Research Article

Genotoxic Effect of Ethyl acetate Fraction of *Cressa cretica* on Chromosomal Aberration on Bone Marrow Cells and Spleen Cells in Mice.

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ABSTRACT

Chromosomes considered an as major structure that carrying the genetic information; hence any damage to the chromosome of healthy cells could lead to very bad consequences like cancer or many heritable diseases if this damage not repaired. Gene toxicity evaluation considered now a day is the most important protocol to evaluate the safety of the chemical compounds that have been used and to measure the ability of these compounds to reduce cancerous mass with minimum side effects on healthy cells. The present study is designed to evaluate genotoxic effect of ethyl acetate fraction of Cressa cretica F.Convolvulaceae on chromosomal integrity at two different doses (100mg/kg and 200mg/kg) on both bone marrow cells and spleen cells in mice for seven successive days, the results was compare their effects with methotrexate which was used as a positive control. The results have been showed that ethyl acetate fraction of Cressa cretica at both doses showed a significant increase in individual and total chromosomal aberration when compared with negative control (dimethylsulfoxide).

Keywords: Cressa cretica, bone marrow cells, spleen cells, ethyl acetate fraction.

INTRODUCTION

Cressa cretica L. belongs to the Convolvulaceae family [1]. It is an erect, small, dwarf shrub, usually grows in sandy and muddy saline habitats along with the species Suaeda maritima, Salicornia europaea, Salsola soda, Limonium vulgare subsp. Serotinum and Crypsis aculeate[2]. The traditional medicine uses of *Cressa cretica* is used to treat a variety of diseases including diabetes, asthma, constipation, joint pain, inflammation, dyspepsia, intestinal worms, flatulence, colic, skin problems, leprosy, urinary discharges and is taken as an expectorant, stomachic, tonic, aphrodisiac, antibilious and alterative. Also in Bahrain the plant is traditionally used as expectorant and antibilious agent[3]. Previous phytochemical investigation showed that Cressa *cretica* contained coumarins, sterols, 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, kaempferol, quercetin 3-O-b-D-glucoside, quercetin-3-O-a-L-rhamno-(1-6)-b-D-glucoside, stigmasterol and b-sitosterol .Cressa cretica yielded five flavonoids that were identified as quercetin, quercetin-3-O-glucoside, kampferol-3-O-glucoside, kampferol-3-O-rhamnoglucoside, and rutin [4]. Pharmacological effects of Cressa *cretica* are Bronchodilatory effect[5], Antitussive effect[6], Nootropic effect,[7] Antidiabetic effect[8] Antibacterial and antifungal effects[9]. The chromosome set of a species remains relatively stable over long periods of time, In fact, within populations there can be found abnormalities involving the structure or number of chromosomes. These alterations arise spontaneously from errors in the normal processes of the cell[10]. Their consequences are usually deleterious, giving rise to individuals who are unhealthy or sterile, though in rare cases alterations provide new adaptive opportunities that allow evolutionary change to occur[11]. During normal course of life cycle, structural rearrangements in chromosomes do occur but less frequently but, under the effect of mutagenic agents such as radiations and conditions of stress like very high temperature, chromosomes are more prone to these changes[12]. Homologous regions seem to be able to find each other and form a synaptonemal complex whether or not they are part of normal chromosomes [13]

MATERIALS AND METHODS

Plant material

The plant had been collected from Baghdad in August, washed thoroughly, chapped 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 (500gm) of the powdered plant was defatted by maceration in 1500 ml of hexane for 24hrs with occasional agitation then filtered. the defatted plant materials was dried introduced in a thimble and extracted using soxhlet apparatus with 1500ml of ethyl acetate solvent (B.p.40-60^{o}C) for 15 hours then cooled, filtered and evaporated under reduced pressure at 40 C^{o} using rotary evaporator[14]. The yield value for ethyl acetate fraction was calculated after evaporation of solvent.

Experimental model

Twenty four Albino Swiss mice (*Mus musculs*) 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 (*ad libitum)***.** The animals were divided into four groups (six mice of each) as follow:

Group 1: Mice were treated with dimethylsulfoxide. This group was served as negative control the dose was given (I.P.) daily for seven successive days.

Group 2: Mice were treated with a single dose (20mg/kg) of methotrexate. This group was served as positive control.

Group 3: Mice were treated (oral) with (100mg/kg) of ethyl acetate extract of *Cressa Cretica* for seven successive days.

Group 4: 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_2SO_4 reagent, 10% NH4OH.

Evaluation of Genotoxicity in Bone marrow

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 processed using aseptic technique for evaluation of mitotic index and total chromosomal aberration as previously reported elsewhere [15] .

