RESEARCH ARTICLE

Assessment of Acute Toxicity of *Convolvulus arvensis* Methanol Extract

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ABSTRACT

Background: Cancer incidence spread widely in the world. The findings of a promising natural product that may function as adjuvant therapy in cancer treatment as a food supplement have a very important impact on tumor development prevention.

Objective: The study's objective is to test the activity of methanol extract of *Convolvulus arvensis*, showing cytotoxic activity against the tumor.

Methods: The plant's leaves of *C. arvensis* were dried, crushed into a fine powder, and extracted with methanol, which revealed cytotoxic activity. Its safety was evaluated on two animal species, mice and rats. Toxicity was assessed in Swiss albino mice by giving them several oral doses of plant extract ranging from 1.0 to 20.0 gm/kg and watching them for four hours and then watching them every 1-hour for one day, then every six hours for another two days (72 hours, acute toxicity). The animals were also given a single dose of 5 gm/kg plant extract, biochemical markers, relative organ weights in laboratory animals (male & female rats), and the body weight was used to determine toxicity.

Results: Methanol extract had an LD50 of 1.08 gm/kg. There were no significant variations in biochemical findings when compared to the control group. Throughout the trial, there are no notable weight changes.

Conclusion: Methanol extract has a low LD50 (1.08 gm/kg), but there is no observed mortality or toxic manifestations during acute toxicity study. The study also approved non-significant variations in body weights, relative organ weights, or biochemical testing between the animals that received the extract and the animals used as control.

Keywords: *Convolvulus arvensis*, Methanol extract, Cytotoxic herb, Acute toxicity

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INTRODUCTION

Convolvulus arvensis L.(C. arvensis) or a bindweed plant belongs to Convolvulaceae family. It is a worldwide distributed plant used widely in past centuries due to its different biological and pharmacological effects.[1,2] Extracts of *C. arvensis* are strongly used as an anticancer agent. A promising study shows that *C. arvensis* can be used as a beneficial and safe chemo-therapy.[1] External part of *C. arvensis* has been utilized as purgative, spasmolytic, and tissue injury healing.[3] The LD_{50} is a standard measure of acute toxicity referred in milligrams of total extracts per kilogram of tested animals' weight. This is the one dosage that kills fifty percent of all tested animals. The lower the lethal 50% dose, the more hazardous agent.[4] As the beginning dose is 10% of LD50, LD50 can be used to determine the needed dose for in vivo experiments and, later, clinical trials.[5] Furthermore, acute toxicity encompasses more than just determining the lethal dose; it also includes determining the alterations that the supplied herb may cause in the animals. Usually, as tested laboratory animals, the rats are utilized to assess the acute toxic activity, and mice are utilized to determine LD_{50} to ensure that at least two different species are evaluated for acute toxicity.[6]

METHODS

Extraction

Before purchase, fresh leaves of *C. arvensis* were harvested and authenticated at Mustansiriyah University's College of Pharmacy's Pharmacognosy and Medicinal Plants Department. The leaves were washed and removed from sunlight to be dried at 25°C, before being milled as a fine substance. A total of 400 g of the plant leaves powder was extracted using the maceration method with methanol at 1:4 w/v ratio (100 gm fine substance /400 cc of dissolvent). Plant leaves fine substance was subdivided into 4 divisions, and the procedure continued overnight for 24 hours in a rotary water bath at 40°C, then filtered the crud extract with Whittman no.1 filter paper.

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A vacuum rotary evaporator was used to concentrate the final crude extract, which was then dried in a weighted petri dish at room temperature and then determined the amount of the final extract. The extraction technique was applied 3 times before the experiment, and the dried extract was kept in a dried umber container at room temperature.[7]

Lethal Dose 50% (LD_{50}) Study

In the toxicity study, 70 Swiss albino mice (35 males and 35 females), their weight was ranging from 20 g to 25 g, each was employed. The mice were divided into one untreated animal group and six tested animal groups (one animal per sex), with each animal group of five members. They were given water and food for one week to adjust to the experimental conditions before the study. The untreated animal group was given 0.3 mL of 2% Tween 80 solution p.o., while the tested animal groups were given *C. arvensis* methanolic extract obtained by dissolving 8 gm in 10 cc of 2% Tween 80 in following dosages: 1, 2.5, 5, 10, 15, and 20 g/kg.[8,9] Following the extract administration, the mice were examined after the first 4 hours, hourly for the next 24 hours, and every 6 hours until 48 hours to look for any mortality or behavioral changes and other physiologic function.[10,11] The Ethical Committee at Mustansiriyah University's College of Pharmacy in Baghdad, Iraq, approved the study.

