

## Full Length Research Paper

## Evaluation of neuropharmacological, analgesic and anti-inflammatory effects of the extract of *Centella asiatica* (Gotu kola) in mice

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Continued research on traditional herbal drugs has become important than ever due to their increasing use and demand as food supplements and nutraceuticals. The standardized extract of *Centella asiatica* (L.) Urban (Apiaceae), commonly known as Gotu kola, was evaluated for neuropharmacological, analgesic and anti-inflammatory effects. Neuropharmacological activities were performed on specifically design apparatus, whereas anti-inflammatory test was carried out by 2% formalin in right hind paw of mice and analgesic effect was observed by 1% acetic acid induced writhing test. During central nervous system (CNS) activities (open field, cage cross, head dip, rearing, traction, light and dark test), the extract exhibited marked CNS depressive effect at 300 mg/kg dose. The results were compared with reference drug diazepam 2 mg/kg. *C. asiatica* extract (300 mg/kg) produced significant anti-inflammatory response in 2nd phase of formalin induced inflammation (61.12%;  $p \leq 0.05$ ). It also reduced no. of writhes up to 40.10% (500 mg/kg). Our findings support the respective traditional use of *C. asiatica* to treat painful and inflammatory conditions along with its supportive sedative and CNS depressant and muscle relaxant effect.

**Key words:** *Centella asiatica*, CNS depressant, sedative, anti-inflammatory.

### INTRODUCTION

*Centella asiatica* belongs to family Apiaceae. Its parts used for medicinal purpose are its leaves along with the petioles. There are many common names used for this plant in different parts of the world but, the most common

name known is Gotu kola. It is a slender, prostrate or creeping perennial aromatic herb with long creeping runners. *C. asiatica* is found in most tropical and subtropical countries, growing in swampy areas, including

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parts of India, Pakistan, Sri Lanka, Madagascar, South Africa and South Pacific and Eastern Europe (Bown, 1995). Other reports may also claim its existence in other parts of the world. Anxiolytic activity can be determined by simple animal model experiments (Crawley and Goodwin, 1980).

*C. asiatica*, often being called the elixir of life, has a long history of utilization in Ayurvedic and Chinese traditional medicines since centuries (Meulenbeld and Wujastyk, 2001). There is a wide range of pharmacological actions exhibited by *C. asiatica* including memory and learning enhancing effects (Nasir, 2011; Mohandas, 2009), antianxiety effect (Wanasuntronwong, 2012; Jana, 2010), attenuating effect in age-related decline in cognitive function and mood disorder (Wattanathorn, 2008), antidepressant effect (Chen, 2003), wound healing activity (Shukla, 1999) etc. The current study was therefore conducted to judge its claimed uses particularly concentrating on its neuropharmacological, analgesic and anti-inflammatory activities.

## MATERIALS AND METHODS

### Plants

The plant material of *C. asiatica* was purchased in 2008 and 2009 from the local market of Karachi, Pakistan, and identified by Prof. Dr. Mansoor Ahmad (I. F.), Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan. A specimen voucher 2008/MQ-Ca1 is deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi.

### Animals

Albino mice of either sex weighing 25 to 30 g were purchased from Animal House of DUHS, Dow University of Health Sciences, Karachi, Pakistan. The test 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 ( $30 \pm 1^\circ\text{C}$  12/12 h light/dark cycle) at least seven days before testing or administering the extract/drug(s).

### Neuropharmacological activities of *C. asiatica* in mice

Neuropharmacological activities of *C. asiatica* in mice were assessed by open field, cage crossing movement, head dip, rearing, traction, light and dark and force induced-swimming tests. All CNS related tests were performed in a calm and peaceful environment. In each test, animals were divided into 4 groups (i.e. Group-A for control, Group-B and Group-C for 300 and 500 mg/kg oral doses of the crude extract, respectively and Group-D for the standard drug). Each group comprised 5 animals. Diazepam as 2 mg/kg orally was used as the standard drug. The crude drug and the standard drug were diluted in distilled water and administered orally. The control animals were treated orally with the same volume of saline as of the crude extract. Upon performing all the tests, the observations were made after 30 to 40 min of oral dose of the test substance.

