

Original Research Article

Neuro-Pharmacological, Analgesic and Anti-Inflammatory Effects of Crude Extract of *Cicuta Virosa*

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A b s t r a c t

Cicutavirosa is commonly known as Water Hemlock and traditionally known as poisonous plants. In the present research work central nervous system (CNS), analgesic and anti-inflammatory effects were assessed in mice and rats. The crude extract showed anxiolytic response at 100 mg/kg which was evaluated through open field, head dip, light and dark, cage cross and swimming test. In open field (73.33±3.28) and light and dark test (time spent in light 5.16±1.07 min.) the movements of mice were increased as compare to standard drugs, whereas in forced swimming test (FST) the mean forced mobility was reduced to 1.16±0.02 seconds. At the dose of 500mg/kg of *C. virosa*, CNS inhibition was observed in cage cross test and dip cage activity. The results of locomotor and exploratory activity were also found reduced in comparison to control and standard Diazepam (2 mg/kg-1). Analgesic activity was evaluated by acetic acid and formalin method. The abdominal constrictions were observed. *C. virosa* at the dose of 50 mg/kg exhibited maximum inhibition of writhes, in first phase (57.67%) second (3.26%) and third phase (0%). The results of analgesic activity was compared with aspirin (orally administered, 300 mg/kg). *C. virosa* also significantly inhibited formalin induced licking and biting response at the dose of 50mg/kg. Anti-inflammatory effect was observed by carrageenan induced edema at 300 and 500 mg/kg doses of *C. virosa*. Maximum inhibition of edema was observed at 500mg/kg (23.07%). The results of neuropharmacological, analgesic and anti-inflammatory activities of *C. virosa* were found significant at P 0.05.

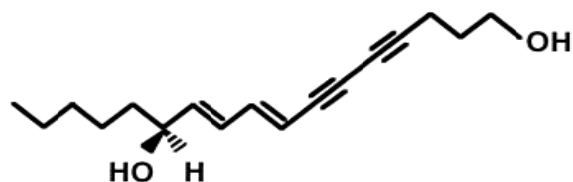
Keywords: Cicutavirosa, anxiolytic, analgesic, anti-inflammatory

Introduction

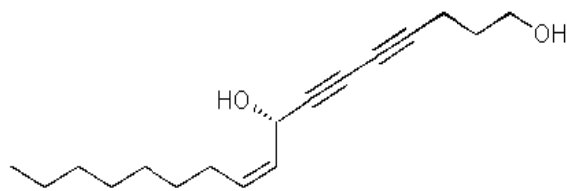
Cicutavirosa commonly known as 'Water Hemlock' belongs to family Apiaceae and contains pseudo alkaloids like Cicutoxin, Isocicutoxin, Cucatine, VirolA, Virol B and VirolC. Roots and tubers of the plant are toxic. The toxic effect of *C. virosa* resembles that of strychnine poisoning. Due to its potent CNS activity it is used in the treatment of epilepsy, meningitis, convulsions, other ailments of the brain, eczema, other skin affections, uraemia and frequent micturition. *C. virosa* is highly toxic and may cause vomiting, acute renal failure, marked metabolic acidosis, drowsiness, seizures and convulsions, or unconsciousness that may lead to death [1 – 3].



Cicutoxin



Virol A



Virol B

Material & Method

Experimental animals

Albino mice (weighing 25-30 gm) of either sex obtained from Animal House, Dow University of Health Sciences, Karachi, Pakistan were used to determine the analgesic, and CNS activities. Animals were kept in colony cages five animals in each group) with access to food and water. They were maintained in a climate and light controlled room 30°C ± 1°C 12/12 Hours light/dark cycle) at least 7 days before testing or administration of the drug.

Animals used for CNS activity were acclimatized first for at least 5 days in the laboratory environment with 12 hours light and 12 hours dark schedule. Animals were housed in standard metal cages and provided food and water *ad-libitum*.