Acute Toxicity Study

A World Health Organization (WHO) guideline was used to estimate acute oral toxicity.[12] The 20 albino rats (ten animals for each sex) were separated into 4 groups: 1 untreated animal group, 1 tested animal group (for every sex group). The extract was administered to the treatment groups orally in one dose of 5 gm/kg BW, while untreated animal groups were given water only. For 14 days, the rats were observed for any poisoning symptoms. Necropsies were performed on the animals who perished during this period. On the 14th day of the experiment, all of the rats were sacrificed.

Experiment Design and Data Analysis

In this investigation, the rationalized complete block design experiment design (RCBD) was employed in this study. The data were presented as a mean and SD. To compare treatment groups, ANOVA was utilized, then a Tukey-test comparison. The differences of the means were statistically significant at a 5% confidence level. SSPS 16.0 was utilized in the statistical analysis, with a significance at $p < 0.05$.

RESULTS

The Lethal dose of 50% (LD50) of a methanol extract of *C. arvensis* was determined on mice.

For male and female mice, Table 1 displays dead and alive mice numbers. Different methanol extract dosages were administered to every animal group. The lethal dose of fifty, or the amount needed to be lethal for half of all mice treated, as shown in Figure 1.

Acute Toxicity for Methanol Extract of *C. arvensis* on Rats

During the 14-days acute toxicity trial, no mortality or symptoms of toxicity were seen when the rat was received one dose of 5 gm/kg BW p.o. Alterations would indicate the toxicity of chemicals in BW and relative organ weight in control animals; however, there were non-significant variations in relative organ weight between tested and untreated rats and no morphological alterations. Table 2 displays the males and females animals body-weight variations through the experiment. Each group comprises five rats, and the data is represented as mean ± standard deviation. Every seven days, the males and females animals' weights were recorded. The relative organ weights of males and females animals are shown in Table 3. After 14 days of acute toxicity testing, biochemical assays were performed on rats who received one dosage of 5 gm/kg of *C. arvensis* methanolic extract and their controls, with the data provided as mean ± standard deviation, several animals equal to five. Non-significant variations between tested and untreated rats ($p > 0.05$) (Table 4).

Table 1: The number and percent of dead animals in tested groups

Group	*Dose (serial dilutions of leave extract) (g/kg)*	*Number of tested laboratory animals (mice)*	*Number of deaths in laboratory animals (mice)*	*Total cumulative deaths (%)*
Control	0	Five male	0	0
		Five female	0	0
1	1	Five male	3	60
		Five female	2	40
2	2.5	Five male	3	60
		Five female	3	60
3	5	Five male	4	80
		Five female	4	80
4	10	Five male	5	100
		Five female	5	100
5	15	Five male	5	100
		Five female	5	100
6	20	Five male	5	100
		Five female	5	100

DISCUSSION

The LD50 of *C. arvensis* methanol extract was found to be 1.08 g/kg in this investigation. Another investigation in a previous study revealed that the LD50 of *C. arvensis* methanol extract was equal to 1.6 g/kg.[13] The difference in LD50 in these studies is not substantial and could be attributed to the difference in land quality where the grass grew, the conditions of extraction method, and the extraction solvent type.

During the experiment, rats given a single oral dose of the aqueous *C. arvensis* methanol extract (5 g/kg) showed no death or toxicity symptoms. There were non-significant variations in body weights between the tested and untreated groups ($p > 0.05$), non-significant variations in relative organ weights between the tested and untreated groups ($p > 0.05$), and no significant differences in biochemical tests between the treated and control groups ($p > 0.05$). Variations in animal weights and organ weights between testing and control animals show the substance's toxicity.[14] Even if there are no morphological changes, there may be a significant variation in organ weight between the tested treatment group and untreated control group.[15] It is strongly suggested that more research be done on this topic.

