NEURO-PHARMACOLOGICAL AND ANALGESIC EFFECTS OF *ARNICA MONTANA* EXTRACT

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ABSTRACT

Anxiety and disorders associated with anxiety are increasing rapidly throughout the world. In the present research *Arnica montana* (Asteraceae) was studied on neuro-pharmacological and analgesic effect in mice. Decreased locomotor and exploratory activities (open field, head dip cage, stationary rod, cage cross, forced swimming and light and dark) were observed in mice at the dose of 100 mg/kg. These results were found significant at P<0.05 when compared with Diazepam 2mg/kg. *A. montana* crude extract also exhibited potent analgesic effect at the doses of 50 and 100 mg/kg (P<0.05). Aspirin 300 mg/kg was used as a reference drug. Highest % of inhibition (57.6%) was observed in the third phase of acetic acid induced writhing test at the dose of 100 mg/kg of *A. montana*. Whereas, significant analgesic response was also observed at the low dose (50mg/kg). The percentage inhibition by formalin induced licking biting was found significant in the 2nd (76.1%) and 3rd (19.8%) phase at 50 mg/kg. These results were suggested that the extract of *A. montana* possess significant analgesic and anxiolytic effect.

Keywords: *Arnica montana*, Anxiolytic, Neuro-pharmacological activities, Diazepam, Analgesic.

INTRODUCTION

Anxiety and its related disorders have become a global problem and affected most of the population world-wide [1]. Benzodiazepines are commonly used for the treatment of anxiety [2-3]. They have pronounced anxiolytic effect along with numerous unpleasant side-effects. The low safety profile of benzodiazepines initiated the researchers to explore natural world and discover new compounds with lesser adverse effects [4-5].

Arnica montana (Wolf's Bane, Leopard's bane), belongs to family Asteraceae. It contains volatile oil, carotenoids, flavonoids, tannins, resins and triterpenic alcohol. *A. montana*, has antiseptic, anti-inflammatory, anti-bacterial, decongestive and anti-fungal properties. It also stimulates the forming the granular tissues and thus accelerating the healing process [6-9].

The arnica flowers are used to treat the pale face skin complexion, wounds, bruises and burns. The treatment of dislocations, bacterial infections, skin cancer, bronchitis, tonsillitis, pharyngitis, flu, lung cirhosis, cystitis, nephritis, kidney infections, coronary insufficiencies, hypertension, angina, cerebral trauma, headaches, paresis, semi-paresis, insomnia, heart palpitations, nightmares, night terrors, moral depressions, neurosis, hysteria etc are the other uses of *A. montana* [10-12].

MATERIAL AND METHOD

Arnica montana plant extract was purchased from Bioron suppliers. The extracts obtained were stored in cool, dry place for further studies. Male albino mice weighing 20-25g were maintained under standard nutritional and environmental conditions throughout the experiment. The animals had access to water and food *ad libitum*. The animals were deprived of food 12 h before experimentation.

Diazepam (2 mg), aspirin (300 mg), formaldehyde and acetic acid were purchased from the local Pharmacy and retail chemical stores. All drugs and *A. montana* extract were dissolved in saline solution which used as a vehicle to a desired concentration. Then, they were filtered through gauze and given to the animals via the intra-gastric feeding tube. All administered substances including the *A. montana* suspension were freshly prepared.

Neuro-pharmacological activities were studied by open field, traction, head dip, rearing test and swimming induced depression test. All tests were performed in a calm and peaceful environment. Animals were divided in to 5 groups (n= 6 in each group). Group I: control group mice, Group II: Positive control treated group. In each test, the positive control group was treated with the standard reference drugs. In order to determine anxiolytic effect, the animals were treated with diazepam (2 mg kg^{-1}). Group III- V: The mice treated with different doses of the test extract respectively (500mg/kg, 300mg/kg, 100 mg/kg), via oral route [13].

Neuro-pharmacological activities

Open field test

Open-field test is a rodent model used for measuring anxiety and exploration as well as locomotion in mice. Open field test area is made of plastic walls and floor divided into nine square of equal area. It is used to evaluate the exploratory activity of animal. The observed parameters in this study are the number of square crossed in 30 minutes [14].

