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COLLABORATIVE RESEARCH

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, leprosy, hydrophobia, mania and other derangements of the nervous system.[1] However in Europe, the plant is documented for its use as diuretic, diaphoretic, expectorant, in dropsy, rheumatism and in hepatic and renal complaints.[2] In Chinese medicine, the herb is used for snake bites, dog bites, in joint ailments and in oedema.[3] In India too *A. arvensis* has been screened for various biological activities[4-7] but not for analgesic and behavioural activity.

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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 on 10^{th}, 20^{th} and 30^{th} day. Readings taken just before administration of the drug/alcohol/normal saline on day 1 of the study were considered as the initial control values for comparison. The experimental protocols were approved by Institutional Animal Ethics Committee of Department of Zoology, Osmania University, Hyderabad (Reg.No.383/01/a/CPCSEA). [All the experiments were conducted in air conditioned room]

Assessment of analgesic activity

Hot plate method

The hot plate latency assay, based on the method of Eddy *et al* [9] was used. The rats were gently placed individually on a hot plate maintained at a constant temperature of 55 ± 2°C, 30 minutes after the administration of drug, alcohol or saline. The time taken (in seconds) by the rats to lick the fore or hind paws was noted which was considered as the reaction time (latency time). Control reaction time of the rats to thermal noxious stimulus was taken on day 1 before the administration of drug, alcohol or normal saline. The percentage of analgesia was calculated as described earlier.[9] None of the rat was kept on the hot plate for more than 15 sec.[10]

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.[9]

Randall–Selitto assay

The analgesic activity of drug against mechanically induced pain was measured by Randall–Selitto assay[11] (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 $2^{nd} – 3^{rd}$ or $3^{rd} – 4^{th}$ 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.[11]

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 (Dolphin™ instrument).[12] 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.[13] 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

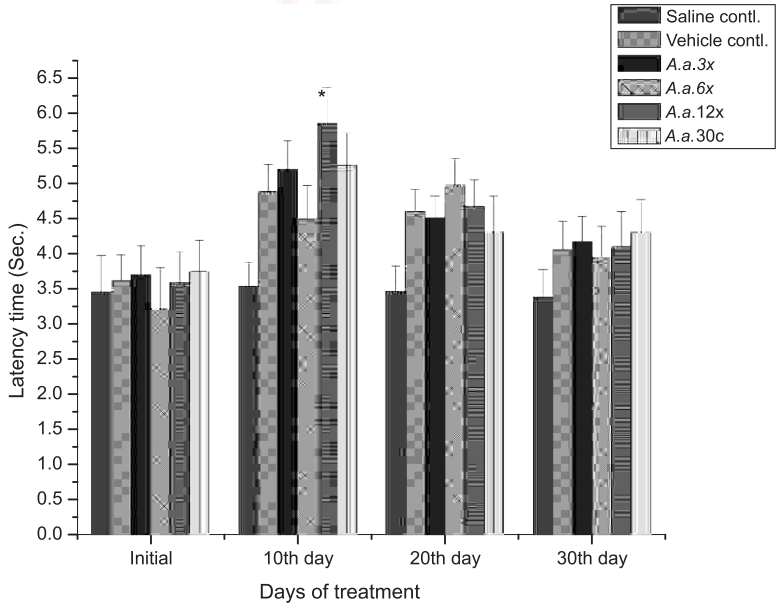
The data were expressed as Mean ± S.E.M. The difference between mean values of groups were statistically analysed by student's't' test. *p*-values < 0.05 were considered as statistically significant[14].

Results

Assessment of analgesic activity

Hot plate method

The results of the analgesic effect of *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 *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 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 20th day and 30th day on continuation of the treatment (Fig.1).

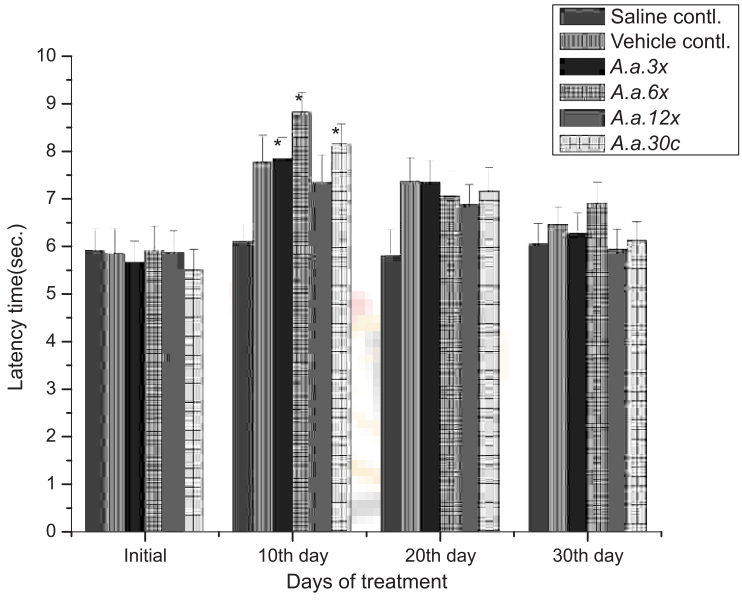


n = 3 animals in each group.
*p<0.05 compared to initial value.