### Open field activity test

The open field apparatus designed in the laboratory consists of 76 x 76 cm square area with opaque walls 42 cm high. The floor is divided by lines into 25 equal squares. Testing was performed in a quiet room under white light as described by Kennett et al. (1985) and Turner (1965). Animals were taken out from their home cage and placed in the central square of the open field box (one at a time). Number of squares crossed with all four paws was counted for 10 min. Activities of the control group and the drug treated group were monitored in a balanced design to avoid order effect.

### Cage crossing movement test

The test was performed on mice in a specifically designed box having rectangular shape. Both control and treated mice were placed into the cage and their cage crossing movements were noted in 10 min. The test is important for the motor activity of the animals. This test was performed according to the method described by Florence et al. (2000).

### Head dip test

This is an exploratory test. A specially designed square shaped head-dip-box having three holes in each side was used in this study. The observation was to count the number of head dips by the animal through these holes in specified time (Sanchez-Mateo et al., 2002; Kasture et al., 2002; Debprasad et al., 2003). The control and drug treated animals were placed individually in the head-dip-box and the observations were made for 10 min.

### Rearing test

Rearing is also an exploratory behavior test. A 1000 ml glass beaker lined with white paper on its bottom was used in this study. The observation was to count the upward movements of the animal locating the body in an erect position in the beaker (Sanchez-Mateo et al., 2002; Kasture et al., 2002; Sakina et al., 1990). The observations were made for 10 min.

### Traction test

The observation was to determine the time taken by the mice to travel an iron rod of one-meter length. At first, the mice were trained to make them able to walk on the iron rod. Any increase or decrease in time taken by the drug treated animals compared to the control animals to travel the iron rod describes the sedative or stimulant activity of the drug, respectively (Sanchez-Mateo et al., 2002; Kasture et al., 2002; Debprasad et al., 2003).

### Light-dark test

The apparatus consisted Plexiglas box with two compartments (20 x 20 cm each) was used. One compartment was illuminated with white light and the other compartment was kept dark. Each animal was placed at the center of the illuminated compartment, facing the dark compartment. The time spent in the illuminated as well as in the dark compartment, besides the number of entries in each space, was recorded for 10 min (Bourin, 2003).

### Force induced-swimming test

Force induced-swimming test was performed according to Sanchez

et al. (2002) and Turner (1965). This test determines the muscle and CNS activity of the crude extract. Mice were placed individually for six minutes in the glass tub filled with water at room temperature up to the level marked. Mouse when placed in the water suddenly starts to move its front and hind paws. The activity time of the animal is determined with the help of a stop watch out of a total observation time of six minutes.

### Pharmacological activity of *C. asiatica* in mice

#### **Assessment of analgesic activity through acetic acid induced-writhing test**

The test was performed according to the modified method of Koster et al. (1959). According to this method writhes were induced by intra-peritoneal administration of the 1% acetic acid solution 10 ml/kg. Thirty minutes prior to the administration of the acetic acid, the animals were treated orally with the test substance. Number of writhes was counted for 30 min immediately after the acetic acid administration. A reduction in the number of writhes as compared to the control animals were considered as an evidence for the presence of analgesia and expressed as percent inhibition of writhing. Mice were divided into four groups (that is, Group-A for control, Group-B and C for the crude extract 300 mg/kg and 500 mg/kg, respectively and Group-D for the standard drug aspirin 300 mg/kg). Each group was comprised of 5 animals, weighing 25 to 30 g. The crude and the standard drugs were diluted with distilled water and administered orally. The control animals were treated orally with the same volume of saline as of the crude extract.

