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**Original Article**

# Acute Toxicity of Convolvulus Arvensis in Mice and its Mechanism of Spasmolysis on Rabbit Jejunum

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## ABSTRACT

**Introduction:** *Convolvulus arvensis* has contrasting results reflecting both the spasmolytic as well as the spasmogenic properties according to studies. It may be because of a wide variety of chemical ingredients in the plants. In addition the mechanism of the effect and the safe therapeutic dose is not clearly understood.

**Objective**: The current study is designed to investigate *Convolvulus arvensis* for the possible antispasmodic activity of the plant extract.

**Material and Methods:** An experimental study was performed for 24 hours in Khyber medical university. Acute toxicity was tested on mice using Lorke's model. 8 groups were made out of which 1 served as a negative control group. Extract of C. arvensis was administered through intraperitoneal route (i.p). Secondly antispasmodic effect of on spontaneous rabbits' jejunal contractions was performed, followed by testing of Antispasmodic effect of on atropine pretreated and KCl-induced jejunal preparations of rabbits respectively.

**Results:** Extract of *C. arvensis* is safe up to 500 mg/kg in experimental animals. Furthermore cumulative addition of crude methanolic extract of *C. arvensis* caused concentration-dependent inhibition in low KCl and high KCl induced contractions. The $EC_{50}$ for the effect of crude methanolic extract of *C. arvensis* on low KCl induced contraction without glibenclamide was (1.79 ± 0.11 mg/mL). The $EC_{50}$ value for the effect of crude methanolic extract of *C. arvensis* on low KCl in the presence of glibenclamide (3 µM) was (2.85±0.12 mg/mL).

**Conclusion:** The observed spasmolytic activity of extract of C. arvensis is through $K^+$ channel activation as well as activation of muscarinic receptors. The crude extract is safe up to 500 mg/kg.

**Key Words:** Spasmolytic, Acute toxicity, EC50, LD50

## Introduction

There are about 250 species of the genus convolvulus in the world. *Convolvulus arvensis* is commonly called field bindweed and wild morning glory. This herbaceous plant commonly found in Malakand region, Middle East, Europe and Himalaya belt of Pakistan. *C. arvensis* has been used in traditional medicines in 1730s [1]. Traditionally, it has been used to treat inflammation, swelling, reducing woods and skin ulcers. The aerial parts of the plant are used for abdominal worms in children and abdominal pain as laxative. It is also used in the treatment of muscular weakness [2]. The plant is also reported to have immunostimulant and diuretic activities. *C. arvensis* leaves are also used in liver complications, asthma and haemostasis[3].Several studies hypothesized a spasmogenic effect of the roots of the plant .

As per the property, the plant was believed to have laxative and purgative properties [4,5]. However, other studies identified an anti-spasmodic or a spasmolytic effect of the plant on the gastrointestinal tract, hence proposing its efficacy in relieving constipation and spasms [6]. Their hence lies a controversy about what the effect of the plant is on the gastrointestinal smooth muscles. The literature gives contrasting results reflecting both the spasmolytic as well as the spasmogenic properties of the plant. It may be because of a wide variety of chemical ingredients in the plants. The chemical constituents include flavonoids, caffeinic acids, saponins [7], lipids, δ-amino levulinic acid as well as alkaloids among several other compounds [8].

The current study is designed to investigate *Convolvulus arvensis* for the possible antispasmodic activity of the plant extract. In addition to the

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ambiguity whether the plant has spasmolytic effect or not, is the question about the underlying mechanism. There is no clear mechanism of antispasmodic effect in the published data [4]. Therefore, we also focused our study on exploring the mechanism behind the anti-spasmodic activity. The study also aimed to observe the acute toxic effects of C.arvensis and to calculate the median lethal dose in order to propose a safe dosage range while putting the plant to a therapeutic dose.

The gross morphology of the mammalian G.I. tract differs among species but it exhibits some basic structural similarities e.g. glandular structures, receptor distributions and the wall layers, which makes it possible to achieve close enough results utilizing animal models like mice and rabbits [9]. Owing to the ease and accuracy mice were chosen for acute toxicity studies, whereas rabbit jejunum was utilized to look for the antispasmodic effect as the smooth muscle structure is similar to human jejunum.

## Material and Methods

An experimental study was conducted for a period of 1 week from 1st March 2021 to 7th March 2021 in Khyber medical University on a sample of 32 mice and 8 rabbits. The acute toxicity was induced and analyzed within 24 hours period. The antispasmodic activity was performed at the spot after dissecting out rabbit jejunum.