Light dark test

Light and dark test is one of the apparatus designed to test anxiolytic behavior in mice. The apparatus consists of a plastic box with two compartments one of which is made of transparent plastic and the other of black colour plastic. Each animal is placed at the center of the transparent compartment and then the number of entries in each space, as well as, time spent in each compartment is recorded for 30 minutes [15-16].

Head-dip test

This study was conducted using wooded apparatus measuring 40×40 cm with 16 evenly spaced holes. Thirty minutes after treatment, the mice were placed singly on a board with 16 evenly spaced holes and the number of times the mice dipped their heads into the holes was noted. The control and drug treated animals were placed individually in the head dip box and the observations were made for 30 minutes [17].

Cage crossing movement

The cage crossing test was performed in a specifically designed mice cage having rectangular shape. Both control and treated mice were placed separately in the cage and their movements were noted for 30 minutes [18].

Forced swimming test

In this test mice are forced to swim in a restricted space from which there is no escape and become immobile. Individual mice were forced to swim in an open cylindrical container containing 7 cm of water at 22.0 ±1.0°C; the duration of immobility or struggling in a period of 6 minutes was recorded. Immobility was evaluated as

when mice ceased to struggle and remained floating in the water, making only necessary movements necessary to keep its head above water. At the end of the session, the animal was removed from water and dried gently [19-20].

Stationary rod test

The stationary rod test was used to assess learning ability and locomotor activity. The test apparatus consisted of horizontal stainless steel rods with the platform. At first the mice were trained and then they were allowed to balance on stationary rod. During the observation time mice maintained balance, travelled and fall from the stationary rod was noted [21].

Analgesic activity (by acetic acid)

The analgesic effect was assessed by acetic acid induced writhing test in mice. The animals were administered orally with the test samples and reference standard drug aspirin (300 mg/kg). 0.6% acetic acid per kg was injected intra-peritoneal. The resultant abdominal writhes consist of a contraction of body muscles and the stretching of hind limb. These abdominal contractions were counted over three periods of 10 min each after injection of acetic acid. The anti-nociceptive activity was considered as the reduction of these writhes in the treated animals. The % inhibition of the test samples was calculated by using the following formula: (number of writhing of control) – (no of writhing of test group)/ (number of writhing of control) ×100 [22].

Analgesic activity (by Formalin)

In this test, 2.5% formalin solution (0.05 ml) was injected in the sub-plantar region of right hind paw of the mice to induce pain. Aspirin was administered (30 min before formalin injection) orally to serve as a control. The *A. montana* extracts (50 and 100 mg/kg) were administered orally 60 min before the reaction. The animals were placed in transparent cage for recording observations and the time spent in licking and biting responses of the injected paw was taken as indicator of pain response. Responses were measured for 30 min after formalin injection. The reduction in the number of licking and biting responses is the inhibition of the pain score and the % inhibition can be calculated by the following expression: (mean of control group) – (mean of treated group)/ (mean of control group) ×100 [23].

Statistical analysis

The results were expressed as mean ± S.E.M. All statistical comparisons were made by means of Student's *t*-test and a *P* value smaller than 0.05 was regarded as significant [24].

RESULTS

Locomotor and exploratory behavior of mice

The anxiolytic activity was assessed using open field, head dip and stationary rod apparatus. The most significant CNS depression effect was observed at the dose of 100 mg/kg of *A. montana* extract. In open field activity the results were found to be 11.33±1.73 counts in 30 minutes. In head dip test, the mice dipped head 24±2.65 times. Number of entries in light compartment is 5.5±1.51 times. The readings of cage cross is 24.67±2.41 times. In forced swimming test (FST) the Mean forced mobility time was 1.78±0.12 seconds. Mean time of mobility on stationary rod was 12.5±0.83 seconds. Locomotor and exploratory activity was reduced in comparison to control and standard drug Diazepam (2 mg/kg^{-1}) (Table 1 and Graph 1).