#### **Assessment of anti-inflammatory activity through formalin test**

Swiss albino mice (25 to 30 g) were divided into different groups of 5 animals each. They were injected with 20  $\mu$ l of 1% formalin in the ventral surface of the right hind paw and the left hind paw was injected with an equal volume of normal saline. Two distinct phases of intensive licking and biting of the right hind paw were observed, during 0 to 10 min (early phase or neurogenic phase) and during 10 to 30 min (late phase or inflammatory phase) after the formalin injection. These phases were scored separately to study the effect of the drug. Vehicle or drugs were administered orally 30 min before the formalin injection (Modified method of Hunskaar and Hole, 1987; Rathi et al., 2003).

## RESULT AND DISCUSSION

The standardized extract of *Centella asiatica* (L.) Urban (Apiaceae/Umbelliferae), exhibited an interesting activity; during the open field activity test (Table 1), at 300 mg/kg, it showed a highly significant reduced the no. of squares covered by animals, mean no. of observations  $\pm$  standard error of mean (SEM) was  $47.83 \pm 3.59$  as compared to the control (0.5 ml saline)  $203.66 \pm 3.99$ . But, at 500 mg/kg dose the extract exhibited comparatively a less effect, mean no. of observations  $\pm$  SEM was  $123.00 \pm 0.89$ . Further, more the effect is compared with 2 mg/kg dose of the standard drug, diazepam, it was also observed that at 300 mg/kg dose of the extract, test drug had mild sedation response along with CNS depression effect. This effect is supported by reported literature that *C. asiatica* reduced the oxidative stress (Gupta, 2003)

which is one of the causes of neurological disturbances (Mousa and Mohammad, 2012). While in cage cross-movement test (Table 1) the extract exhibited a dose dependent CNS depression effect.

At 300 mg/kg of the extract there was a decreased in mean no. of observations,  $28.16 \pm 2.64$  as compared to the control group  $40.16 \pm 2.27$ , at 500 mg/kg the mean no. of observations was further decreased,  $15.25 \pm 1.94$ . The results of the head-dip test (Table 1) were again interesting; the extract showed a highly significant reduction in exploratory behaviour. At 300 mg/kg the result was  $20.33 \pm 3.11$  as compared to the control  $59.16 \pm 0.98$ . Same response was found with rearing test. In Traction test (Table 1) *C. asiatica*, time taken to travel along the iron rod by the test animal was recorded. The mean time taken by the test animal to travel along the iron rod, with a dose of 300 mg/kg of the extract, was 15 s compared to 7 s of the control group, showed a muscle relaxing effect and it was more than the effect produced at 500 mg/kg dose. However, the time taken by the standard drug (2 mg/kg of diazepam) was 21 s with falling down activity. Upon conducting the light and dark activity test (Table 2), it was noticed that the test animals spent more time in the dark portion of the test apparatus after administration of the extract. With 300 and 500 mg/kg doses of the extract, the test animals spent more time (8 min) in the dark compartment, pointing towards the CNS depressive effect of the extract. There was no significant dose-related activity found during this test, but there was a close-match between the effects produced by the standard drug 2 mg/kg ( $7.54 \pm 0.00$ ) and 300 mg/kg ( $8.02 \pm 0.01$ ) of the extract. In case of force-induced swimming test (Table 2) conducted on *Centella asiatica* (Gotu kola), the mobility time taken by the test animal at a dose of 500 mg/kg of the extract was 2.46 min. as compared to the control group, 3.21 min. The results of force induced-swimming test with *C. asiatica* intimate that the drug exhibits a moderate muscle relaxing effect.

The analgesic activity of *C. asiatica* was evaluated through acetic induced-writhing test and the results were given in Table 3, Figure 1. The intra-peritoneal injection of the acetic acid was applied to produce experimental writhes in the test animal. Two doses, 300 and 500 mg/kg, of the extract were given orally and, aspirin at a dose of 300 mg/kg orally was used as the standard drug. In case of the control animals treated with saline 0.5 ml orally, the number of writhes were  $72.16 \pm 4.13$  that decreased to  $46.25 \pm 3.36$  (inhibition% = 35.90) and  $43.22 \pm 1.82$  (inhibition% = 40.10) with 300 and 500 mg/kg oral doses of *C. asiatica*, respectively showed a dose dependent analgesic effect. While aspirin 300 mg/kg orally reduced the writhes number to  $31.16 \pm 0.40$  (inhibition% = 56.83) exhibiting significant analgesic effect which was more than produced by 500 mg/kg of the extract. The results revealed that *C. asiatica* exhibits an analgesic activity in acetic acid-induced writhing test.

