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Antinociceptive Effects of *Spartium junceum* L. Extract on Mouse Formalin Test

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Abstract: Medicinal plants are an important source of medications. *Spartium junceum* L. (Spanish broom) are used as an antinociceptive medication in Iranian folk medicine. So, we conducted to design an experimental trial study to assess and compare the antinociceptive effect of hydoalcoholic extracts of *S. junceum* L. flowers with morphine sulphate by using formalin test. The results showed the test extracts produced antinociceptive effect on both early and late phases. However, on the late phase, it showed antinociceptive effects equal to morphine sulphate, 3 mg kg^{-1}. The present study supports the traditional use of it in Yazd province as an antinociceptive medication. However, further investigations are required to study the efficacy and safety of this herbal medication in man.

Key words: *S. junceum* · antinociceptive · formalin test · mice

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Thus study of plant species that traditionally have been used as pain killers should still be seen as a strategy in research for new analgesics drugs. The *Spartium junceum* L. (Spanish broom) belongs to the Fabaceae family, is a small shrub indigenous in the Mediterranean countries and cultivated as an ornamental plant. Its flowers are large, yellow and of agreeable scent [1]. *S. junceum* L. contains flavonoids and saponins. Two new flavonoids and a saponin were isolated from its flowers in a study by Bilia *et al.* [2]. Another study showed that *S. junceum* L. seeds contain a large amount of lectin-like proteins that did not appear to be an active lectin [3]. An herbal tea (known as Zahraa) widely consumed in Syria contains 6-14 species components including *S. junceum* L. [4].

Flowers of *S. junceum* L. are used for the treatment of gastric ulcers in Turkish folk medicine [5]. It has been showed in a study by Yesilada *et al.*, [6] that *S. junceum* L. did not have anti-helicobacter pylori activity. Flavonoid–rich fractions from the flowers *S. junceum* L. showed potent antioxidant activity reported by Yesilada *et al.* [7].

It's known to stimulate uterine contractions and GI tract, help body to dispose excess fluid by increasing amount of urine and cause vomiting [8]. It has been studied for its antifertility activity in mammalian male [9]. Another study showed injection of *S. junceum* L. extract in adult male rats reduced the rate of fertility and acrosin enzyme activity [10]. Only one study reported the antinociceptive and anti-inflammatory effects of this plant [11]. Keeping this in view, the present study has been done to evaluate the antinociceptive effect of the hydroalcoholic extract of this plant using formalin test. Among the several models of persistent nociception, formalin test has been well established as a valid model to study the antinociceptive effects [12-14].

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the Ethics committee of the Yazd Shahid Sadoughi University, Yazd, Iran.

Plant material: Fresh flowers of *S. junceum* L. were collected from Yazd, Yazd Province, Iran in May 2005 at an attitude of 1100 m. The plant was authenticated by Herbal museum of the Faculty of Agriculture, Azad Meybod University, Meybod, Yazd, Iran. A voucher specimen of

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the plant has been deposited in the University's herbarium.

Preparation of *S. junceum* Flower Hydroalcoholic Extract: One kilogram (1 kg) of fresh flowers of *S. junceum* L. were washed, chopped and air-dried under shade and then powdered and stored in an airtight container. The powder was soaked in aqueous ethanol (50% v v^{-1}) at room temperature for 24 h. The extract was concentrated to dryness under reduced pressure at room temperature to constant weight (yield: 6.5%). Aliquot portions of the extract 6.5% were weighed and dissolved in distilled water for using on each day of the experiment.

Animal Material: Male Balb C mice (Mus Domesticus) weighing 30-35 g were obtained from the Yazd University animal house in Iran. They were kept at standard environmental conditions (12/12 h light/dark cycle) and were allowed free access to food (standard pellet diet) and water ad libitum. The mice were randomly divided into groups of eight as control, sham and test subjects.

Preparation of Doses: The doses of 10, 20, 30 and 40 mg kg^{-1} of the extract were used. The doses were selected based on the extract dry weight. Normal saline 0.9 % was used as solvent. All doses in a volume of 2 ml were administered interperitoneally (IP) 30 min before formalin injection to animals.

Formalin Test: Each animal was allowed 15 min to explore the chamber before injection and its behavior was rated according to the scale described below. These data constituted the pain-free baseline. Each mouse received 25 μl formalin 1% subcutaneously into the dorsal surface of the right hind paw using a microsyringe. Each animal was placed individually on a flat glass floor under the chamber and a mirror was arranged at an angle of 45°C under the glass to allow clear observation of the paw of the animal. The mouse was observed continuously by a blinded observer for 60 min. Formalin injection was associated with an early phase (0-10 min) and late nociceptive phase (15-60). The antinociceptive activity of the compound was determined using the method described by Dubuisson and Denis [12].

Six groups, each containing eight mice, were run:

- A control group received formalin and 2 ml normal saline (IP).
- A 10 mg kg^{-1} extract test group
- A 20 mg kg^{-1} extract test group
- A 30 mg kg^{-1} extract test group
- A 40 mg kg-1 extract test group
- A sham group received 3 mg kg^{-1} morphine sulphate

Pain intensity was rated according to the following numerical scale:

- Both forepaws are placed on the floor and weight is evenly distributed.
- The injection paw rests lightly on the floor or on another part of the animal's body and little or no weight is placed upon it.
- The injected paw is elevated and not in contact with any surface. The uninjected paw is placed firmly on the floor.
- The injected paw is licked, bitten or shaken, while the uninjected paw is not.

