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Research Article

STUDY OF THE CYTOTOXIC AND GENOTOXIC EFFECTS FOR FRACTIONIATED EXTRACTS OF *CONVOLULUS ARVENSIS* ON BONE MARROW IN MICE

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ABSTRACT

Objective: The aerial parts of *convolvulus arvensis* examined for their cytotoxicity and genotoxicity actions for two doses of four organic solvents fractions according to their polarity.

Methods: The aerial parts of the crud plant were extracted with 80% aqueous ethanol and fractionated using petroleum ether, chloroform, ethyl acetate and *n*-butanol which were then examined on bone marrow of mice by measuring the mitotic index (MI) and chromosomal aberrations (CA) for two doses of the each fraction dissolved in DMSO and used cyclophosphamide as standard for positive control while DMSO only remain as negative control.

Results: The results showed that the chloroform and *n*-butanol fractions give significant decrease in MI and increased CA for both doses. The petroleum ether in low doses didn't give any significant effect while the high dose had. The ethyl acetate fraction of low dose increased MI and decreased CA while the high dose gives the inverse action.

Conclusion: The results could be explained due to different active constituent that present in each fraction mainly alkaloids, flavanoids and saponins for chloroform, ethyl acetate and petroleum ether respectively. For this reason it is possible that the plant extracts can have a therapeutic effect to destroy the cancerous cells. However, further studies on active components and their effects on cell divisions are needed.

Keywords: *Convolvulus arvensis*, Extraction, Fractionation, Genotoxicity and Chromosomal aberrations

INTRODUCTION

Field bindweed (*Convolvulus arvensis* L, Family: *Convolvulaceae*) is one of the most common noxious weed and one of the most difficult to control. The genus *Convolvulus* contains about 250 species. Many of the species of this genus are weeds, which can swamp other valuable plants by climbing over them. The weed is native to Europe, North Africa and Asia while it most likely arrived in the America as contaminant in farm and garden seeds. It's successful in many types of climates, including temperate, tropical, and Mediterranean, but is most troublesome for agriculture throughout the temperate zone. Fifty-four countries report field bindweed as a weed in 32 different crops [1]. Phytochemical studies on the aerial parts of this plant showed the presence of various compounds such as saponins, terpinoids, steroids, tropane alkaloids (Pseudotropine, tropine, tropinone, meso-cuscohygrine, Hygrine, calystegine and atropine), flavonoids (Kaempferol, Quercetin and rutin), phenolic acids and different quantities of essential elements. In addition to that, the whole plant parts contain also starch, mucilage, fat and protein with different quantities depending on part that distributed [2]. *Convolvulus arvensis* was recorded to use traditionally as a laxative and diuretic plant in different worldwide. It's also used in skin disorders as anti-furunculosis, anti-dandruff and against spider bites [3,4]. Recent study showed that the plant has anticancer activity against different types of cancer cell culture [5]. The plant showed also anti-tumor effect in mice [6]. The aim of this study is to assess the cytotoxic and genotoxic effects through measuring the Mitotic index and Chromosomal aberrations on bone marrow cells of mice respectively of the *Convolvulus arvensis* organic fractions' of different doses.

METRIALS AND METHODS

Plant material

The aerial parts of Field bindweed (*Convolvulus arvensis* L, Family: *Convolvulaceae*) used were collected from Medicinal Plants Garden in Department of Pharmacognosy and Medicinal plants, College of Pharmacy-University of Baghdad. The plant was identified by the Iraqi National Herbarium at Abu-Ghraib and a voucher sample was kept in the department of Pharmacognosy and medicinal plants in the college.

200 grams of the grounded aerial parts were extracted with 1.5 liter of 80% aqueous ethanol using soxhlet for 10 hours. The extract was filtered and evaporated using rotary evaporator at 45 ^{o}C to a volume about 100 ml, then was fractionated by partitioning with petroleum ether, chloroform, ethyl acetate and n- Butanol successfully using (150ml x 3times) for each solvent. The first three fractions were dried over anhydrous sodium sulfate, filtered and evaporated to dryness, while the n-butanol fraction was evaporated directly.

For each fraction, two solutions were prepared through dissolving 0.5g and 1g from each extract in 5 mL of Dimethylsulfoxide DMSO to be given as low dose of 200mg/kg and high dose 400mg/kg respectively. Cyclophosphamide was given as a standard for positive control in dose of 15mg /kg from a solution of 0.75% concentration in DMSO while negative control group receive only the DMSO vehicle.

Laboratory Animals

Albino Swiss mice (Mus musculs) approximately 3 to 5 months old were used supplied by the Biotechnology Research Centre (Al-Nahrain University). Their weights were between 23-27 grams. The animals were maintained at a temperature of 23 – 25°C, and they had free excess to food (standard pellets) and water (ad libitum). Sixty animals were divided into ten groups (six mice of each) as follow:

Group1: treated with DMSO solution only as negative control group.

Group2: treated with cyclophosphamide in DMSO as positive control group.

Group3: treated with (400mg/kg) of chloroform fraction of *Convolvulus arvensis*.

Group4: treated with (200mg/kg) of chloroform fraction of *Convolvulus arvensis*.

Group5: treated with (400mg/kg) of ethyl acetate fraction of *Convolvulus arvensis*.

