

ORIGINAL ARTICLE

WEIGHT AND CROWN-RUMP LENGTH REDUCTION, GROSS MALFORMATION AND PREGNANCY OUTCOME IN *CARTHAMUS TINCTORIUS* L-TREATED MICE

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Background – The flowers of the plant *Carthamus tinctorius* L are used to provide color and flavor in the food industry. They are cheaper than saffron and therefore a water extract of these flowers is used instead of saffron. *C. tinctorius* also initiates bleeding in delayed menstruation. Therefore, in early pregnancy when the menstrual cycle is delayed, the use of *C. tinctorius* flowers may lead to malformation of the embryo. This experimental study was designed to investigate probable malformations due to *C. tinctorius*.

Methods – One hundred and thirty Balb/C mice were maintained under standard laboratory conditions; then two females were housed with one male overnight, and successful mating was confirmed by vaginal plug. Pregnant females were divided into two groups. In the experimental group (120 mice), aqueous extract (1, 10, 25 or 50 mg/kg) was injected intraperitoneally, on the 7th or 8th day of pregnancy as a single dose or on the 9th and 10th days as multidoses. Distilled water was injected into the control group (10 mice). The pregnant mice delivered on the 18th day of pregnancy and the fetuses were examined for external malformation. Each fetus was weighed, the crown-rump length was measured, and the number of live and resorbed fetuses was recorded.

Results – Water extract of *C. tinctorius* flowers caused death and decreased crown-rump length and weight. Congenital malformations included exencephaly, spina bifida and tail and limb necrosis also were seen.

Conclusion – The water extract of *C. tinctorius* flowers has many toxic effects on early development of the embryo and its use in pregnant women must be avoided.

Keywords • *Carthamus tinctorius* • embryotoxicity • teratogenicity

Introduction

In many countries, the use of plants and natural products is popular as an alternative to classic medical care. Lack of information about the toxic effects of such plants allows them to be used for medical treatment. *Carthamus tinctorius* L, with the persian name of Golrang (safflower), belongs to the family *Compositae* and has been known for thousands of years. Many *Compositae* plants have a folk use as an abortion

promoter. It is native to the Middle-East and nowadays is also widely cultivated throughout Europe and the USA.¹ Its flowers are used not only in medicine, but also for flavor and color in food and as an additive in beverages.² The plant's water extract is used in dysmenorrheal conditions and as a sedative, laxative and antiinflammatory agent in traditional medicine.³ Safflower oil is a rich source of linoleic acid and unsaturated fatty acids, and can reduce serum cholesterol levels.¹ The mutagenic effect of *Carthami* flowers has been reported in the literature,^{4, 5} and sterols, and erythro-alkane-6,8-diols, which have anti-inflammatory and antitumor-promoting activity, are present in

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Carthamus extract.⁶ *C. tinctorius* extract in doses of 0.2 – 2 mg/kg/day during 0 – 8 days of gestation induces microscopic changes in the mouse embryo that leads to histological changes in the nervous system.⁷ Because the reported study was limited to a specific tissue, the present study investigated morphological malformations in the whole mice embryos. The study aimed to evaluate the relationship between different doses of *C. tinctorius* in mice on different days of the organogenetic period and to compare the effect of extract on critical days of organogenesis.

Materials and Methods

Plant materials

The flowers were obtained from herbal medicinal stores (Herbarium No. 93, Faculty of Pharmacy, Tehran University of Medical Sciences). The water extract was obtained using the Soxhlet procedure and dried on a rotatory evaporator. The extract was obtained with 39% yield.

Teratogenic effect of *C. tinctorius* L in pregnant mice

Young female Balb/C mice (130) were allowed to acclimatize to standard conditions (20 ± 4 °C and 12-hour light-dark cycle) for 30 days. Two females weighing 30 – 35 g were then placed with one male of the same strain. Pregnancy was confirmed by the presence of vaginal plugs. The