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 terpeniods 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)

Gene toxicity of different concentrations of ethyl acetate fraction of *Cressa Cretica*

In table (1) ethyl acetate fraction of *Cressa Cretica* at a dose 100mg/kg and 200mg/kg showed significant increase in total chromosomal aberration and chromatid break in bone marrow cells when compared to negative control ($p<0.05$), other types of individual chromosomal aberration showed not significant increase in bone marrow cells when compared to negative control ($p>0.05$), at the same time ethyl acetate fraction of *Cressa Cretica* at a dose 100mg/kg and 200mg/kg showed significant decrease in total chromosomal aberration , chromatid break, chromatid gap, acentric chromosome, dicentric chromosome deletion and ring chromosome in bone marrow cells when compared to positive control ($p<0.05$), other types of individual chromosomal aberration showed not significant decrease in bone marrow cells when compared to positive control ($p>0.05$)

in table (2), ethyl acetate fraction of *Cressa Cretica* at a dose 100mg/kg and 200mg/kg showed significant increase in total chromosomal aberration ,chromosomal gap, ring chromosome and chromatid break in spleen cells when compared to negative control ($p<0.05$),other individual chromosomal aberration show not significant increase in spleen cells when compared to negative control($p>0.05$) at the same time ethyl acetate fraction of *Cressa Cretica* at a dose 100mg/kg and 200mg/kg showed significant decrease in total chromosomal aberration , chromatid break, chromatid gap, acentric chromosome, dicentric chromosome deletion and in spleen cells when compared to positive control ($p<0.05$), other types of individual chromosomal aberration showed not significant decrease in spleen cells when compared to positive control ($p>0.05$) Polyphenols are a heterogeneous group of secondary metabolites. They have in common the presence in their structure of one or more phenol groups [16]. 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 [17], and even *in vitro*, *in vivo* and *in silico* clastogenic activity[18] . These pro-oxidant activities cause the formation of reactive oxygen species (ROS) and inhibition of antioxidants systems[19]. This can generate oxidative damage to cells and tissues and biomolecules such as proteins, DNA and lipids[20]. The presence of another type of active constituents like sterol and trepenoids according to the phytochemical study that done in the present study, these active constituents are reported to be anti-oxidant effects [21].

The in equal potency between the antioxidants and pro-oxidants active constituents creat a condition that shift toward the more potent ingredient, in the present study , the prooxident coumarin had predoniment effect [22]

Table 1: Incidence of individual and total chromosomal aberration in bone marrow of albino mice treated with different doses of the ethyl acetate extract of Cressa Cretica compared to methotrexate and dimethylsulfoxide

Bone marrow	Chromatid break	Chromatid gap	Deletion	Dicentric chromosome	Acentric chromosome	Ring chromosome	Chromosome break	Chromosome gap	Total chromosomal aberration
Dimethylsulfoxide (DMSO) (Negative Control)	0.094±0.03	0.094±0.03	0.22 ±0.02	0.222 ±0.02	0.206 ±0.02	0.023 ±0.02	0.086 ±0.01	0.04 ±0.01	0.121 ±0.01
Methotrexate (MTX) (Positive Control) 20mg/kg	0.216±0.02*a	0.216±0.02*a	0.363±0.01*a	0.628±0.03*a	0.866±0.03*a	0.084±0.01a	0.138±0.01*a	0.108±0.01*a	0.322±0.01*a
Ethyl acetate fraction 100mg/kg	0.174±0.02*Ab	0.128±0.05Ab	0.23±0.03Ab	0.242±0.04Ab	0.23±0.05Ab	0.068±0.06Ab	0.108±0.05Aa	0.06±0.01Aa	0.145±0.06*Ab
Ethyl acetate fraction 200mg/kg	0.172±0.01*Ab	0.158±0.05*Ab	0.24±0.04*Ab	0.263±0.07Ab	0.28±0.08Ab	0.092±0.03*Ab	0.148±0.03Aa	0.08±0.01Aa	0.157±0.03*Ab

Data are expressed as mean±S.D; n=6 animals in each group;

-*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)

Table 2: Incidence of individual and total chromosomal aberration in spleen cells of albino mice treated with different doses of the ethyl acetate extract of Cressa Cretica compared to methotrexate and dimethylsulfoxide

Spleen cells	Chromatid break	Chromatid gap	Deletion	Dicentric chromosome	Acentric chromosome	Ring chromosome	Chromosome break	Chromosome gap	Total chromosomal aberration
Dimethylsulfoxide (DMSO) (Negative Control)	0.048±0.01	0.048±0.01	0.194±0.03	0.194±0.03	0.228±0.03	0.042±0.03	0.072±0.03	0.022±0.01	0.108±0.01
Methotrexate (MTX) (Positive Control) 20mg/kg	0.2±0.01*a	0.2±0.01*a	0.272±0.01*a	0.49±0.03*a	0.752±0.03*a	0.066±0.01*a	0.121±0.01*a	0.102±0.01*a	0.272±0.01*a
Ethyl acetate fraction 100mg/kg	0.098±0.05*Ab	0.074±0.03Ab	0.204±0.01Aa	0.23±0.07Ab	0.252±0.04Ab	0.122±0.04*Aa	0.082±0.04Aa	0.08±0.01*Aa	0.134±0.01*Ab
Ethyl acetate fraction 200mg/kg	0.125±0.03*Ab	0.095±0.02Ab	0.228±0.02Aa	0.25±0.06*Ab	0.271±0.03*Ab	0.107±0.03*Aa	0.119±0.03Aa	0.076±0.01*Aa	0.141±0.04*Ab

Data are expressed as mean±S.D; n=6 animals in each group;

-*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).

CONCLUSION

The ethyl acetate extract of Cressa Cretica shows pronounced gene toxicity with increasing the dose when administered orally to mice when compared to negative control due to presence of genotoxic substance (coumarins) .

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