**Animals Model:** Swiss albino mice (healthy, 25-30 g body weight) of either sex (male and female) were used in the study to assess the acute toxicity, as mice model can easily show prominent signs of toxicity[17]. Local breed of healthy rabbits having 2-2.5kg body weight were used in antispasmodic activity and pharmacological screenings as the rabbit intestine owing to its receptors and structure give precise results[18]. All animals were kept in the animal house of IBMS KMU, at the temperature range of 20-25$^{o}$C with 12 h dark and 12 h light exposures. Recommended standard diet was provided to all animals.

**Extraction of Plant's Materials:** The plant material (5.0 kg) of *C. arvensis* was cleaned and put to shade drying on 40$^{o}$C. The shade dried materials were thoroughly grinded to powder form and then soaked in commercial grade (80%) methanol at room temperature for 20 days with occasional shaking. Later, it was filtered through a Muslim cloth and ordinary filter paper. This process was repeated three times. The filtrates were combined. The combined filtrates were evaporated using a rotary evaporator under reduced pressure on 40$^{o}$C till a thick and semi-solid mass of brownish black color extract was obtained (free of organic solvent). This extract was refrigerated using ordinary refrigerator.

**Acute toxicity of C. arvensis:** The crude methanolic extract of *C. arvensis* was administered through intraperitoneal route (i.p) using mice model. Mice were divided into eight groups (four mice in each group). Each group contained both male and female mice. In order to determine the lethal dose, the preliminary screening of acute toxicity was performed in two stages. In the first stage, four groups were made (A, B, C and D) that received, respectively 1 mg/kg, 10 mg/kg, 100 mg/kg and 1000 mg/kg of the body weight of crude methanolic extract of *C. arvensis* once daily. The experiments were followed by a second stage, in which three groups were made (E, F and G) that received 250 mg/kg, 500 mg/kg and 750 mg/kg of body weight of crude extract once daily. Group H served as a negative control group that received 3 mL/kg/day of normal saline. The numbers of death of experimental mice were noted for 24 h in each group. Median lethal dose ($LD_{50}$) was calculated using Graph Pad prism.The morphological characteristics of each group were also observed for 24 h.

**Antispasmodic activity of *C. arvensis***

**Preparation of test and physiological solutions:** Different solutions are needed to perform different pharmacological screenings. The required solution was Normal Tyrode's solution. Tyrode's solution was prepared in distilled water. While preparing the normal Tyrode's solution, calcium chloride was added at the end to avoid possible physical appearance.

**Antispasmodic effect of the *C. arvensis* crude extract on spontaneous rabbits' jejunal contractions:** The rabbits were slaughtered after keeping on water only diet overnight. Their abdomens were dissected carefully and the jejunums were removed without bruising or strain injury. The mesentery was carefully removed from jejunums. The jejunums were cut into small portions of 1.5 cm - 2.0 cm. Upon removal of jejunum, it was transferred to Petri dish containing freshly prepared Tyrode's solution. Carbogen gas was continuously supplied to maintain the tissue viability. The jejunum pieces were then gently mounted in the organ bath having 10 mL of Tyrode's solution. The organ bath temperature was

adjusted to 37±1°C. The tissues were stabilized for minimum 30 to 40 minutes. After stabilization, the tissues were then tested with the crude methanolic extract of *C. arvensis*. Different concentrations of extract were used i.e. 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0, 10.0 and 15.0 mg/mL. The process was repeated at least three times using different animals. Responses were recorded.

**Antispasmodic effect of the *C. arvensis* crude extract on atropine pretreated jejunal preparations of rabbits:** The jejunal portions were treated with 0.03 µM of atropine. After incubation period of 15- 20 minutes extract dose was applied in accordance to the standardized protocol to check for possible mechanism through cholinergic receptors for spasmolytic response. The extract was tested in similar concentrations as tried for possible effects on spontaneous rabbits' jejunal preparations. The process was repeated at least three times using different animals.