Preparation of test Material

Method of preparing the water extract is already described in experimental part of Farah-Saeed's thesis (2014) [4]. The dose of extract was prepared in 0.5 ml distilled water i.e. 300 mg/kg and 500 mg/kg/0.5 ml.

Assessment of neuro-pharmacological activity

CNS activity was studied by open field test, traction test, head dip test, rearing test, and swimming induced depression test. All the CNS related tests were performed with the protocol described by Irwin (1964) [5].

In each test, animals were divided into 4 groups (Group-A for control, Group-B and Group-C for 300mg/kg oral doses of crude extract respectively, and Group-D for standard). Each group comprised of 5 animals. Diazepam as 2mg/kg orally was used as standard. The crude drug and the diazepam were diluted in distilled water and administered orally. The control animals were treated orally with same volume of saline as the crude extract. In all the tests, observations were made after 30 to 40 minutes of oral dose of the test substance.

Open Field Activity

The open field apparatus designed in the laboratory as described by Irwin (1964) i.e. consists of 76 x 76 cm square area with opaque walls 42 cm high. The floor is divided by lines into 25 equal squares. 25 to 30 gm weight mice were used in this experiment. Test was performed as described by Kennett *et al.*, (1985) and Turner (1965) [6 – 7]. Animals taken out from their home cages and were placed in the center square of the open field (one at a time). Number of squares crossed with all four paws was counted for 30 minutes. Activities of control mice and drug treated mice were monitored in a balanced design to avoid order effect.

Light dark test

Light and dark test is one of the apparatus designed to observe anxiolytic behavior in mice [8]. 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 min.

Forced induced Swimming Test

Forced induced swimming test was performed according to Sanchez *et al.*, (2002) [9] and Turner *et al.*, (1965) [7]. This test determines the muscle and CNS activity of the crude extract. Mice weighing 25 to 30 gm were placed individually for six minutes in the glass tub filled with water at room temperature up to the marked level. Mouse when placed in water suddenly starts to move its front and hind paws. The activity time of animal is determined with the help of stopwatch out of total observation time of six minutes.

Acetic acid-induced writhing in mice (Analgesic activity)

The abdominal constrictions resulting from intra-peritoneal (i.p.) injection of 3% acetic acid consisting of the contraction of abdominal muscle together with the stretching of hind limbs was carried out according to the standard procedure (Correa *et al.*, 1996; Ojewole 2005, Nwafor *et al.*, 2007) [10 – 12]. The animals were divided into six groups of six mice per group. Group 1 served as control while groups 2-4 were pretreated with respective drug *extract* (i.p.) respectively. Group 5 was administered with acetyl salicylic acid (Aspirin) [(300mg/kg; i.p (Sigma, USA)] and 30min later, was treated with extract (48mg/kg i.p.) while group 6 received acetylsalicylic acid (300mg/kg i.p) only. The number of writhing movements by each mouse was counted for 30 min. Anti-nociceptive (analgesia) expressed as the reduction in the number of abdominal constrictions between control animals (acetic acid treated mice) and mice pretreated with the extract.

Formalin – induced hind paw licking in mice (Analgesic activity)

The procedure used was essentially similar to that described previously by Sanchez – Mateo *et al.*, (2006) [13]. The animals were used to analyze the first phase of formalin-induced licking. 2ml formalin solution (0.9% formaldehyde) made up in 98ml distilled water was injected subcutaneously under the surface of the right hand paw. The amount of time spent licking the injected paw was timed and was considered as indicative of pain. The first of the nociceptive response normally peaks 5 min after injection and the second phase 15-30min after formalin injection, representing the neurogenic and inflammatory pains responses respectively [14]. The animals in groups 2-4 were pretreated with extract orally for this study, and then were injected formalin in paw.

