



## The Evaluation of Ethyl Acetate Fraction of *Cressa cretica* Effect on Mitotic Index and Micronucleous Frequency in Mice

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### ABSTRACT

*Cressa cretica* L. belongs to the Convolvulaceae family. it has been reported to possess antibilious, anti-tubercular, antiviral, antibacterial, antidiabetic, expectorant, anthelmintic, stomachic aphrodisiac, enriches the blood, constipation, leprosy asthma, urinary discharges, also it exhibits cytotoxic and anti-inflammatory activity. The present study designed to evaluate the genotoxicity of ethyl acetate fraction of *Cressa cretica* L, extract administered orally, in two different doses [100mg/kg and 200mg/kg] on both bone marrow and spleen cells in mice for seven successive days, and comparing their effects with methotrexate (positive control) and dimethylsulfoxide [DMSO](negative control). The results have been showed that ethyl acetate fraction of *Cressa cretica* at a dose 100mg/kg and 200mg/kg showed a significant decrease of mitotic index in bone marrow cells and spleen cells in comparison with DMSO, meanwhile it showed a significant increase of micronucleus appearance in bone marrow cells, in conclusion ethyl acetate fraction of *Cressa cretica* L showed powerful effect on mitotic index and micronucleus appearance on bone marrow cells and spleen cells in mice.

**Keywords:** *Cressa Cretica*, Ethyl acetate, Mitotic index, micronucleus appearance.

### INTRODUCTION

Most cells grow, perform the activities needed to survive, and divide to create new cells. These basic processes, known collectively as the cell cycle, are repeated throughout the life of a cell<sup>1</sup>. The major goal of this series of events is the doubling of the cell, for instance, for the replacement of damaged cells also for development from a single-cell zygote to fertile adult requires many rounds of cell division<sup>2,3</sup>.

The cell cycle consists of two different phases, inter phase and the Meta phase, Interphase is the longest phase, lasting about 90% of a cell's life, and during this time, the cell is performing for the body. Maybe the cell's function is to help in absorb nutrients or protect from disease, and during inter phase, that's what it's doing. The other 10% of the time the cell is in M phase, or, mitotic phase, which is when the cell is dividing<sup>4</sup>. For many years, exogenous sources of damage have been thought to be the primary cause of DNA mutations leading to cancer. However, a study achieved on 2001 proposed that endogenous sources of DNA damage also contribute significantly to mutations that lead to malignancy<sup>5</sup>. Mitotic index defined as the ratio between the numbers of cells in a population undergoing mitosis (at metaphase) to the number of cells not undergoing mitosis. The purpose of the mitotic index is to measure cellular proliferation<sup>6</sup>. The mitotic index is an important prognostic factor predicting both overall survival and response to chemotherapy in most types of cancer<sup>7</sup>. It may lose much of its predictive value for elderly populations, for example a low mitotic index loses any prognostic value for women

over 70 years old with breast cancer. An elevated mitotic index indicates more cells are dividing, and thus obvious in cancer cells, the mitotic index may be elevated during necessary processes to life, such as the normal growth of plants or animals, as well as cellular repair the site of an injury<sup>8</sup>. A micronucleus assay is an assay used in toxicological screening for potential genotoxic compounds. The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens<sup>9</sup>. There are two major versions of this test, one in vivo and the other in vitro<sup>10</sup>. The in vivo test normally uses mouse bone marrow or mouse peripheral blood. When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the otherwise nucleated cytoplasm<sup>11</sup>. *Cressa cretica* L. belongs to the Convolvulaceae family<sup>12</sup>. Flowering and fruiting time is from the end of June to the end of August. During September, the plant gradually withers. In the end of September and beginning of October, coincides with the opening days of more abundant and higher quantum of precipitation, when the moisture content in the soil increases and finally the whole area becomes inundated again. The plant contained coumarins, sterols, alkaloids, tannins, glycosides, protein, carbohydrate, flavonoids, unidentified sugars and high salt content; nine compounds included three coumarins, four flavonoids along with two phytosterols from *Cressa cretica*. Their structure established as coumarin, umbelliferone, daphnetin, quercetin, rutin, kaempferol, quercetin, stigmasterol and b-sitosterol.<sup>13</sup> It has been reported to possess



antibilious, antitubercular, antiviral, antibacterial antidiabetic, expectorant, anthelmintic, stomachic aphrodisiac, enriches the blood, constipation, leprosy asthma, urinary discharges, cytotoxic and had anti-inflammatory activity.<sup>12</sup>

## MATERIALS AND METHODS

### Plant material

The plant had been collected from Baghdad in August, washed thoroughly, chopped into bits, and allowed to dry under shade. The dried plant was blended into fine powder using electric blender.