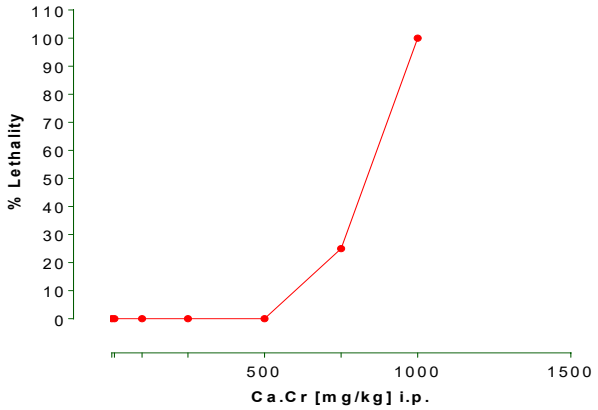
**Antispasmodic effect of the *C. arvensis* crude extract on KCl-induced jejunal contractions in rabbits:** Small portions (1.5-2.0 cm) of the rabbit's jejunum were mounted in the organ bath. Tissue stabilization was achieved to allow them to contract spontaneously for 30 minutes. High KCl (80 mM) produces sustained contractions. Then crude extract was applied in concentrations between 0.01 to 15 mg/ml in similar concentrations as above. The process was repeated three times and the effects were observed. Since the 80 mM KCl- induced contractions were not relaxed 100%. The test drug was tried in a similar fashion in low (20 mM) concentration induced contractions.

# Results

**Acute toxicity:** The summarized results of acute toxicity study are shown in Table 1. According to Table 1, the test extract of *C. arvensis* is safe up to 500 mg/kg in experimental animals. There were no significant signs and symptoms of toxicity during acute toxicity testing with different doses, i.e. 1 mg/kg, 10 mg/kg, 100 mg/kg, 250 mg/kg, 500mg/kg, 750 mg/kg and 1000 mg/kg as has been shown in Table 2. The present study illustrates that $LD_{50}$ value is 833 mg/kg. The data is plotted as % lethality of toxic effect is shown in Figure 1.

**Table 1: Acute Toxicity of crud methanolic extract of *C. arvensis* using mice model**

| Dose (mg/kg body weight) (*n*=4 in each group) | | | | |
|---|---|---|---|---|
| **1st Stage** | Group 1 (1 mg/kg) | Group 2 (10 mg/kg) | Group 3 (100 mg/kg) | Group 4 (1000 mg/kg) |
| | All alive | All alive | All alive | All died |
| **2nd Stage** | Group 1 (250 mg/kg) | Group 2 (500 mg/kg) | Group 3 (750mg/kg) | ___ |
| | All alive | All alive | One died | ___ |




*Ca.Cr=Convolvulous Arvenesis Concenration

**Figure 1: Acute toxicity study of crude methanolic extract of *C. arvensis* on mice**

The symptoms of acute toxicity were measured and summarized (Table 2).

**Table 2: Signs and symptoms during acute toxicity tests**

| Signs and Symptoms | Intensity on test dose (01mg/kg) | Intensity on test dose (10mg/kg) | Intensity on test dose (100mg/kg) | Intensity on test dose (250mg/kg) | Intensity on test dose (500mg/kg) | Intensity on test dose (750mg/kg) | Intensity on test dose (1000mg/kg) |
|---|---|---|---|---|---|---|---|
| Increase motor activity | No | No | No | No | No | No | No |
| Clonic convulsions | No | No | No | No | No | No | No |
| Tremors | No | No | No | No | No | No | No |
| Straub reaction | No | No | No | No | No | No | No |
| Tonic extensor | No | No | No | No | No | No | No |
| Piloerection | No | No | No | No | Yes | Yes | Yes |
| Opisthotonos | No | No | No | No | No | No | No |
| Catatonia | No | No | No | No | Yes | Yes | Yes |
| Hyperesthesia | No | No | No | No | No | No | No |
| Decrease motor activity | No | No | No | Yes | Yes | Yes | Yes |
| Loss of light reflex | No | No | No | No | No | No | No |
| Sedation | No | No | No | Yes | Yes | Yes | Yes |
| Ataxia | No | No | No | No | No | No | No |
| Hypnosis | No | No | No | No | No | No | No |
| Anesthesia | No | No | No | No | No | No | No |
| Analgesia | No | No | Yes | Yes | Yes | Yes | Yes |
| Arching and rolling | No | No | No | No | No | No | No |
| Lacrimation | No | No | No | No | No | No | No |
| Ptosis | No | No | No | No | No | No | No |
| Exophthalmos | No | No | No | No | No | No | No |
| Diarrhea | No | No | No | No | No | No | No |
| Writhing | No | No | No | No | Yes | Yes | Yes |
| Salvation<br>a) Watery<br>b) Viscid | No | No | No | No | No | No | No |
| Skin colour<br>a) Blanching<br>b) Flushing<br>c) Cyanosis | No | No | No | No | No | No | No |
| Respiration<br>a) Depression<br>b) Stimulation<br>c) Failure | No | No | No | No | Yes Stimulation | Yes Stimulation | Yes Stimulation |