Anti-inflammatory Activity



The anti-inflammatory activity was evaluated by the Carrageenan induced paw edema method. Wistar rats (male), weighing 180-200 g respectively, were used. The rats were divided into ten groups of six animals each. Freshly prepared aqueous suspension of Carrageenan(2%) was injected in the plantar aponeurosis of the right hind paw of each rat. One group was kept as a control, second group was given standard drug (Aspirin) and the animals of other groups were pre-treated with test drugs (300mg/kg and 500mg/kg doses) given orally 30 minutes before Carrageenan injection. The compound and standard drug Aspirin were administered orally to the animals, respectively. The animals of group I was treated with as Control. The group II animals were treated with Aspirin 300 mg/kg as a standard drug. The animals of the groups III to X were given test compounds. Thirty minutes after the administration of test compounds and Aspirin, paw edema was induced in albino rats by injecting 0.1 mL of Carrageenan suspension, into sub- plantar region of the left hind paw of each rat. The paw volume of all animals in all groups was measured at 0, 60, 120 and 180 minutes intervals, after Carrageenan administration. The differences in the paw volumes (i.e. edema volumes) of each

animal of all the groups were measured by vernier caliper and compared with the changes in the edema volumes of control and the drug treated animals [15 -16]. The results were expressed as percentage reduction in edema volume, which can be calculated by using the formula:

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Results

The anxiolytic activity was assessed by using open field, head dip, light and dark, cage cross and swimming (Table 1, Graph 1).

Analgesic activity was assessed by acetic acid and formalin method (Table 2 & 3, Graph 2, 3, 4 & 5).

Maximum percentage inhibition in paw volume was 22.22% at 1.5 hours in case of Aspirin. *C. virosa* (300mg/kg) exhibited anti-inflammatory effect 14.47% at 1.5 hours and *C. virosa* (500mg/kg) revealed 23.07% inhibition of paw volume at 3.5 hours (Table 4; Graph 6).

Table 1: Shows the neuro-pharmacological effects of 500, 300 and 100 mg/kg *Cicuta virosa* extract on mice in comparison with control and Diazepam

Treatment	Open field activity	Dip cage activity	Light and dark activity	Cage cross activity	FST (mobility time)
Control	387.5±14.64	274.5±5.65	21.5±3.46	75.5±3.76	2.52±0.11
<i>Cicutaviroso</i> 500mg/kg	145.33±17.35	6.5±0.83	6.67±2.03	13.83±3.75	1.45±0.14
<i>Cicutaviroso</i> 300mg/kg	113.83±5.11	26.83±3.48	11.16±1.27	36.33±2.90	1.44±0.02
<i>Cicutaviroso</i> 100mg/kg	73.33±3.28	12.5±1.37	5.16±1.07	35.16±1.98	1.16±0.02
Standard-Diazepam 2mg/kg	12.5±0.83	11.5±0.83	1±0.4	19.5±0.83	1.60±0.06

FST = Forced swimming test

Table 2: Shows the assessment of analgesic activity of *Cicuta virosa* at 50 and 25 mg/kg by Acetic acid method in comparison to Control and Aspirin in mice

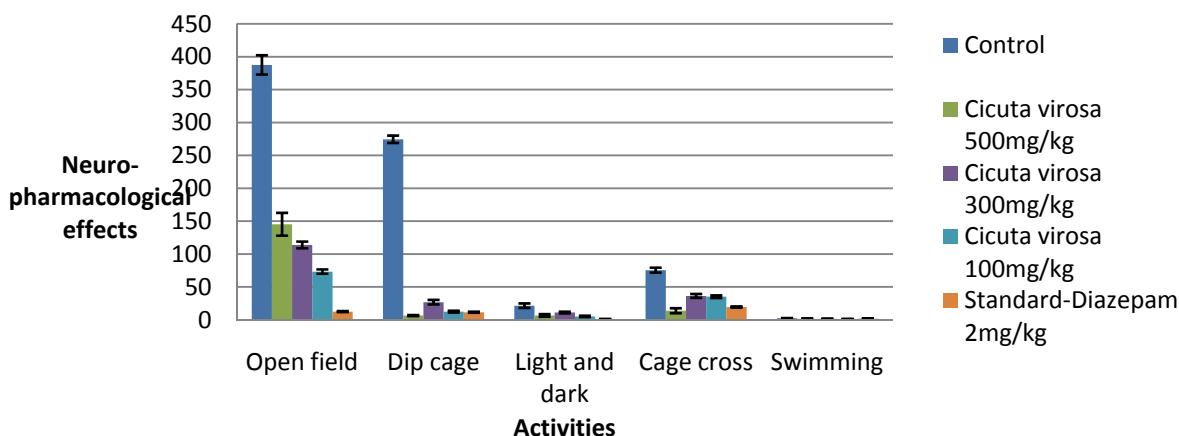
TREATMENT Dose mg/kg	PHASE 1		PHASE 2		PHASE 3	
	Mean No. of Writhes	% of Inhibition	Mean No. of Writhes	% of Inhibition	Mean No. of Writhes	% of Inhibition
Control (0.5 ml saline)	19.5±0.83	-	15.5±0.83	-	14.16±1.03	-
Aspirin(300mg)	20.33±0.73	9.89%	25.5±0.83	66.34%	13.5±0.83	42.10%
<i>C. virosa</i> (50mg)	7.83±0.86	57.67%	14.83±1.50	3.26%	9.5±1.22	0%
<i>C. virosa</i> (25mg)	8.33±1.34	46.25%	11.5±0.83	4.17%	9±0.93	28.57%