day of detection of a vaginal plug was designated Day 0. Pregnant mice were separated and placed in a different cage and were divided into control (10 mice) and experimental groups. The experimental group was further subdivided into groups of 10 based on the dose of water extract administered (1, 10, 25 or 50 mg/kg) and the day of exposure (7th, 8th, or 9th and 10th day). Each mouse in the experimental group received a single intraperitoneal (IP) dose on the 7th or 8th day or a single IP dose on the 9th and 10th days of pregnancy while the control mice received distilled water in the same manner. Pregnant females were sacrificed on Day 18 of pregnancy. Their abdominal cavities were opened and uterine horns were removed and placed in physiological saline. Embryos were rapidly dissected from the uterus and extraembryonic tissue was immersed in 10% formaldehyde solution. The number of resorbed, live and dead fetuses were recorded. The survivors were weighed and the crown-rump length was measured. Fetuses were evaluated in terms of gross malformations. For detailed studies, specimens were observed under a Zeiss stereomicroscope. Cleft palate effect was studied randomly in abnormal fetuses. Data were analyzed by one-way analysis of variance (ANOVA) and Fisher's test.

Results

The number of resorbed fetuses, fetal weight and crown-rump length are presented in Table 1.

Table 1. Effect of different doses of an aqueous extract of *Carthamus tinctorius* given by intraperitoneal injection during the organogenic period in pregnant mice on the number of resorbed fetuses and the weight and length of embryos.

Time of administration	Dose (mg/kg)	No. of fetuses	No. of resorbed fetuses	Weight (mean \pm SD; g)	Length (mean \pm SD; mm)
Control	0	82	4	1.36 \pm 0.20	21.76 \pm 1.56
Day 7	50	25	16	0.87 \pm 0.20*	18.11 \pm 1.81*
Day 7	25	22	8	1.01 \pm 0.14*	19.31 \pm 1.03*
Day 7	10	50	12	1.12 \pm 0.16*	20.20 \pm 1.45*
Day 7	1	38	3	1.23 \pm 0.13*	21.32 \pm 1.04
Day 8	50	23	12	0.80 \pm 0.18*	18.54 \pm 2.21*
Day 8	25	24	8	1.05 \pm 0.12*	19.96 \pm 0.97*
Day 8	10	43	10	1.11 \pm 0.19*	20.29 \pm 1.22*
Day 8	1	33	0	1.24 \pm 0.08*	21.51 \pm 0.75
Day 9 and 10	50	23	23	—	—
Day 9 and 10	25	23	15	0.90 \pm 0.09*	18.63 \pm 0.89*
Day 9 and 10	10	38	13	1.02 \pm 0.11*	19.27 \pm 1.18*
Day 9 and 10	1	41	0	1.24 \pm 0.14*	21.83 \pm 1.45

The results of ANOVA were as follow: $p < 0.05$, compared with control group; F-ratio = 25.34; DF = 340 (weight), F-ratio = 20.92; DF = 340 (length). *Significantly different from control group.

The most effective dose produced the most resorbed fetuses and greatest loss of weight and length, which were significantly different from those in the control group (ANOVA, $p < 0.05$). There was no significant length reduction at the minimum dose (ANOVA, $p < 0.05$). A dose of 50 mg/kg was toxic and caused 50 – 100% resorption of fetuses and 30 – 60% mortality. The incidence of external malformations increased significantly with increasing dose (Table 2) (Fisher's exact test, $p < 0.02$). Various external malformations, such as exencephaly, spina bifida and dermatological malformations, were observed in groups treated with doses of 50, 25 and 10 mg/kg on Days 7 and 8. The minimum dose (1 mg/kg) on Day 8 did not cause any external malformation, but on Days 7, 9 and 10, there were a few external malformations; feet and hand necrosis was seen in one case. There was evidence of hemorrhage on the skin of fetuses and also signs of echymosis, wrinkling and ichthyosis. The distal ends of limbs and the tails of embryos showed signs of ischemia and necrosis. Ocular defects, such as cataract and Peter's anomaly (keratolenticular adhesion), were seen in some embryos. The aqueous extract of *C. tinctorius* flowers given to pregnant mice throughout the organogenic period caused abortion or death of dams at higher doses. At doses of 10 and 1 mg/kg, there was no bleeding or death of pregnant mice (Table 3).

Discussion

Many *Compositae* plants have a folk use as an abortion promoter. The water extract of *C. tinctorius* is used not only in medicine but also as a food colorant and flavoring. In our study, the number of absorbed fetuses and the percentage of post-implantation loss were dose dependent. These results indicate that the doses used in our study have some lethal effects. They also show that the doses used in pregnancy are important for reproductive performance.