Figure 1-Analgesic effect of 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).



n = 3 animals in each group.
*p< 0.05 compared to initial value.

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).

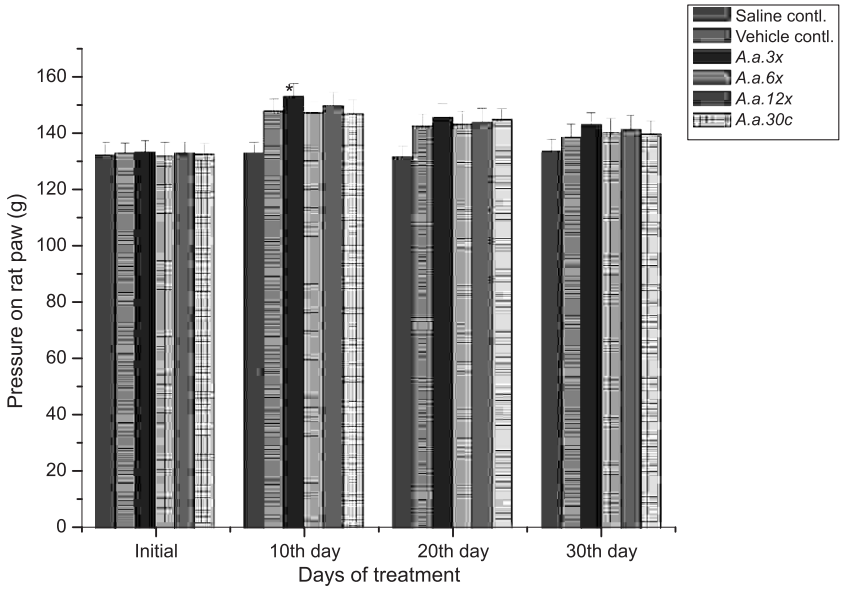
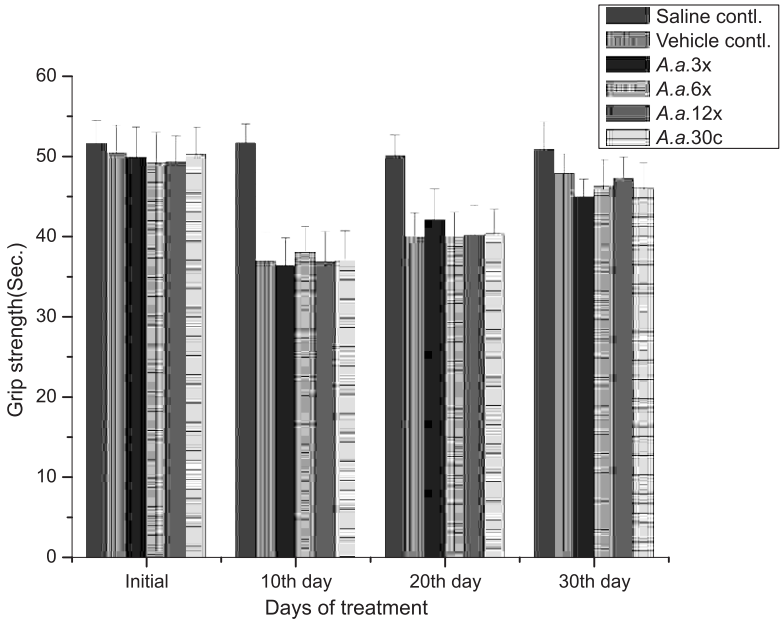


Figure 3-Analgesic effect of *A. arvensis* (0.5ml/rat/day) on Randall-Selitto assay(Mean ± S.E.M.)

Assessment of behavioural activity

Rota-rod assay

Fig.4 shows the effect of different potencies of *A. arvensis* on motor coordination activity of rats using grip strength test. The average grip strength of the rats determined 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 on the rotating rod was 'more or less' same (49.11 to 51.55 seconds). On the other hand, there was a decrease in the grip strength of the rats when measured 30 minutes after the administration of the different potencies (3x, 6x, 12x and 30C) of *A. arvensis* or alcohol at a dose of 0.5 ml/rat/day for 30 days. On the 10th day of experiment, drug or alcohol treated rats fell between 32.81 to 37.98 seconds from the rota rod when they were subjected to test after 30 minutes of drug administration. However, the decrease in grip strength in drug treated rats was not statistically significant when compared to that of normal saline treated rats. Afterwards, there was a progressive reversal in the grip strengths of drug or alcohol treated rats on further continuation of the treatment as the rats stayed for longer duration but still for less duration on the rota rod that was observed on day 1 before administration of drug when tested on 20th and 30th day of experiment (Fig.4).