Table 3: Shows the assessment of analgesic activity of *Cicuta virosa* at 50 and 25 mg/kg by Formalin method in comparison to Control and Aspirin in mice

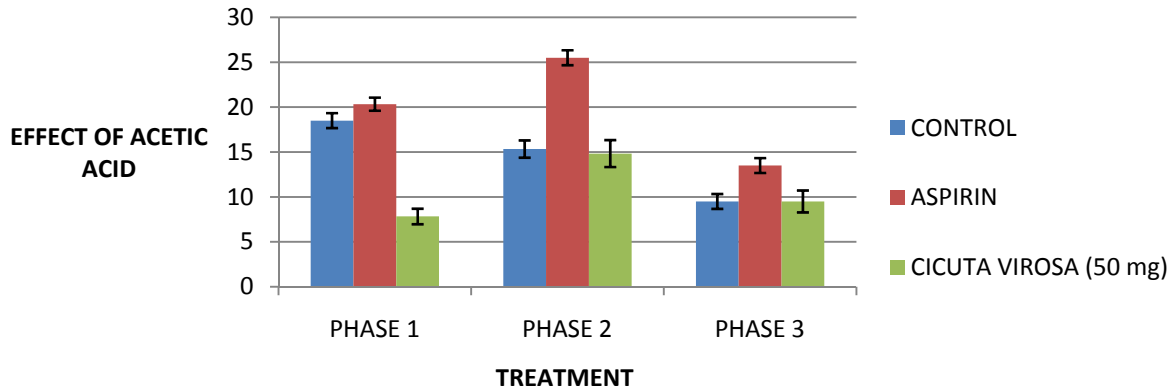
TREATMENT Dose mg/kg	PHASE 1		PHASE 2		PHASE 3	
	Mean No. of biting & licking	% of Inhibition	Mean No. of biting & licking	% of Inhibition	Mean No. of biting & licking	% of Inhibition
Control (0.5 ml saline)	19.16±1.03	-	10.67±0.54	-	5.83±1.03	-
Aspirin(300mg)	41.5±1.08	32.88%	5.83±0.65	61.54%	18.16±1.03	41.41%
<i>C. virosa</i> (50mg)	28.5±4.20	53.90%	28.5±4.20	96.70%	16.83±2.18	45.70%
<i>C. virosa</i> (25mg)	11.16±3.03	81.08%	0.5±0.37	96%	1.83±0.65	93.34%

Table 4: Shows the effect of *Cicuta virosa* extract at the doses of 300 and 500 mg/kg on 2% carrageenan induced paw edema in rats in comparison with Control and Aspirin

