Clastogenic Effect of *Carthamus lanatus* L. (Asteraceae)

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The clastogenic effect of total dichloromethane, methanol and water extracts, four bioactive fractions and three individual constituents from *Carthamus lanatus* aerial parts were evaluated in mice by bone marrow chromosome aberration assay with mitomycin C as positive control. Significant differences in the percentage of aberrant mitosis of the extracts were observed. The dichloromethane extract exhibited a considerable clastogenic effect and the water extract a negligible one. Different types of chromosome aberrations and time-dependant effects for the active fractions and individual compounds were found.

Key words: Carthamus lanatus, Clastogenic Effect

Introduction

Carthamus lanatus L. (Asteraceae) is known as a plant of phytopharmaceutical importance with sedative, anti-tumor and interferon-inducing activities (Benedi *et al.*, 1986; Yasuhuko *et al.*, 1979). Recently, a variety of biological activities of *C. lanatus*, including antioxidant, antibacterial, antifungal activity and cytotoxicity were shown (Taskova *et al.*, 2002, 2003; Mitova *et al.*, 2003). Until now, the clastogenic effect of the species has not been studied. Some data about the closely related *C. tinctorius* were reported. Ames test and *Salmonella* microsome reversion assay (Morimoto *et al.*, 1982) showed a mutagenic effect of the water extract of *C. tinctorius* flowers, which was confirmed by Esmaili-rad *et al.* (1995). Yin *et al.* (1991) demonstrated that the water extract was negative in the Ames test but positive in the chromosomal aberration and micronucleus assay in mice. The results of Nobakht *et al.* (2000) indicated harmful effects on cellular growth and differentiation during the embryonic development.

In the present paper, the clastogenic effect of three total extracts of *C. lanatus*, as well as their bioactive fractions and main individual constituents, was evaluated in mice using a bone marrow chromosome aberration assay.

Experimental

Plant material

Aerial parts of *Carthamus lanatus* L. (Asteraceae) were collected in July at the Losen village region, Sofia. A voucher specimen (No 156639) is deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM). The plant was collected and identified by Dr. Rilka Taskova.

Extraction and isolation of fractions and constituents

Dichloromethane extract

Dry and ground aerial parts of *C. lanatus* (1.5 kg) were extracted with dichloromethane (15 l). The dry residue of the extract (29 g) was partitioned between upper (13 g) and lower (13 g) layer of hexane/methanol/water (19:19:2, v/v/v). 5 g of the lower layer were separated on silica gel (Merck) column with hexane and hexane/ethylacetate (20:1 to 1:10). Crude fractions 53–57 (31 mg; sterol mixture), fractions 62–64 (90 mg; fraction B; R_f of oleanolic acid on TLC) and fractions 77–91 (2.7 g; α-bisabolol fucopyranoside) were separated and further purified by SEP-Pak C_{18} cartridges for rapid sample preparation (Waters, Milford, USA) with methanol.

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MeOH extract

Dry ground aerial parts (1.5 kg) were extracted with methanol. The dry residue of the extract (160 g) was successively extracted with diethyl ether (18 g), ethyl acetate (4 g) and butanol (9 g); water part (110 g). The ethyl acetate and butanol fractions were further separated by droplet counter current chromatography and column chromatography on silica gel (Merck) to yield the flavonoid constituents. The methanol extract, the ethyl acetate fraction, its main constituent, luteolin 7-*O*-glucoside, and a minor bioactive constituent, rutin, were tested.

Water extract

100 g dried aerial parts were extracted with hot water, concentrated (15 g) and separated on Sephadex G-25 (Pharmacia) with water. The water extract and the low-molecular water fraction ($M < 10^3$) (610 mg) were subjected to the analysis.

Animals

Male and female ICR mice weighing 20 g ± 1.5 g were obtained from the Base for Experimental Animals, BAS, Slivnitza. The animals were kept at standard conditions at 20 °C and 12 h light-dark cycle, having free access to food and water. Experiments were performed in accordance with the current guidelines for the care of laboratory animals.

Chromosome aberrations

The investigation was performed according to Preston *et al.* (1987). The samples were administered intraperitoneally (*i.p.*) at a dose of 1 mg/kg. Mitomycin C (Kyowa, Tokyo, Japan) (1 mg/kg) was used as a positive control. The control animals were injected with 0.2 ml solvent solution (DMSO) or 0.9 % NaCl.

The bone marrow chromosome aberration assay was performed on groups of animals consisted of 5 males and 5 females, which were treated with the samples, the DMSO solvent, 0.9 % NaCl and the positive control, respectively. The animals were injected *i.p.* with colchicine at a dose of 0.4 mg/kg 24 h and 48 h after administration of the samples, of DMSO, of mitomycin C (MMC) or 0.9 % NaCl, respectively, and 1 h before isolation of the bone marrow cells. The sampling times were chosen to ensure the cell analysis in case of a considerable mitotic delay. Mice were euthanized, bone marrow cells flushed from femur and hypotonized in 0.075 M KCl at 37 °C for 20 min. The cells were fixed in methanol/acetic acid (3:1), dropped on cold slides and air-dried. The slides were stained with 5 % Giemsa solution (Sigma, St. Louis, USA). At least 50 well-spread metaphases were analysed per experimental animal at random.

Statistical analysis

Three-way analysis of variance (ANOVA) with fixed effects, followed by two-group Student's *t*-test and *post hoc* pairwise comparison test of Dunnett with a control was performed using BMDP4V, BMDP3D and BMDP7D programs (Dixon *et al.*, 1990).