Acetic Acid Test

Analgesic activity is widely assessed by the method of acetic acid induced abdominal constrictions. 0.6% (10 ml/kg i.p) acetic acid solution was administered in mice and the abdominal constrictions (writhes) were observed after 05 minutes. The writhes were counted for three phases, each of 10 minutes respectively. The inhibition of acetic acid induced writhes by Aspirin was as follows; 1st; 66.7%, 2nd; 32.2%, 3rd phase; 35.5%. Where as *A. montana*, at the dose of 100 mg/kg exhibited maximum inhibition (57.6 %) in 3rd phase. (Table 2 and Graph 2).

Formalin Test

The results exhibited prominent analgesic effect in comparison with aspirin. The analgesic effect of *A. montana* showed maximum inhibition of the licking and biting response (76.1%) induced by formalin at the dose of 50 mg/kg in 2nd phase. Aspirin in 1st, 2nd and 3rd phase showed was 21.7%, 84.3% and 22.8% inhibition respectively (Table 3 and Graph 3).

Table 1: Neuro-pharmacological activities of *Arnica montana*

Treatment	Open field	Head Dip cage	Light and dark	Cage cross	FST (mobility time)	Stationary rod test
Control	209.83±3.97	109.5±3.67	17.5±1.99	68.33±3.68	1.741±0.05	17±1.05
A .montana 500mg/kg	125±7.94	65.33±4.19	15.83±1.55	26.33±4.46	0.66±0.16	22.16±1.27
A. montana 300mg/kg	187.67±2.98	67.83±2.90	21±2.28	97.83±2.76	2.38±0.03	18.16±1.03
A. montana 100mg/kg	11.33±1.73	24±2.65	5.5±1.51	24.67±2.41	1.78±0.12	12.5±0.83
Standard-Diazepam 2mg/kg	12.5±0.83	11.5±0.83	1±0.4	19.5±0.83	1.60±0.06	11.33±1.73 (fall)

Results were expressed along mean ± standard error mean. Significance of data were evaluated at P < 0.05 – student t test
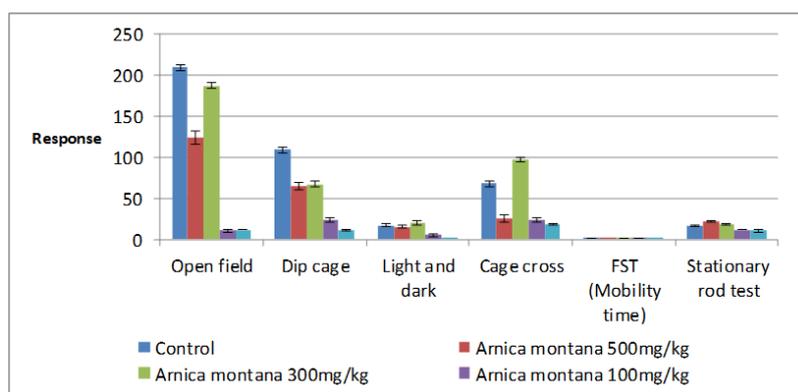
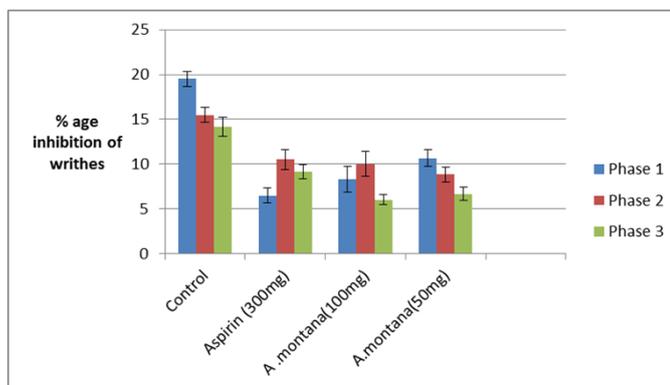**Graph 1: Neuro-pharmacological activities of *A. montana***