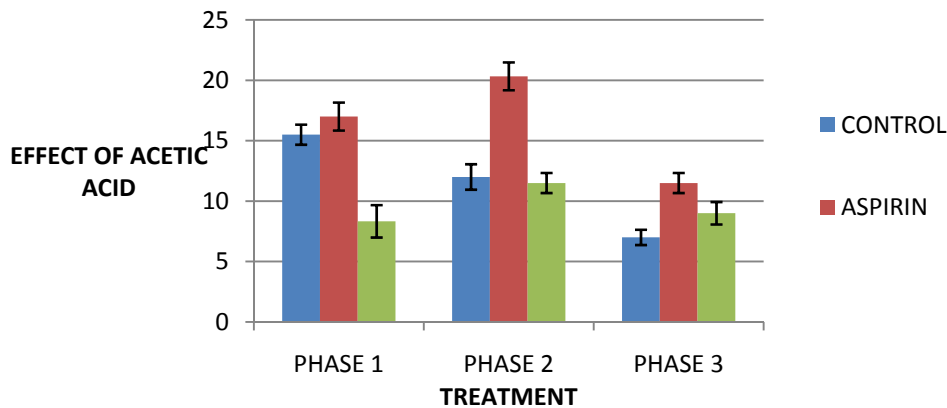
Time (Hrs)	Control	<i>C. virosa</i> (500mg/kg)		<i>C. virosa</i> (300mg/kg)		Aspirin (300 mg/kg)	
	(edema Diameter in mm)	(edema Diameter in mm)	Percentage Inhibition (%)	(edema Diameter in mm)	Percentage Inhibition (%)	(edema Diameter in mm)	Percentage Inhibition (%)
0	6±0.0083	6±0.0063	0%	5.50±0.0046	0%	6±0.0063	0%
0.5	7.73±0.0061	7.24±0.0036	0%	6.49±0.0073	10.34%	6.75±0.0063	12.90%
1.0	8.23±0.0065	7.45±0.0063	9.69%	7.5±0.0063	6.25%	6.85±0.0063	16.97%
1.5	9±0.0083	7.57±0.0052	16%	7.27±0.0063	14.47%	7±0.0083	22.22%
2.5	9.24±0.0046	7.73±0.0052	16.21%	7.74±0.0046	11.42%	7.27±0.004	21.51%
3.5	9.75±0.0063	7.51±0.0073	23.07%	7.75±0.0052	13.89%	8±0.0077	17.94%
4.5	8.74±0.0065	7.23±0.0073	17.14%	7.75±0.0077	11.42%	7.74±0.0036	11.42%
24	7±0.0096	6.23±0.0061	10.71%	6±0.0063	14.28%	6.75±0.0063	3.57%



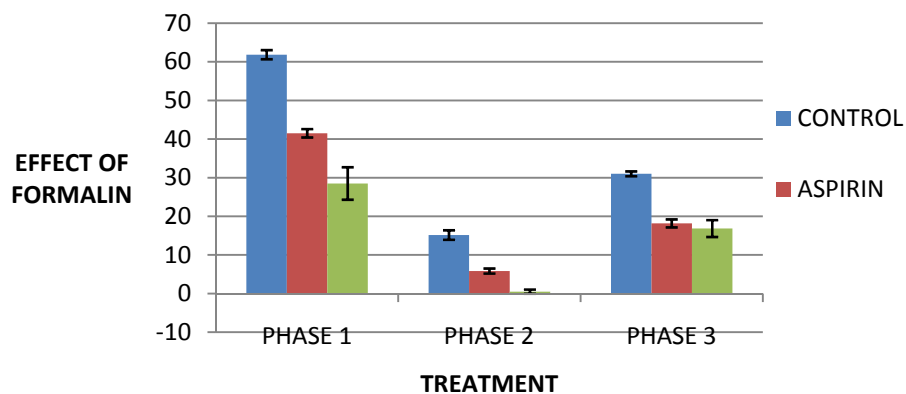
Graph 1: Shows the neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *C. virosa* extract on mice in comparison with control and standard drug.



Graph 2: Shows the analgesic effect of *C. virosa* (50mg) in comparison with control and standard drug in acetic acid induced writhes activity.