### Preparation of extract

Five hundred grams of the powdered plant was defatted by maceration in 1500 ml of hexane for 24 hours with occasional agitation then filtered. The defatted plant materials were dried introduced in a thimble and extracted using soxhlet apparatus using 1500ml of ethyl acetate (B.p.40-60 °C) for 15 hours then cooled, filtered and evaporated under reduced pressure at 40 °C using rotary evaporator<sup>14</sup>.

The yield values for ethyl acetate fraction have been obtained.

### Experimental model

Twenty four Albino Swiss mice (*Mus musculus*) were used for each experiment. They were supplied by National Center for Drug Control and Research. Their weights were 20-25 gram. They were divided into four groups; each was kept in a separate plastic cage. The animals were maintained at a temperature of 23 – 25°C, and they had free excess to food (standard pellets) and water.

each) as follow:

Group1: six mice were treated with dimethylsulfoxide (DMSO). This group was served as negative control the dose was given (I.P.) daily for seven successive days.

Group2: six mice were treated with a single dose (20mg/kg) of methotrexate (MTX). This group was served as positive control.

Group3: six mice were treated (oral) with (100mg/kg) of ethyl acetate extract of *Cressa Cretica* for seven successive days.

Group4: six mice were treated (oral) with (200mg/kg) of ethyl acetate extract of *Cressa Cretica* for seven successive days.

Mice were sacrificed by (spinal dislocation). Samples of bone marrow cells and spleen cells were taken and genotoxic analyses were carried out as described latter.

### Phytochemical Investigation

Preliminary phytochemical investigation was carried out for ethyl acetate fraction using, Dragendorff's spray reagent and 5% ethanolic KOH spray reagent, vaniline/H<sub>2</sub>SO<sub>4</sub> reagent, 10% NH<sub>4</sub>OH.

Evaluation of mitotic index in Bone marrow cells and spleen cells

After seven days of treatment, all animals were injected intraperitoneally with 1mg/kg colchicines, and then two hours later they are scarified by cervical dislocation. Bone marrow samples was aspirated from the femur bone and spleen cells have been collected processed using aseptic technique for evaluation of mitotic index as previously reported elsewhere<sup>15</sup>.

Evaluation of micronucleus assay in Bone marrow cells

After chemical treatment, mice were killed and femoral marrow cells were smeared on clean glass slides, fixed with methanol for 5 min at room temperature, and stained with Giemsa<sup>16</sup>

### Statistical Analysis

Data are expressed as Mean ± SD; unless otherwise indicated, statistical analyses were performed using unpaired *t*-test. If the overall F value was found statistically significant ( $P < 0.05$ ), further comparisons among groups were made according to post *hoc* Tukey's test. All statistical analyses were performed using SPSS GraphPad InStat 3 (GraphPad Software Inc., La Jolla, CA, USA) software.

## RESULTS AND DISCUSSION

### Phytochemical Investigations

Phytochemical investigations revealed the presence coumarins, steriols and terpenoids compounds. The stepwise fractionations offering a separation of active constituents during extraction, this separation procedure give a pronounced different genotoxic effect on bone marrow cell and spleen cell. The yield value was ( 5.5gram)

Mitotic index and micronucleus appearance of different concentrations of ethyl acetate fraction of *Cressa Cretica* In table 1 shows that, ethyl acetate extract of *Cressa cretica* at both dases 100mg/kg and 200mg/kg caused significant decrease of mitotic index in both bone marrow cells and spleen cells when compared to negative control (DMSO) ( $P < 0.05$ ), which is a parameter that give indication about cell division when compared to negative [DMSO], at the same time , these two doses show significant reduction in mitotic index in both bone marrow cells and spleen cells when compared to positive control ( $P < 0.05$ ). Methotrexate caused significant decrease ( $P < 0.05$ ) of mitotic index compare to negative control and extract in bone marrow and spleen cell.

In Table 2, figure (1), shows that, ethyl acetate extract of *Cressa cretica* at both doses caused significant increase in micronucleus appearance in bone marrow cells when compared to negative control (DMSO) ( $P < 0.05$ ). Methotrexate caused significant increase ( $P < 0.05$ ) in micronucleus appearance compare to negative control and extract in bone marrow.