**Table 1.** Neuropharmacological activities of *C. asiatica* (Gotu kola) in mice

Treatment	Open field (Mean no. of observations $\pm$ SEM)	Cage crossing (Mean no. of observations $\pm$ SEM)	Hed-dip (Mean no. of Observations $\pm$ SEM)	Rearing (Mean no. of Observations $\pm$ SEM)	Traction (Mean no. of Observations $\pm$ SEM)
Control	203.66 $\pm$ 3.99	40.16 $\pm$ 2.27	59.16 $\pm$ 0.98	32.33 $\pm$ 1.63	7.21 $\pm$ 0.33
<i>C. asiatica</i> extract 300 mg/kg	47.83 $\pm$ 3.59	28.16 $\pm$ 2.64	20.33 $\pm$ 3.11	15.91 $\pm$ 2.04	15.58 $\pm$ 1.16
<i>C. asiatica</i> extract 500 mg/kg	123.00 $\pm$ 0.89	15.2 $\pm$ 1.94	28.50 $\pm$ 0.97	19.50 $\pm$ 1.09	9.5 $\pm$ 0.31
Diazepam (std.) 2 mg/kg	96.83 $\pm$ 4.09	17.33 $\pm$ 1.12	19.83 $\pm$ 1.30	12.16 $\pm$ 0.51	21.5 $\pm$ 0.24

All values are mean  $\pm$  SEM; n=5 \* = Significant results ( $P<0.05$ )\*\* = highly significant results ( $P<0.01$ ).

**Table 2.** Neuropharmacological activities of *C. asiatica* (Gotu kola) in mice.

Treatment	Light and dark test		Force induced-swimming test	
	Time in minutes in light compartment (Mean no. of observations $\pm$ SEM)	Time in minutes in dark compartment (Mean no. of observations $\pm$ SEM)	Mobility time (Mean no. of observations $\pm$ SEM)	Immobility time (Mean no. of observations $\pm$ SEM)
Contol 0.5 ml saline	3.34 $\pm$ 0.00	6.26 $\pm$ 0.00	3.21 $\pm$ 0.00	2.39 $\pm$ 0.00
<i>C. asiatica</i> extract 300 mg/kg	1.58 $\pm$ 0.00	8.02 $\pm$ 0.01	3.26 $\pm$ 0.01	2.24 $\pm$ 0.20
<i>C. asiatica</i> extract 500 mg/kg	1.31 $\pm$ 0.00	8.29 $\pm$ 0.04	2.46 $\pm$ 0.02	3.14 $\pm$ 0.01
Diazepam (standard) 2 mg/kg	2.06 $\pm$ 0.00	7.54 $\pm$ 0.05	1.14 $\pm$ 0.00	4.46 $\pm$ 0.00

All values are mean  $\pm$  SEM; n=5 \* = Significant results ( $P<0.05$ )\*\* = highly significant results ( $P<0.01$ ).

**Table 3.** Analgesic activity of *C. asiatica* (Gotu kola) on acetic acid induced writhing in mice.

Treatment	Dose mg/kg orally	Mean no. of writhes $\pm$ SEM	Inhibition %
Control	0.5 ml saline	72.16 $\pm$ 4.139	00
<i>C. asiatica</i> extract	300 mg/kg	46.25 $\pm$ 3.361	35.90
	500 mg/kg	43.22 $\pm$ 1.828	40.10*
Aspirin	300 mg/kg	31.16 $\pm$ 0.401	56.83*

All values are mean  $\pm$  SEM; n=5 \* = Significant results ( $P<0.05$ )\*\* = highly significant results ( $P<0.01$ ).