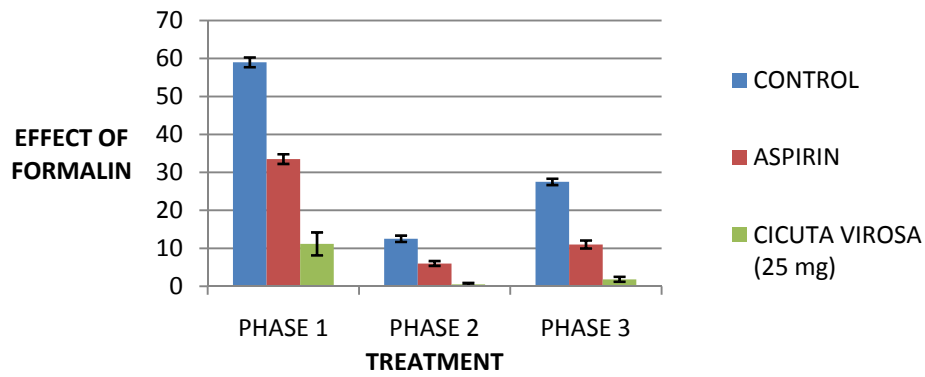


Graph 3: Shows the analgesic effect of *C. virosa* (25 mg) in comparison with control and standard drug in acetic acid induced writhes activity.

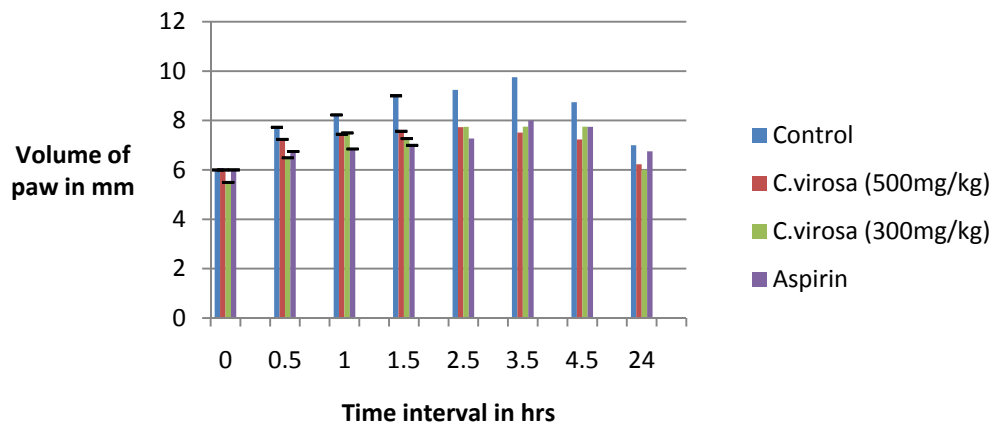


Graph 4: Shows the analgesic effect of *C. virosa* (50 mg) in comparison with control and standard drug in formalin induced paw edema.





Graph 5: Shows the analgesic effect of *C. virosa* (25 mg) in comparison with control and standard drug in formalin induced paw edema.



Graph 6: Shows the anti-inflammatory effect of 300 and 500 mg/kg of *C. virosa* extract in comparison with control and standard drug in carrageenan induced paw inflammation.



Flowers Head of *Cicutavirosa*



Discussion

There are more than 20 known species of *C. virosa* and all of them are extremely toxic. This plant is native to North America, North and Central Europe, Northern Asia. It contains Cicutoxin which is an unsaturated long chain alcohol, GABA receptor antagonist and Potassium channel blocker [17 – 19]. The mechanism of action of *C. virosa* involves antagonism of gamma amino butyric acid at its receptor on the neuronal chloride channel, imbalance of acetylcholine homeostasis, mimicry of excitatory amino acid, sodium channel alteration, or hypoglycaemia [17]. Toxicological and pharmacological studies were carried out on *C. virosa* by several researchers, for example [2,18,20]. The effects of Cicutoxin, the poisonous constituent of *C. virosa* on T-lymphocyte proliferation was studied by Struß *et al.* (1996) [2]. Virol A another toxic component of *C. virosa* was found to selectively inhibit the GABA_A receptor Cl⁻ channel complexes at neuronal level in mammals [1]. Violent tetanic convulsions caused by *C. virosa* toxicity resemble strychnine poisoning [21].

