A preliminary study to evaluate analgesic and behavioural activities of the homoeopathic drug, Anagallis arvensis in rats


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In Homoeopathy, Anagallis arvensis is used in the treatment of skin rashes, warts and urinary tract infections, but not for the treatment of diseases of central nervous system unlike its use in Indian medicine for mania and other derangements of the nervous system. In the present preliminary study, the effect of different potencies (3x, 6x, 12x and 30C) of A. arvensis administered at a daily dose of 0.5 ml/rat/day had been examined and were tested for their analgesic (hot plate, ice plate and Randall-Selitto assay) and behavioural (rota rod and open field assay) activities 30 minutes after administration of drug on 10th, 20th and 30th day of the study. All the four different potencies (3x, 6x, 12x and 30C) of A. arvensis had increased the latency time required to raise and to lick the hind paw for thermal sensation on hot plate test and for cold sensation on ice plate test. They had also increased the quantum of threshold pressure to mechanically induced pain on Randall-Selitto assay but depressed the motor coordination and locomotor activity. The analgesic and behavioural effects of these potencies on 10th day was maximum but subsided on 20th day and 30th day of the study. The preliminary results suggest that A. arvensis may be screened for CNS depressive property on appropriate animal model in order to arrive at a meaningful conclusion.

Keywords: homeopathic medicine; A. arvensis; potencies; analgesic activity; behavioural effect; albino rats

Introduction

Anagallis arvensis Linn. also known as red pimpernel, is a beautiful annual trailing plant. It is extensively used in traditional medicine for the treatment of various ailments such as gout, dropsical affections, epileptic attacks, cerebral affections, leprous, hydrophobia, mania and other derangements of the nervous system. However in Europe, the plant is documented for its use as diuretic, diaphoretic, expectorant, in dropsy, rheumatism and in hepatic and renal complaints. In Chinese medicine, the herb is used for snake bites, dog bites, in joint ailments and in oedema. In India too A. arvensis has been screened for various biological activities but not for analgesic and behavioural activity.

The study was therefore undertaken at Dept. of Zoology, Osmania University, from 2006-2009, to evaluate these effects at different (3x, 6x, 12x and 30C) potencies of A. arvensis in experimental animal models.

Materials and Methods

The raw material (whole plant) of A. arvensis was procured by Survey of Medicinal Plants and Collection Unit, Udagamandalam, Tamil Nadu and sent to M/S. Bahola Laboratories, Puducherry, Tamil Nadu which had prepared different potencies (3x, 6x, 12x and 30C) of A. arvensis from the same batch and sent to us for further experimental study.

Animals

Healthy albino rats weighing between 120-140gm were procured from M/S Jagan animal’s breeder and supplier, Hyderabad and housed (12 / 12 hours, light/dark cycles, Room temperature 22-24°C.) in...
polypropylene cages (47 x 34 x 20 cm) lined with husk which was renewed on every alternate day. They were fed balanced pelleted diet and drinking water ad-libitum. Initially, the animals were acclimatized to standard laboratory conditions for 10 days and thereafter they were accustomed to respective test procedures by giving them three test trials at 10 minutes intervals on each day for three days before subjected to experimental protocol.

**Experimental design**

A total of 90 rats were taken and grouped into 5 batches of 18 each which were further divided into 6 sub-batches of 3 each. The different potencies (3x, 6x, 12x and 30C) of A. arvensis were orally administered daily at a dose of 0.5 ml/rat/day for 30 days. Two groups of parallel control, one receiving equivalent volume of alcohol (91.5% v/v; used as a vehicle for preparation of different potencies of the test drug) and other receiving equivalent volume of normal saline were also run simultaneously. The test potencies of A. arvensis and alcohol were diluted with distilled water in a ratio of 1:4 so that each rat should not receive total volume of more than 2 ml per day. The response of drug was measured after 30 minutes of its administration of drug, alcohol or saline. The time taken (in seconds) to lick the fore or hind paws to cold sensation was noted. Control reaction time (latency time) of the rats to cold sensation was taken on day 1 before the administration of drug, alcohol or normal saline. The percentage of analgesia was calculated as described in hot plate technique.

**Ice plate method**

For ice plate latency assay, rats were gently placed individually on the ice cubes (0 - 4°C) filled in a container (20 x 20 x 20 cm) and covered with a plastic cover, 30 minutes after administration of drug, alcohol or saline. The rats were visualized through a transparent wall and the time taken (in seconds) to lick the fore or hind paws to cold sensation was noted. Control reaction time (latency time) of the rats to cold sensation was taken on day 1 before the administration of drug, alcohol or normal saline. The percentage of analgesia was calculated as described in hot plate technique.

**Randall–Selitto assay**

The analgesic activity of drug against mechanically induced pain was measured by Randall–Selitto assay (Randall–Selitto apparatus, Ugo Basile, Italy). After 30 minutes of drug, alcohol or saline administration, the rats were gently held in the hand. Afterwards, the paw of the right foot of the rat was placed on the rubber base of the apparatus and pressure (in ponds; expressed in gm) was applied either on 2nd – 3rd or 3rd – 4th metatarsal region through a pointed tip and increased gradually until vocalization elicited which was considered as threshold pressure to mechanical induced pain. Control threshold pressure to mechanical induced pain was taken on day 1 before the administration of drug, alcohol or normal saline. The percentage of analgesia was calculated as described in Randall - Selitto assay.