**Table 1:** Incidence of mitotic index in bone marrow and spleen cells of albino mice treated with different doses of the ethyl acetate extract of *Cressa Cretica* compared to methotrexate and dimethylsulfoxide

Treatment Groups	Mitotic Index	
	Bone Marrow Cells	Spleen Cells
Dimethylsulfoxide (DMSO) (Negative control)	5.06±0.33	3.34±0.27
Methotrexate (MTX) (positive control) 20mg/kg	2.18±0.16 <sup>*a</sup>	1.1±0.27 <sup>*a</sup>
Ethyl acetate extract of <i>Cressa Cretica</i> at dose 100mg/kg	3.14± 0.75 <sup>*Ab</sup>	2.84±0.58 <sup>*Ab</sup>
Ethyl acetate extract of <i>Cressa Cretica</i> at dose 200mg/kg	2.9±0.53 <sup>*Ab</sup>	2.64±0.64 <sup>*Ab</sup>

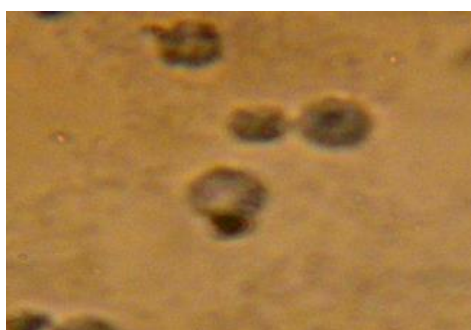
Data are expressed as mean±S.D; n=6 animals in each group; <sup>\*</sup>significantly different compared to DMSO (negative control) ( $P<0.05$ ); -Values with non-identical small letters superscripts (a,b) consider significant different when compared between groups ( $P<0.05$ ); -Values with non-identical capital letters superscripts (A, B) consider significant different when among compared tests doses ( $P<0.05$ );

Finally there was no significant difference ( $P>0.05$ ) between ethyl acetate extract doses in mitotic index and micronucleus appearance.

**Table 2:** Incidence of micronucleus appearance in bone marrow cells of albino mice treated with different doses of the ethyl acetate extract of *Cressa cretica* compared to methotrexate and dimethylsulfoxide

Micronucleous appearance	
Treatment group	Bone Marrow Cells
Dimethylsulfoxide (DMSO) (Negative control)	7.1±0.72
Methotrexate (MTX) (positive control) 20mg/kg	30.41±3.14 <sup>*a</sup>
Ethyl acetate extract of <i>Cressa Cretica</i> at dose 100mg/kg	8.84± 0.95 <sup>*Ab</sup>
Ethyl acetate extract of <i>Cressa Cretica</i> at dose 200mg/kg	9.32±0.85 <sup>*Ab</sup>

Data are expressed as mean±S.D; n=6 animals in each group; <sup>\*</sup>significantly different compared to DMSO (negative control) ( $P<0.05$ ); -Values with non-identical small letters superscripts (a,b) consider significant different when compared between groups ( $P<0.05$ ); - Values with non-identical capital letters superscripts (A,B) consider significant different when compared among tests doses ( $P<0.05$ ).

**Figure 1:** Micronucleus appeared after treatment with ethyl acetate extract of *Cressa Cretica*

In Tables 1 and 2 indicate that, methotrexate effects on mitotic index cells was significantly lower when compared to ethyl acetate extract in both doses 100mg/kg and 200 mg/kg. Meanwhile they showed significant increase in micronucleus appearance in both doses as compare to extract. Regarding to the results obtained the explanations that ethyl acetate fraction of *Cressa Cretica* contain coumarins, steriols and terpenoids compounds.

Polyphenols are a heterogeneous group of secondary metabolites. They have in common the presence in their structure of one or more phenol groups<sup>17</sup>. It can be divided in flavonoids and non-flavonoids, such as coumarins and simple phenols. Several studies showed the activity of coumarins has been linked to the possible prevention of diseases such as cardiovascular, cancer, neurodegenerative, However, many other studies of these compounds have been presented of coumarin with pro-oxidant activity<sup>18</sup>, and even *in vitro*, *in vivo* and *in silico* clastogenic activity<sup>19</sup>. This pro-oxidant activity causes the formation of reactive oxygen species (ROS) and inhibition of antioxidants systems<sup>20</sup>. This can generate oxidative damage to cells and tissues and biomolecules such as proteins, DNA and lipids which in turn decrease cell division in order to repair the damaged that happened, this explain the significant decrease in mitotic index<sup>21</sup>. The presence of another type of active constituents like sterol and terpenoids according to the phytochemical study that done in the present study, these active constituents are reported to be anti-oxidant effects<sup>22</sup>. The in equal potency between the antioxidants

and pro-oxidants active constituents create a condition that shift toward the more potent ingredient, in the present study, the pro-oxidant coumarin had predominant effect<sup>23</sup>

## CONCLUSION

Ethyl acetate fraction of *Cressa cretica L* showed powerful effect on mitotic index and micronucleus appearance in bone marrow and spleen cells in mice.

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