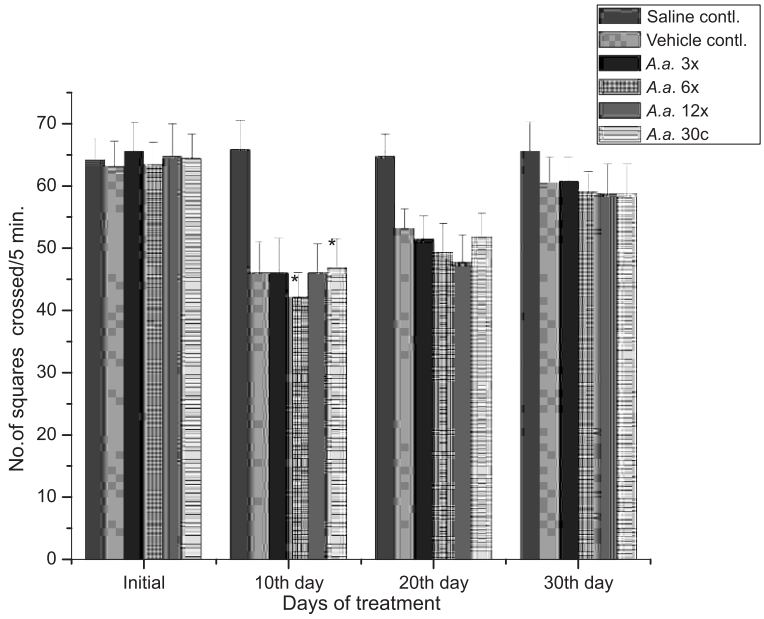


n = 3 animals in each group.

Figure 4-Behavioural effect of *A. arvensis* (0.5ml/rat/day) on Rota - rod assay (Mean ± S.E.M.)

Open field test

Fig. 5 shows the effect of different potencies of *A. arvensis* on locomotor activity of rats using open field test. The average locomotor activity as measured in terms of crossing of the squares of a open field apparatus during 5 minutes of observations 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 (63.00 to 65 squares in 5 minutes) (Fig.5). There was a decrease in the locomotor activity of the rats (42.00 to 46.66 squares in 5 minutes) when measured on 10th day of the experiment, 30 minutes after administration of different potencies (3x, 6x, 12x and 30C) of *A. arvensis* or alcohol at a dose of 0.5 ml/rat/day. The difference in locomotor activity was significant ($p<0.05$) with those rats treated with 6x and 30C potency of the drug when compared to that of initial locomotor activity taken just before administration of drug on day 1 of the study. However, such depressant effect of drug or alcohol on locomotor activity slowly vanished off even on continuation of drug/alcohol treatment when tested subsequently on 20th and 30th day of the study.



n = 3 animals in each group.
*p<0.05 compared to initial value.

Figure 5-Behavioural effect of *A. arvensis* (0.5ml/rat/day) in the open field test (Mean ± S.E.M.)

Discussion and Conclusion

As mentioned earlier the preliminary screening was undertaken to have some assessment about the analgesic and behavioural activities of different (3x, 6x, 12x and 30C) potencies of *Anagallis arvensis* in albino rats.

The analgesic activities of different potencies (3x, 6x, 12x and 30C) of *A. arvensis* in rats were evaluated by using Hot plate assay, Ice plate assay and Randall-Selitto assay. The data obtained in respect of it's analgesic effects revealed that all the four potencies of *A. arvensis* had increased the latency times for both thermal noxious stimulus and cold sensation and had also increased the quantum of threshold pressure to mechanical induced pain when measured on 10th day of study, 30 minutes after the administration of the drug. The level of significance varied not only between the potencies of the drug but also between different sets of experiments when compared to that of normal saline group whereas, the analgesic responses obtained for different potencies (3x, 6x, 12x and 30C) of *A. arvensis* and for alcohol were not significantly different.

Likewise, the behavioural activities of different potencies (3x, 6x, 12x and 30C) of *A. arvensis* in rats were evaluated by using rota rod (for motor coordination) and open field test (for locomotor activity). The present results showed that different (3x, 6x, 12x and 30C) potencies of *A. arvensis* had decreased the grip strength and locomotor activity when measured on 10th day of study, 30 minutes after the administration of the drug. Similar depressant effects on grip strength and locomotor activity were also observed when equivalent volume of alcohol was administered to the rats.

Observation of increase in the latency time to noxious thermal stimulus and/or cold sensation and increased in the quantum of threshold pressure to mechanical induced pain and decreased locomotor activity and motor coordination by the drug is the sign of CNS depression.[15] Wearing off the depression on prolonged and continuous use of the drug is either due to decreased sensitivity of the central nervous system or because of its increased metabolising enzymatic activity in the liver, need to be worked out by designing newer set of experiment. It is worth while to mention that alcohol when used as a vehicle to prepare

different potencies (3x, 6x, 12x and 30C) of the drug *A. arvensis,* is well known to have these effects on its prolonged use.

The over all results of the present preliminary study suggests that the homoeopathic formulation of *A. Arvensis* possess central nervous system depression property. However, more experimentation, detailed physico-chemical and experimental analysis are required in order to arrive at a definite conclusion.

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