The mouse was observed for 60 minutes after the injection of formalin and the amount of time (sec) spent in each scale (0, 1, 2 and 3) was recorded.

Ratings are averaged over 3 min blocks. Numerical ratings are calculated from the following formula:

$$\text{Pain rating} = \frac{T1+2T2+3T3}{180}$$

Where T1, T2 and T3 are the durations (in sec) spent in categories 1, 2 or 3, respectively during each 3 min block.

Statistical Analysis: Comparison between groups were made by one-way analysis of variance (ANOVA) followed by Tukey test. Differences with $P<0.005$ between experimental groups were considered statistically significant. SPSS was used to analyze the data.

RESULTS

Figure 1 shows the baseline and pain rating curves for the six groups of mice. Ratings are averaged over 3 min blocks. The left hand side of the Fig. 1 shows the baseline data. The average rating given to these do not favor one forepaw over the other. The formalin injected paw in control group mice were shaken, licked or bitten and generally kept elevated.

The results showed *S. junceum* L. extracts had antinociceptive effect on both early and late phases, which showed more antinociceptive effect on late

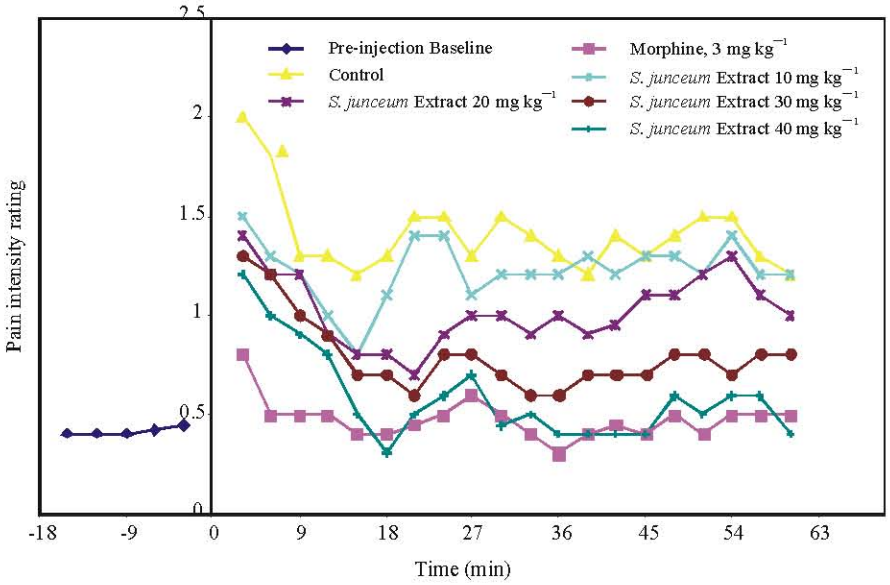

Fig. 1: Quantitative data from the 6 groups of mice used. On the ordinate is pain intensity; lower values signify less pain. On the abscissa is time. The zero time point represents the pain rating in the first 5 seconds following injection. The remaining points represent weighted averages over 3 min blocks

phase that early phase. The results of Tukey test showed a significant difference ($P<0.005$) between 20, 30 and 40 mg kg^{-1} doses of *S. junceum* L. extracts with control group on the early and late phases. On the early phase, there was a significant difference ($P<0.005$) between *S. junceum* L. extract and morphine sulphate 3 mg kg^{-1}. However, on the late phase the differences were not significant. It means that on early phase morphine sulphate 3 mg kg^{-1} had more antinociceptive effect than all of the *S. junceum* L. extracts and on late phase the all *S. junceum* L. extracts showed the same antinociceptive effect as morphine sulphate 3mg kg^{-1}. Increasing the dose to 20, 30 and 40 mg kg^{-1} produced more analgesic effect. This is apparent from Fig. 1, which shows a deepening of the early dips in the curves and absence of high rating for the remainders of the session, especially for the high dose of the extract.

DISCUSSION

S. junceum L. has been used as a traditional medicine in some countries such as Iran, Turkey and Syria as anti-ulcerative and sedative [4, 5, 8]. Its flowers contain flavonoids, as antioxidant materials, saponins and lectin-like proteins [2,3,5]. Spartitrioside is the major saponin compound *of S. junceum* L. [1] which is known as an anti-ulcerogenic ingredient of the plant. It showed the diuretic and emetic effects [8]. There is a report describing that *S. junceum* L. extract has antinfrtility effect in adult male rats [10]. Only one recently reported study showed the antinocieptive and anti-inflammatory effects of *S. junceum* L. [11].

In the present study, which used a model of acute (phasic) analgesiometric test, an antinociceptive action for the hydroalcoholic extract of *S. junceum* L. was found.

The correlation between the antinociceptive effect of the *S. junceum* L. extracts and 3 mg kg^{-1} morphine sulphate indicates that the effect might be related to opioid receptors. The antinociceptive effect of *S. junceum* L. extracts parallels the traditional use of it in a restricted part of Iran, Yazd province. The mechanisms of action of *S. junceum* L. extract remain to be elucidated by further studies. Then, the efficacy and safety of this herbal medication in man is required to be investigated.

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