In conclusion, this extract is dangerous due to its low LD50 (1.08 g/kg), yet no mortality or toxicity symptoms in the acute toxic study, non-significant variations in body weights, relative organ weights, and biochemical assays between tested and untreated groups.

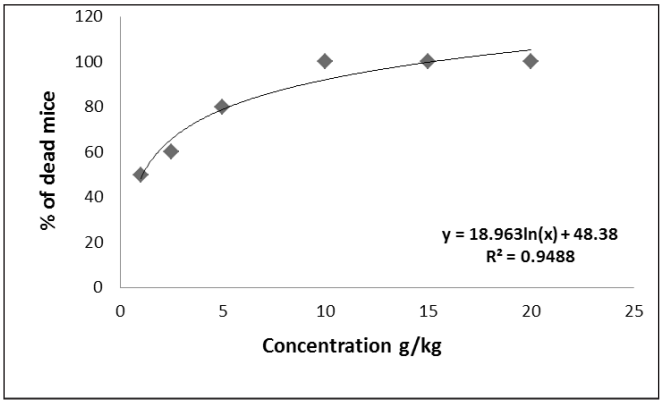

Figure 1: Lethal dose 50% (LD_{50}) of methanol extract of *C. arvensis* in laboratory animals (mice), measured by the equation [y=18.963ln(x)+48.38], where LD_{50} equals 1.08 gm/kg.

Table 2: The weight (g) of treated and control mice throughout a 14 days acute toxicity trial. There was no significant weight loss in the animals ($p > 0.05$). The weight fluctuations are given by mean ± standard deviation, n = 5.

	Weight of the tested laboratory animals (g)			
Time (day)	*Male group*	*Control male group*	*Female group*	*Control female group*
Day zero	205 ± 7.8	203.8 ± 5.8	195 ± 9.2	197.4 ± 5.2
Day 7	198 ± 8.4	210.4 ± 7.5	187 ± 6.5	202.7 ± 4.5
Day 14	187 ± 11.3	219.7 ± 11.7	179 ± 10.2	211.5 ± 9.6

Table 3: Males and females animals, as well as their controls, after 2 weeks of acute poisoning, relative organ weight (g), n = 5. The relative organ weight of tested rats & control rats was non-significantly different ($p > 0.05$)

	Organs	*Treatment*	*Control*
Male Rats	Liver	5.233 ± 0.66	5.878 ± 0.75
	Kidney	0.809 ± 0.02	0.887 ± 0.04
	Spleen	0.628 ± 0.08	0.642 ± 0.07
	Heart	0.710 ± 0.03	0.754 ± 0.05
Female Rats	Liver	5.117 ± 0.35	5.984 ± 0.48
	Kidney	0.878 ± 0.07	0.904 ± 0.03
	Spleen	0.524 ± 0.04	0.608 ± 0.05
	Heart	0.693 ± 0.02	0.722 ± 0.04

Table 4: Biochemical tests after 14 days of receiving 5 g/kg single dose of methanol extract of *C. arvensis*.

	Male groups		*Female groups*	
Biochemical test	*Treatment*	*Control*	*Treatment*	*Control*
ALT (GPT) (U/L)	38.7 ± 3.05	34.2 ± 2.34	37.3 ± 2.45	32.6 ± 3.27
AST (GOT) (U/L)	37.8 ± 4.08	29.3 ± 6.23	35.7 ± 4.67	31.2 ± 3.87
ALP (U/L)	164.5 ± 6.73	170 ± 3.54	150.4 ± 3.48	154 ± 5.25
Blood Urea (mg/dL)	34.3 ± 5.65	29.7 ± 3.72	31.4 ± 4.28	26.3 ± 5.35
Sr.creatinine (mg/dL)	0.54 ± 0.05	0.49 ± 0.0	0.50 ± 0.06	0.47 ± 0.04
Blood Sugar (mg/dL)	96.5 ± 8.82	117.3 ± 4.29	101.9 ± 7.68	118.7 ± 5.56

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