Table 2: Analgesic activity of *A. montana* (by acetic acid)

| Treatment Dose mg/kg	Phase 1		Phase 2		Phase 3	
	Mean No. of Writhes	% of Inhibition	Mean No. of Writhes	% of Inhibition	Mean No. of Writhes	% of Inhibition
Control 0.5 ml saline	19.5±0.83	-	15.5±0.83	-	14.16±1.03	-
Aspirin (300mg)	6.5±0.83**	66.7	10.5±1.08	32.2	9.16±0.77	35.3
A. Montana (100 mg)	8.33±1.43*	57.4	10±1.41*	35.4	6±0.56**	57.6
A. Montana (50 mg)	10.67±0.96	45.1	8.83±0.86**	43	6.67±0.73*	52.8

Results were expressed along mean ± standard error mean. Significance of data were evaluated at $P < 0.05$ – student t test

Graph 2 Analgesic Activity of *A. montana* (by Acetic Acid)

Table 3: Analgesic activity of *A. montana* (by formalin)

Treatment Dose mg/kg	Phase 1		Phase 2		Phase3	
	Mean No. of biting & licking	% of Inhibition	Mean No. of biting & licking	% of Inhibition	Mean No. of biting & licking	% of Inhibition
Control 0.5 ml saline	19.16±1.03	-	10.67±0.54	-	5.83±1.03	-
Aspirin(300mg)	23.33±1.15	21.7	1.67±0.61	84.3	4.5±0.83**	22.8
A. montana (100 mg)	13.16±3.06**	45.5	1.16±0.52	73.1	6.16±1.86	5.6
A. montana(50 mg)	19±3.42	0.83	0.16±0.18**	76.1	4.67±1.40*	19.8

Results were expressed along mean ± standard error mean. Significance of data were evaluated at $P < 0.05$ – student t test
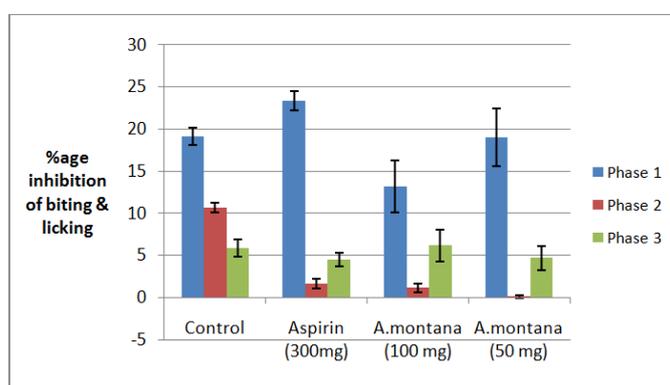
Graph 3: Analgesic Activity of *A. montana* (by Formalin)

DISCUSSION

Stress is the causative factor behind anxiety and depression. It is estimated that by 2020, these disorders will become the second number cause of disability [25]. In present study the neuro-pharmacological screening like open field activity, stationary rod activity test and head dip activity of *A. montana* were evaluated. The extract decreased the exploratory and locomotor activities in mice. Our observations indicated that *A. montana* treated mice spent more time in light compartment and therefore, had anxiolytic effect. The light and dark box test in mice is use to assess anxiolytic activity [26]. The lesser number of entries in the light box reflects the sedative and anxiolytic effect of the extract [26].

The development of immobility, during swimming test indicated that the cessation of affective/motivational behavior. Plants rich in tannins and flavonoid content have been found to be of therapeutic significance in treating many CNS disorders [27]. *A. montana* also contains tannins and flavonoids and maybe due to the presence of these constituents it exhibited pronounced anxiolytic effect.

The present study revealed that *A. montana* possess significant analgesic effect. The medicinal uses of *A. montana* are well established and have been effectively used since ancient time for the treatment of strains, sprains and bruises. *A. montana* contains helenalin, a sesquiterpene lactone that is a major ingredient having anti-inflammatory effect due to which it is mostly used for the

treatment of bruises. It can be suggested that analgesic effect may be due to the presence of volatile oil.

CONCLUSION

Our present study revealed that *A. montana* possesses significant analgesic and anxiolytic effect, along with prominent decrease in neuro-pharmacological activities due to its active chemical constituents. However, further studies are required to confirm the mechanism of action behind the effects observed in our study.

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