The homoeopathic remedy *Cicuta* is prepared from freshly expressed sap of the plant and is dilute with equal amount of alcohol. Dilution nullifies the harmful effects of the plant on humans.

There was significantly reduced pharmacological activity in the mice treated with 100mg/kg, 300mg/kg and 500mg/kg of *C. virosa* in comparison with the control group mice. In open field activity, at the dose of 100mg/kg of *C. virosa* the activity was reduced to 73.33±3.28 counts in comparison with the control group 387.5±14.64 counts. In dip cage activity the maximum inhibition was observed at 500mg/kg dose, mice dipped head 6.5±0.83 times in comparison with the standard drug 274.5±5.65 times and standard drug, Diazepam (2mg/kg) 11.5±0.83 times. In light and dark activity, the number of entries in light compartment were 5.16±1.07 times at the dose of 100mg of *C. virosa* as compared to the control group 21.5±3.46 times. In cage cross activity, at the dose of 500 mg /kg of *C. virosa* mice crossed cage 13.83±3.75 times in comparison to the control group in which response was 75.5±3.76 times. *C. virosa* (100mg) revealed the least mobility time in forced swimming test, that is 1.16±0.02 seconds in comparison with the control group 2.52±0.11 seconds and standard group, Diazepam (2 mg/kg) was 1.60±0.06 seconds. All the above mentioned results confirmed the anxiolytic effect of *C. virosa* extract.

C. virosa (50mg/kg) revealed 57.67% of inhibition of writhes induced by acetic acid as compared to the standard drug, Aspirin (300mg/kg) 66.34% of inhibition. *C. virosa* (25mg/kg) revealed pronounced inhibition of biting and licking response induced by Formalin, that is, 93.34% in third phase in comparison with the

control and standard drug group. Above mentioned results established the presence of potent analgesic activity in *C. virosa* extract.

Maximum inhibition of Carrageenan induced inflammation was 23.07% at the end of 3.5 hours at the dose of 500mg/kg of *C. virosa* in comparison to the maximum inhibition of Carrageenan induced inflammation at the dose of 300 mg/kg of Aspirin 22.22%. Our results indicated strong anti-inflammatory activity of *C. virosa* extract.

Our results are in conformity to the research work done by Julian & Launay (1966) [22]. In one of the series of experiments conducted by them on rodents, in mice *C. virosa* in 3DH dilution delayed the onset of action of Reserpine. While in Guinea pigs preventive effect of *C. virosa* was observed in 5CH, 15CH and 30 CH dilutions. Julian & Launay (1966) demonstrated by their experiments that exposure to chronic sub-acute *C. virosa* delays the onset of catalepsy induced by Reserpine as well as reduced its duration. In rats, only sub-acute exposure to *C. virosa* exhibited protective effect. Our results also indicated the anxiolytic, analgesic and anti-inflammatory effect of *C. virosa* extract that may be used for the treatment of epilepsy, convulsions and spasms effectively.

Conclusion

Our research work revealed the anxiolytic, analgesic and anti-inflammatory potentials of *C. virosa* extract. Further work need to be done for better understanding of its mechanism of action and safety for its effective therapeutic utilization in treating epilepsy, seizures and convulsions associated with various pathologies.

List of Abbreviations

CNS (Central nervous system), FST (Fast swimming test), i.p. (intraperitoneal), *C. virosa* (*Cicuta virosa*), CH (Centismal Hahnemannienne), DH (Decimal Hahnemannienne).

Competing Interests

No Financial or non-financial competing interests.

Author's Contributions

Farah-Saeed contributed in both the experimental and preparation of manuscript. Prof. Dr. Mansoor Ahmad did the final checking and review of the manuscript. Dr. Mehjabeen and Dr. Noor Jahan contributed in experimental work.

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