**Assessment of behavioural activity**

**Rota-rod performance assay**

To observe behavioural strategy adopted by the rats to maintain motor coordination, grip strengths of the rats were measured by using the automated rota rod apparatus (DolphinTM instrument). The rotor was divided into three compartments which allowed three rats to test simultaneously at a time. Rats were placed on the rotor with the body axis perpendicular to the rotor's long axis with the head directed opposite to the direction of rotating rod. In the beginning, each rat was trained on the rota rod driven at a constant speed of 5 rpm until rat achieved the criteria of remaining on the rotating spindle for about 60 seconds. The control grip strengths of the rats were measured on day 1 just before administration of the drug, alcohol or normal saline by placing the rats on the rotating spindle and recording the duration of time as soon as rat falls from
the spindle. On the day of experiment, the duration of grip strength was recorded 30 minutes after the administration of drug, alcohol or normal saline.

Open field test

For recording the locomotor activity of the rats, the method of open field test was used.\(^1\) The floor of the apparatus which was made up of wooden box (96 x 96 x 6 cm) was divided in to 36 equal squares. The latter was coloured black and white alternatively. The apparatus was illuminated with low intensity diffuse light (40 W) placed at a height of 100 cm at the time of experiment, and it was cleaned by using 5% alcohol every time after each test trial. Initially, the rats were made accustomed to the environment of the apparatus by placing them gently in the centre of the floor and allowing them to walk freely for 5 minutes daily for 3 - 4 days. Control locomotor activity of the rats were recorded on day 1 just before administration of drug, alcohol or normal saline by placing the rats individually in the apparatus and by counting the number of squares crossed by the rats in 5 minutes. On the day of experiment, the locomotor activities of the rats were recorded 30 minutes after administration of drug, alcohol or normal saline.

Statistical analysis

The data were expressed as Mean ± S.E.M. The difference between mean values of groups were statistically analysed by student's t test. \(p\)-values < 0.05 were considered as statistically significant\(^1\).4

Results

Assessment of analgesic activity

Hot plate method

The results of the analgesic effect of \textit{A. arvensis} using hot plate assay are summarised in Fig.1. The initial latency time recorded on day 1 before administration of drug, alcohol or normal saline and 30 minutes after the administration of normal saline on different days of experimentation to noxious thermal stimulus was more or less constant (3.38 to 3.75 sec). On the other hand, there was an increase in the latency time (4.49 to 5.88 sec.) to thermal noxious stimulus when measured 30 minutes after the administration of different potencies (3x, 6x, 12x and 30C) of \textit{A. arvensis} or alcohol at a dose of 0.5 ml/rat/day on 10\(^{th}\) day. The difference was significant (\(p<0.05\)) only with those rats treated with 12x potency when compared to that of normal saline treated rats. Afterwards, the increase in the duration of latency time to thermal noxious stimulus was tapered off gradually on 20\(^{th}\) day and 30\(^{th}\) day on continuation of the treatment (Fig.1).

\textbf{Figure 1}-Analgesic effect of \textit{A. arvensis} (0.5ml/rat/day) on Hot plate assay (Mean ± S.E.M.)
Ice plate method

Fig.2 shows the results of the analgesic effect of different potencies of *A. arvensis* using ice plate assay. The initial latency time to cold sensation recorded on day 1 before administration of drug, alcohol or normal saline and 30 minutes after the administration of normal saline on different days of experimentation was more or less same (5.49 to 6.09 sec). Similar to the effect on hot plate, there was an increase in the latency time (8.14 to 8.81 sec) to cold sensation when measured 30 minutes after the administration of different potencies (3x, 6x, 12x and 30C) of *A. arvensis* or alcohol at a dose of 0.5 ml/rat/day on 10th day. The difference in the increase in latency time to cold sensation was significant (p<0.05) with those groups which were treated with 3x, 6x, and 30C potencies of *A. arvensis*. Thereafter, the increase in the duration of latency time to cold sensation was tapered off gradually on 20th day and 30th day on continuation of the treatment (Fig.2).

**Figure 2**-Analgesic effect of *A. arvensis* (0.5ml/rat/day) on Ice plate assay (Mean ± S.E.M.)

Randall–Selitto assay

Fig.3 shows the results of the analgesic effect of different potencies of *A. arvensis* on Randall-Selitto assay. The quantum of threshold pressure required to elicit vocalization to applied mechanical pain was more or less same (131.33 to 133.33 g) on day 1 before administration of drug, alcohol or normal saline and 30 minutes after the administration of normal saline on different days of experimentation. There was an increase in the quantum of applied threshold pressure (146.66 to 152.66g) required to elicit vocalization to mechanical pain when measured 30 minutes after the administration of different potencies (3x, 6x, 12x and 30C) of *A. arvensis* or alcohol at a dose of 0.5 ml/rat/day on 10th day. The difference was significant (p<0.05) only with those rats treated with 3x potency when compared to that of normal saline treated rats. Afterwards, the increase in the quantum of threshold pressure required to elicit vocalization to applied mechanical pain did not persist but gradually tapered off on 20th day and 30th day of experiments on further continuation of the treatment (Fig.3).