Ilkay (2012) also reported that *C. asiatica* exhibited wound healing property due to triterpene (Ilkay, 2012).

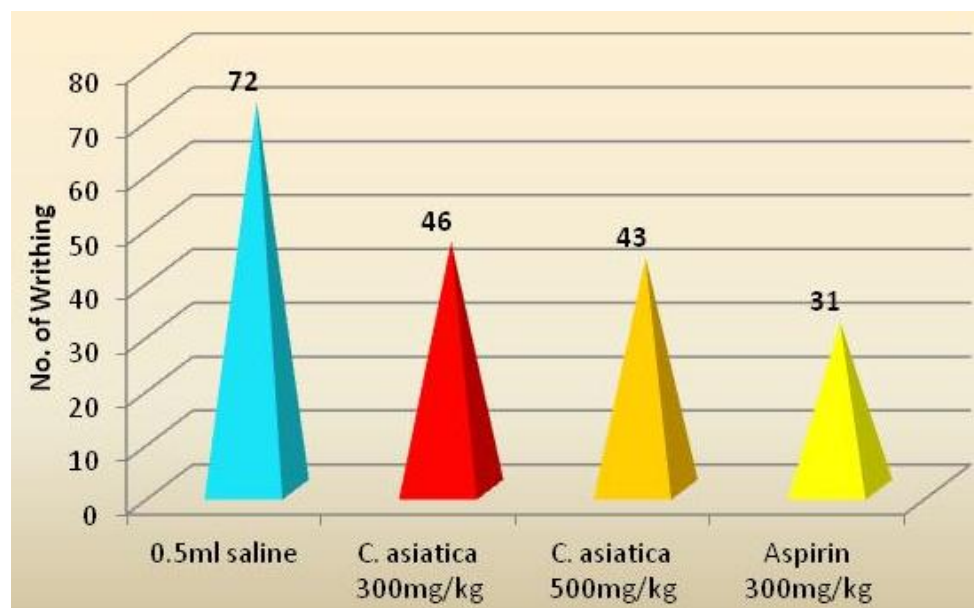
The anti-inflammatory activity of *C. asiatica* was performed through formalin test. The results obtained were elaborated in Table 4, Figure 2.

The extract of *C. asiatica* did not show a significant anti-inflammatory activity during the first phase of the observations conducted. In the

**Table 4.** Anti-inflammatory activity of *C. asiatica* (Gotu kola) through formalin test in mice

Dose mg/kg	First Phase, mean no. of observations±SEM			Second phase, mean No. of observations±SEM		
	Number of licking and biting	Time spent (s)	Inhibition (%)	Number of licking and biting	Time spent (s)	Inhibition (%)
0.5ml Saline Control	46.33 ± 1.69	73.16 ± 5.56	-	65.16 ± 4.13	177.6 ± 1.27	-
<i>C. asiatica</i> extract 300 mg/kg	45.83 ± 2.29	97.5 ± 3.17	1.08	25.33 ± 3.92	55.30 ± 4.84	61.12*
<i>C. asiatica</i> extract 500 mg/kg	37.50 ± 3.27	87.50 ± 1.72	19.1	33.77 ± 1.48	56.35 ± 2.19	48.17
Aspirin 300 mg/kg	20.66 ± 0.86*	32.50 ± 0.51*	55.4*	18.1 ± 1.08	29.66 ± 1.21	72.22**

All values are mean ± SEM; n=5 \* = Significant results (P<0.05) \*\* = highly significant results (P<0.01).