Key: No = Not Observed, Yes = Observed or present

**Antispasmodic effect of the crude extracts of *C. arvensis* on spontaneous rabbits' jejunal contractions:** In experiments on rabbit's

jejunal preparations, it was observed that crude Methanolic extract of *C. arvensis* has relaxation properties in high concentrations. The results of crude methanolic extract of *C. arvensis* on rabbits' jejunal preparations in the presence and absence of atropine (0.03 µM). According to our investigations, the results showed spasmolytic effects on spontaneous jejunal contractions without atropine. The spasmolytic effect was 188±2.8% of control, maximum on concentration 5 mg/ml of *C. arvensis* extract. While on concentration 15 mg/mL, the spontaneous activity was completely relaxed in absence of atropine. The $EC_{50}$ value on spontaneous was recorded (11.97±0.9 mg/mL). In further investigations, in the presence of 0.03 µM concentration of atropine, the methanolic extract of *C. arvensis* was investigated. No spasmolytic result was observed and $EC_{50}$ value was recorded in the presence of atropine that is (4.28±0.4 mg/mL). Consequently, there was left shift as the spontaneous activities were relaxed on 10 mg/mL instead of 15 mg/mL. This concludes that *C. arvensis* follows the cholinergic pathway for spasmolytic mechanisms as atropine is a standard anticholinergic drug. Representative graph tracings in the presence and absence of atropine (0.03 µM) are shown in Figure 2.

The % responses in the presence of test concentrations of *C. arvensis* are plotted in Figure 2. A left shift in $EC_{50}$ strongly shows the involvements of cholinergic receptors for spasmolytic response. This *C. arvensis* can be a good source of cholinergic agonists. The extract may also be used for possible gastroprokinatic activities.

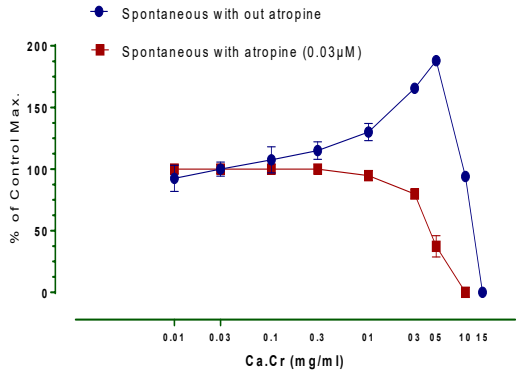



**Figure 2: Effect of crude methanolic extract of *C. arvensis* on spontaneous rabbit jejunal preparations in the absence and presence of atropine (0.03 µM) (All values are mean±SEM, *n*=4)**

Potassium chloride (KCl) induced contractions were used to observe the effect of crude extract of *C. arvensis* on gut motility using isolated rabbits' jejunal model. The cumulative addition of crude methanolic extract of *C. arvensis* caused concentration-dependent inhibition in low KCl (20 mM) and high KCl(80 mM) induced contractions. Representative graph tracings are shown in Figure 3a low KCl and 3b high KCl induced contractions. On high KCl ,the crude extract of *C. arvensis* was tested which caused contractions; that relaxed high KCl induced contractions only up to 28.5% (*n*=4), therefore, calculations of the $EC_{50}$ (high $K^+$)was not possible as it did not relax contractions in rabbit's jejunal preparations up to 50% or more than 50% of control maximum. When tested for low KCl induced contractions, it was observed that total relaxation of tissue contractions occurred in concentration of 5mg/mL as shown in Figure 3b. The $EC_{50}$ for the effect of crude methanolic extract of *C. arvensis* on low KCl induced contraction was (1.79 ± 0.11 mg/mL).

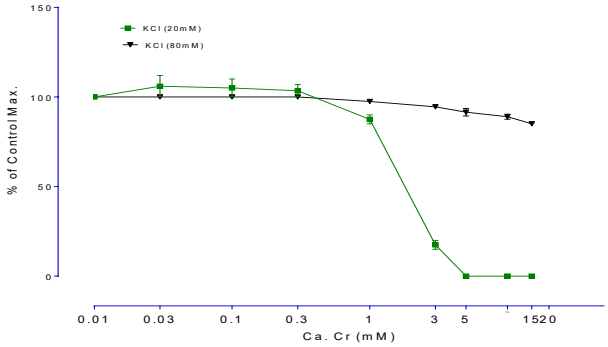



**Figure 3a :Effect of crude methanolic extract of *C. arvensis* on low KCl (20 mM) and high KCl (80 mM) induced contractions in rabbit's jejunum preparations(All values are mean±SEM, *n*=4)**