Our purpose was to determine whether there was a correlation between the use of *Carthamus* extract and the occurrence of abortion or malformation at the doses of flower extract used in folk medicine. Thus, the doses of flower extract used varied from a minimum dose to a maximum dose that could cause abortion or toxicity in dams. This corresponded to doses of 1 mg/kg and 50 mg/kg on different days of the organogenic period. In a previous study, Nobakht et al showed that at doses of 1.6 and 2 mg/kg/day (12.8 mg – 16 mg total), embryos were absorbed, while at lower doses (1.2 mg/kg/day, 9.6 mg total), changes in cellular orientation and degeneration were observed.⁷ In this study, we used a single IP dose of *C. tinctorius* flower extract to compare its effect on different days (7, 8, 9 and 10) of organogenesis. A dose of 50 mg/kg on Days 9 and 10 caused

Table 2. Incidence of external malformations in the fetuses of mice given different doses of aqueous extract of *Carthamus tinctorius* by intraperitoneal injection during the organogenic period.

Time of administration	Dose (mg/kg)	No. of fetuses	Surviving fetuses		Exencephaly [†]		Spina bifida [‡]		Dermatological manifestations [§]	
			No.	%	No.	%	No.	%	No.	%
Control	0	82	78	95.1	—	—	—	—	—	—
Day 7	50	25	9	36.0	7	77.9	8	88.9	4	44.5
Day 7	25	22	14	63.6	5	35.7	4	28.6	3	21.4
Day 7	10	50	38	76	6	15.8	5	13.2	5	13.6
Day 7	1	38	35	92.1	0	0	1	2.9	1	2.9
Day 8	50	23	11	47.8	4	36.4	6	54.6	4	36.4
Day 8	25	24	16	66.7	3	18.8	3	18.8	3	18.8
Day 8	10	43	33	76.7	3	9.1	4	12.1	4	12.1
Day 8	1	33	33	100	—	—	—	—	—	—
Day 9 & 10	50	23	0	0	—	—	—	—	—	—
Day 9 & 10	25	23	8	34.8	—	—	3	37.5	2	25
Day 9 & 10	10	38	25	65.8	—	—	3	12	4	16
Day 9 & 10	1	41	41	100	—	—	—	—	1	2.4

* Fisher's exact test; $p < 0.02$, compared with control group. All fetuses of dams that received 50 mg/kg on Days 9 and 10 were resorbed. [†] Exencephaly observed in all groups except those that received injections on Days 9 and 10 ($p < 0.02$); [‡] All groups significantly different from control group except those that received 1 mg/kg on Day 7 ($p < 0.02$); [§] All groups significantly different from control group except those that received 1 mg/kg ($p < 0.02$).

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Table 3. Effect of different doses of aqueous extract of *Carthamus tinctorius* given by intraperitoneal injection during the organogenic period on the number of deaths and vaginal bleeding in dams.

Time of administration	Dose (mg/kg)	No. of mice	Bleeding		Death of dams	
			No.	%	No.	%
Control	0	10	0	0	0	0
Day 7	50	10	7	70	3	30
Day 7	25	10	5	50	2	20
Day 7	10	5	0	0	0	0
Day 7	1	5	0	0	0	0
Day 8	50	10	6	60	4	40
Day 8	25	10	4	40	1	10
Day 8	10	5	0	0	0	0
Day 8	1	5	0	0	0	0
Day 9 and 10	50	10	7	70	6	60
Day 9 and 10	25	10	6	60	3	30
Day 9 and 10	10	5	0	0	0	0
Day 9 and 10	1	5	0	0	0	0

100% absorption of fetuses and was also toxic for dams. A dose of 10 mg/kg on all days of administration caused no bleeding in dams but did cause absorption, weight and length lose, and exencephaly, spina bifida and dermatological manifestations. Exencephaly was not observed after treatment on Days 9 and 10, possibly because the nervous tube is formed on Days 7 and 8 and is complete by Day 9. One case, which received 1 mg/kg on Day 8 had embryos with no tail, which confirms the mutagenic effects of *C. tinctorius* flower extract published by others.^{4, 5} Overall, our results show that *C. tinctorius* flower extract could cause serious teratogenic effects on mice embryos. As a result, women who are pregnant or who might become so should carefully control their intake of *Carthamus* flowers (Golrang, safflower), both as an alternative medicine and as a food additive.

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