافق. اختبر الفئران، غمر في الماء الساخن والبارد. أظهرت النتائج قدرة النبات (400 - 700 ملغم/كلغ) وبصورة معنوية (P < 0.01)، كذلك أظهرت الدراسة قابلية النبات على زيادة وقت التفاعل الحشائي إلى اللوحة الساخنة ورفع قابلية تحمل الحيوانات لكل من الماء البارد والحار وبصورة معنوية. نستنتج من الدراسة أن المستخلص المائي لنبات الشيح / الجزء الهوائي له تأثير معنوي كعلاج مسكن للألل، وهذا النتائج يعتمد على جرعة النبات.