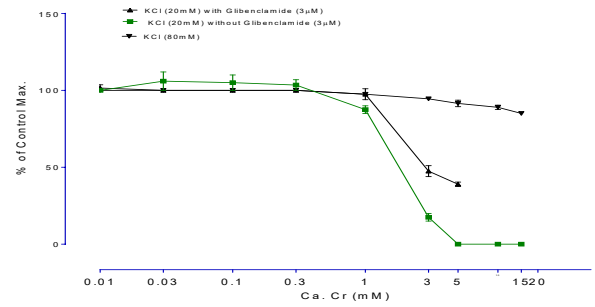



**Figure 3b: Effects of crude methanolic extract of *C. arvensis* on rabbit jejunal preparations on low KCl (20mM) induced contractions in the absence and presence of glibenclamide (3 µM) and high KCl (80 mM) (All values are mean±SEM, *n*=4)**

## Discussion

Phytochemical studies on this plant have been limited to the presence of caffenic acid and carbohydrates as major constituents and report that the *C. arvensis* showed no Antidiarrhoeal effect [4]. The flavonoids12, saponin13, fats, alkaloids, and δ-amino levulinic acid. Field bindweed also contains alkaloids including tropane, tropinine, tropine, pseudotropine and the pyrollidine alkaloids including cuscohygrine and hygrine [10].

Inhibitory action of Convolvulus arvensis on the smooth muscles of the intestine has been under study. This action was reversible in the presence of calcium that highlighted that the plant did not block the calcium channels. In addition the relaxant mechanism was observed not to be related to the blockade of the para-sympathetic or ganglionic receptors, and neither was the outcome of agonism of the adrenergic receptors since addition of both acetyl-choline and nicotine reversed the relaxant action. It was therefore concluded that the plant produced the relaxant action via another mechanism that was specific to the plan itself [11,12].

According to our investigations, the results showed promising spasmolytic effects on spontaneous jejunal contractions without atropine. The spasmolytic effect was 188±2.8% of control maximum on concentration 5 mg/ml of *C. arvensis* extract. While on concentration 15 mg/mL, the spontaneous activity was completely relaxed in absence of atropine. The $EC_{50}$ value on spontaneous was recorded (11.97±0.9 mg/mL). In further investigations, in the presence of 0.03 µM concentration of atropine, the methanolic extract of *C. arvensis* was investigated [13].

The utility of the plant extracts in diarrhea is of great benefit owing to diarrhea being a grave cause of motality as well as morbidity, more commonly in children and the developing states. Plant extracts including C.arvensis have hence caught the interest in this regards and a number of researches have proven their benefit in diarrhea [14].

Toxicity studies of the morning glory plant are deficient. Literature on the safe, toxic and lethal dosage ranges has not been established clearly. In our study the lethal dose started after 750mg/kg and at a dose as high as 1000m/kg, all the mice died. In another study where C.arvensis was fed to the mice, the animals developed necrotic lesions in the liver, gastritric ulcers and inflammation. Mice receiving lower dose for a period of 6 to 8 week did not have any ulcerations of the liver and stomach [15].

The current study also investigated the outcomes of the acute toxicity of the plant. At 500mg/kg and above piloerection and catatonia was observed. The mice were sedated and had a decrease in the motor activity. Writhing and respiratory stimulation also started at the same dose and above. Weight loss with colic was observed in the horses feeding on the pastures of northern Colorado. The causative agents were investigated to be tropane alkaloids of C.arvensis. The similar preparations of the plant were fed to the mice experimentally led to fibrosis of the intestine and affected motility in a dose dependent manner [16].

The signs of the toxicity depend upon the amount of the plant taken. The main ingredient proposed to be responsible for the adverse effect is the alkaloid content. Moreover, the effects of the plant on motility of the gastro-intestinal tract is thought to be related to pseudotropine, that is one of the main alkaloids present in C.arvensis [17]. Pseudotropine is also a probable causative agent for the intestinal fibrosis.

## Conclusion

The observed spasmolytic activity of extract of C. arvensis is through $K^+$ channel activation as well as activation of muscarinic receptors that may explain uses of C. arvensis in various gastrointestinal disorders. The crude extract is safe up to 500 mg/kg. The study results do not share the findings with a number of studies that propose contrary results. It is therefore, recommended that further studies are carried out to explore the effect on GI muscles.

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

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