**Figure 1.** Analgesic activity of *C. asiatica* (Gotu kola) on acetic acid induced writhing in mice.

first phase, the standard drug, aspirin 300 mg/kg, produced significant analgesic effect as the no. of licking and biting was decreased to 20.66 ± 0.86 times as compared to the control group, 46.33 ±

1.69 times. Whereas, in the second phase, at the doses 300 and 500 mg/kg, there was a significant anti-inflammatory effect exhibited by 300 mg/kg dose of the extract as the no. of licking and biting

was decreased to 25.33 ± 3.92 times (inhibition of licking and biting response was 61.12%) as compared to the control group 65.16 ± 4.12 times. Interestingly, the no. of licking and biting was

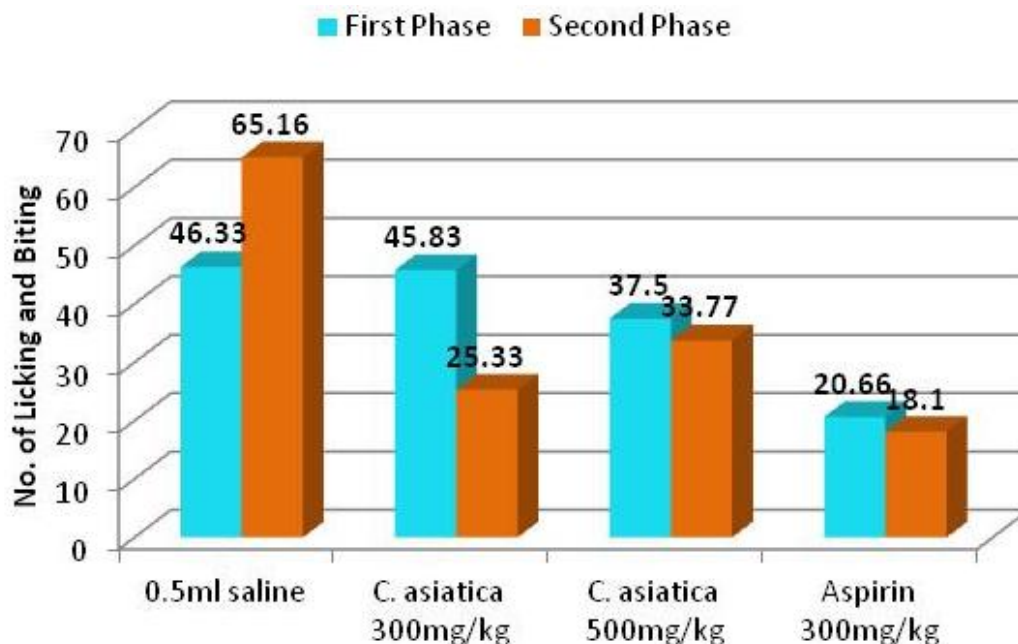


Figure 2. Anti-inflammatory activity of *C. asiatica* (Gotu kola) through formalin test in mice.

decreased to  $33.77 \pm 1.48$  times (inhibition% = 48.17) at 500 mg/kg of the extract as compared to the control group  $65.16 \pm 4.12$  times; a less anti-inflammatory activity in this phase upon increasing the dose to 500 mg/kg from 300 mg/kg. On the other side, the standard drug, aspirin 300 mg/kg, exhibited highly significant anti-inflammatory response in the second phase, as the no. of licking and biting was decreased to  $18.1 \pm 1.07$  times (inhibition% = 72.22) as compared to  $65.16 \pm 4.12$  times of the control. In the second phase, the anti-inflammatory effect of *C. asiatica*, at 300 mg/kg dose was significant, but it was less as compared to the standard drug aspirin (300 mg/kg). The analgesic and anti-inflammatory effect of *C. asiatica* is due to the presence of glycosides which possess its potential role in inhibition of prostaglandins and cyclooxygenase pathway (George and Joseph, 2009).

## Conclusion

Our studies support the traditional use of *C. asiatica* (Gotu Kola) as analgesic and antinociceptive and anti-inflammatory agent along with its supportive CNS depression effect. Neuropharmacological studies leaves room for in-depth investigations on low dose and high dose effects of the drug in some areas.

## Conflict of Interests

The authors have not declared any conflict of interests.

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