Group6: treated with (200mg/kg) of ethyl acetate fraction of *Convolvulus arvensis*

Group7: treated with (400mg/kg) of *n*-butanol fraction of *Convolvulus arvensis*

Group8: treated with (200mg/kg) of *n*-butanol fraction of *Convolvulus arvensis*

Group9: treated with (400mg/kg) of petroleum ether fraction of *Convolvulus arvensis*

Group10: treated with (200mg/kg) of petroleum ether fraction of *Convolvulus arvensis*

All the treatments for all of the solutions above were given to all animals intra-peritoneal as an equal volume of 2ml/kg dose for seven successive days. After 24 hr of given dose, the animals were injected with 0.05% colchicines solution at dose of 2ml/kg intra-peritoneal before 2 hours of killing the animals to block the cells in metaphase.

Preparation of slides

The slides prepared essentially to the modified method of Preston, *et. al.* [7]. The animals were killed by cervical dislocation and both femora were immediately removed. The cells in bone marrow were flushed by pushing 2-3 times of 0.075 M hypotonic solution KCl into the marrow cavity of femur. Then the tubes were put in water bath at (37ºC) with shaking from time to time and centrifuged at 2000 rpm for 10 min. The supernatant was removed; the pellet was re-suspended and fixed in 5 ml of Carnoy's fixative (methanol: acetic acid = 3:1) for 20 min. Repeat the centrifuge and fixed cells in two or three times. Finally, the cells were dropped on a clean wet slide from a height of 3feet at rate of (4-5) drops to give the chance for the chromosomes to spread well and stained with 5% Giemsa stain, left for 15 minutes and then wash with D.W.

Measuring of mitotic index and chromosomal aberrations

The number of dividing cells, including late prophases and metaphases were counted. The mitotic index (MI) was calculated in which the total number of the cells counted as 1000 cell for each slide according to following formula [8]:

$$\text{Motic Index (MI)} = \frac{\text{No. of divided cells}}{\text{Total No. of cells}} \times 100$$

The analysis of chromosomal aberrations (CA) as deletion, rings, acentric, dicentric chromosomes, and breaks with their total score were part of the methodology used counted as100 cell for each slide.

Statistical analysis

The results were presented as mean ± standard deviation (SD). The statistical analysis includes unpaired *t*-test. The significance level of all tests was taken as P value<0.05

RESULTS AND DISCUSSION

The results of mitotic index and chromosomal aberrations for all groups were showed in table 1. The results indicated that the positive control group of cyclophosphamide showed significant decrease in mitotic index and highly increase in chromosomal aberrations. These results were expected due to the cytotoxic and anti tumor effects of cyclophosphamide that's for this reason used as standard anti-cancer agent [9].

Both groups of chloroform fraction showed significant changes in MI and CA as compared with control groups. The MI decreased scientifically while CA increased significantly also as compared with negative control group that received vehicle only. These results might be due the presence of alkaloids especially tropane type in the chloroform fraction that have these actions [10,11] or might be related to some lipophilic glycoside compounds extracted in this fraction as indicated in our work by the primary phytochemical studies [12]. The plant showed cytotoxic effects on human tumor cell line in vitro study [5, 12]. However there were no significant differences in results between both doses of chloroform fraction, and they were still having significant lower MI and higher CA as compared with negative control group of vehicle only.

The petroleum ether fraction in low dose gave no significant difference in MI as compared with the negative control group but the higher dose showed significant decreases with the negative control although still significantly higher than cyclophosphamide group. The two groups showed significant increases in CA as compared with negative control group even though they were significantly lower than that of positive control group. These results might be correlated with other studies which indicates the presences of terpenoids in the petroleum ether fraction and gives cytotoxic actions [13, 14].

The low dose group of ethyl acetate fraction, which should has mainly flavanoids shows increment of MI significantly above the negative control group with decrease of CA below significantly also. This result that different from other groups is compatible with previous studies which shows that the flavanoids such as quercetine or rutin presents in ethyl acetate fraction exhibits these effects [15,16]. However, the high dose group of ethyl acetate with both groups of n-butanol shows inverse effects which might be due to the direct genotoxicity of the plant constituents in these fractions [17].

Table 1: Effects of the plant fractions groups on Mitotic Index (MI) and Total Chromosomal aberrations (TCA) in mice bone marrow

Groups	MI %	T	CA	
Negative Control	5.617+0.794	*	0.543+0.057	*
Positive Control	1.824+0.463	*†	2.151+0.094	*†
Chloroform 400	3.267+0.468	*†	0.905+0.083	*†
Chloroform 200	3.417+0.677	*†	0.928+0.043	*†
Ethyl acetate 400	3.150+1.269	*†	0.698+ 0.142	*†
Ethyl acetate 200	7.384+0.564	*†	0.405+0.030	*†
n-Butanol 400	3.350+0.472	*†	0.803+0.050	*†
n-Butanol 200	4.034+0.769	*†	0.871+ 0.037	*†
Petroleum ether 400	2.817+0.366	†	0.873+0.057	*†
Petroleum ether 200	5.434+0.509		0.836+0.042	

- Values are mean ± SD of six mice (N=6) from each group;
- * *P*<0.05 show significant when compare with negative control
- † *P*<0.05 show significant when compare with positive control

CONCLUSION

The results of this study suggest that, although *C. arvensis* can be utilized in folk medicine, serious damages on cells by incorrectly usage can be observed. Our results concerning the decrease in MI and the increase CA frequencies induced by the plants extracts lead to the conclusion that is able to induce both cytotoxic and genotoxic effects. For this reason it is possible that the plant extracts can have a therapeutic effect to destroy the cancerous cells. However, further studies on active components and their effects on cell divisions are needed.

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