Results and Discussion

Three total extracts with different polarity (CH_2Cl_2, MeOH and H_2O extracts), four bioactive fractions and three main constituents were subjected to analysis of chromosomal aberrations in mitotic chromosomes of mouse bone marrow cells at a dose of 1 mg/kg. The tested fractions and constituents were chosen on the ground of proven biological activities. The sterol fraction and fraction B showed significant cytotoxicity assessed by the brine shrimp assay (Mitova *et al.*, 2003). The ethyl acetate fraction and its main constituent, luteolin 7-*O*-glucoside, exhibited cytotoxic and antioxidant activities (Taskova *et al.*, 2002, 2003). The low-molecular water fraction possessed cytotoxic activity and antimicrobial activity against *Staphylococcus aureus* (Taskova *et al.*, 2002).

The obtained data were presented in protocols according to Preston *et al.* (1987) and summarized in Table I. The solvent, dimethyl sulfoxide (DMSO) (0.01 ml/g) caused statistically insignificant increase of the percentage of aberrant mitosis *vs.* the control (0.9 % NaCl). The positive control, mitomycin C (MMC) (1 mg/kg) caused 12.8 ± 0.8 % aberrations 24 h after administering. After 48 h the percentage decreased to 5.0 ± 0.33, which is in agreement with the time-dependent frequencies of aberrations after a single injection of 1 mg/kg MMC described by Hayashi *et al.* (1984).

Table I. Frequencies of chromosome aberrations in affected mouse bone marrow cells after *i. p.* treatment of fractions and constituents of *Carthamus lanatus* aerial parts[a].

Sample[b]	Interval [h]	Type of aberrations Breaks	Fragments	c/c	t/t	Percentage of cells with aberrations X ± s.e.m.	Statistical significance[c]	Statistical significance[d]
Dichloromethane extract	24	16	8	24	2	10.0 ± 0.86	**	
	48	12	4	18	8	8.4 ± 0.97	**	*
Sterol fraction	24	11	9	6	0	6.5 ± 0.73	**	**
	48	3	4	9	0	3.2 ± 0.53	*	
Fraction B	24	4	0	10	0	2.8 ± 0.32		
	48	13	0	7	0	4.0 ± 0.61	**	**
α-Bisabolol fucopyranoside	24	9	5	0	0	2.6 ± 0.42		**
	48	7	15	0	2	5.6 ± 0.40		
MeOH extract	24	6	8	16	2	6.8 ± 0.43	**	**
	48	4	1	22	1	5.6 ± 0.39	**	
Ethyl acetate fraction	24	8	0	16	0	4.8 ± 0.53	**	**
	48	7	0	13	0	2.6 ± 0.42		
Luteolin 7-*O*-glucoside	24	8	3	18	0	5.4 ± 1.16	**	**
	48	14	6	13	0	6.8 ± 1.08	**	
Rutin	24	0	0	0	0	0.6 ± 0.30		**
	48	5	0	15	0	4.2 ± 0.46	**	
Water extract	24	4	0	11	1	3.2 ± 0.49	**	**
	48	2	0	8	0	2.0 ± 0.63		**
Low-molecular water fraction	24	5	0	7	0	2.8 ± 0.44		
	48	1	0	8	0	1.8 ± 0.36		
Solvent: DMSO	24	1	1	4	0	1.4 ± 0.30	control	**
	48	2	0	2	0	0.8 ± 0.32	control	**
Control: 0.9% NaCl	24	1	0	2	0	0.6 ± 0.30		**
	48	0	0	3	0	0.6 ± 0.30		**
Mitomycin C	24	31	24	9	0	12.8 ± 0.8	**	control
	48	10	13	0	0	5.0 ± 0.33	**	control

[a] Number of methaphases scored 500.
[b] Dose of 1 mg/kg.
[c] Dunnet control group comparison test with DMSO as a control group.
[d] Dunnet control group comparison test with the positive control as a control group.
(** $p < 0.01$; * $p < 0.05$; $p > 0.05$ – not significant).

Significant differences in the percentage of aberrant mitosis of the studied samples, the types of chromosome aberrations and time-dependant effects after treatment were observed. The dichloromethane extract showed the highest clastogenic effect. Relatively high percentage of aberrations was found also for the methanol extract, the sterol fraction and luteolin 7-*O*-glucoside. Both, the water extract and its low-molecular water fraction, produced low percentage of aberrations.

The scored chromosome aberrations were predominantly breaks and centromeric-centromeric fission (c/c) and more rarely fragments and telomeric-telomeric fission (t/t). For example, fraction B, the ethyl acetate and low molecular fractions, and rutin caused only breaks and c/c fission; the sterol fraction gave also fragments; the water extract caused breaks, c/c and t/t fissions.

Time-dependent frequencies of the aberrant metaphases were observed. The effect of the crude extracts and fractions (except fraction B) followed the relationship observed for MMC, lower values at 48 h *vs.* 24 h after dosing. Fraction B and the individual compounds caused increasing in the aberrant mitosis 48 h after administering. It could be presumed that the studied glycosides (α-bisabolol fucopyranoside, luteolin 7-*O*-glucoside and rutin) metabolize in the mouse organism to the corresponding aglycones or other metabolites with stronger clastogenic effects. For example, rutin did not damage the chromosomes after 24 h, but after 48 h caused a significant increase of aberrant mito-

sis (Table I, $p < 0.01$). Rutin metabolizes to quercetin, which is known to inhibit the growth of cells in various human cancers (Yoshida *et al.*, 1990; Ranelletti *et al.*, 1992; Scambia *et al.*, 1991) and has selective cytotoxicity in malignant cells (Jagadeeswaran *et al.*, 2000).

In conclusion, all tested samples showed lower percentage of aberrations *vs.* mitomycin C and in this sense are less harmful for mammalian cells. The water extract and the low-molecular water fraction did not show a statistically significant increase in aberrations, either at 24 h or 48 h, which suggests that the plant does not pose a great hazard when used as herbal medicine.

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