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Safety Profile of Carthamus Tinctorius L. in Lactation: Brain, Renal and Hepatotoxicity

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ABSTRACT

Background and Objective: Safflower (*Carthamus tinctorius* L.) is used as dye and flavor in food industry. However, its effects on the infant during lactation has not been yet determined. The present study was conducted with the aim of investigating the possible effects of taking this herb during lactation on brain, liver, kidney and hematologic parameters of newborn mice.

Methodology: In this experimental study, 32 pregnant Balb/C mice were randomly divided into four groups of 8. Following the delivery, group 1 (control group) received normal saline injection, and group 2 to 4 received daily intraperitoneal injection of 10, 20 and 40mg/kg methanolic safflower extract for 25 days (until the end of lactation period), respectively. The newborns' hematological parameters were assessed at the end of the study period. Liver, kidney and brain tissue samples of male newborns were histopathologically studied after staining with Hematoxylin & Eosin. Data were analyzed using ANOVA and Scheffe's tests.

Results: Use of safflower did not cause any significant difference in the number of White Blood Cells, Red Blood Cells, Hemoglobin and Hematocrit in treatment group in comparison with the control group. In histopathological study, mild to severe injuries were observed in kidney, liver and brain tissues of newborn mice treated with the extract.

Conclusions: The results of the present study revealed that taking Safflower extract during lactation period may be toxic for infants and cause some damage in the liver, kidney and brain tissue. Therefore, it is better for lactating mothers to refrain from its use.

KEY WORDS: Histopathology study; Medical Plants; Safflower; *Carthamus tinctorius;* Teratogenicity.

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INTRODUCTION

Many mothers are required to use drugs during breast feeding. However, all drugs absorb into breast milk to some extent, exceptions are heparin and insulin which are too large to cross biological membranes. With rare exceptions, drug transfer from maternal plasma to milk is by passive diffusion across biological membranes. The transfer of components is greatest in the presence of low maternal plasma protein binding and high lipid solubility. Factors such as the dose received via breast milk, and the pharmacokinetics and effect of the drug in the infant need to be taken into consideration.[1]

Drug clearance in the infant is an important consideration, because they have a limited ability to clear drugs. Within a few days of delivery, term infants have glomerular filtration rates approximately one-third of adult values after adjusting for difference in body surface area, and premature infants have even more impaired clearance. Metabolic processes such as phase one oxidation and phase two glucuronidation are also impaired in neonates. Drugs subject to high first-pass metabolism may have higher oral availability in infants due to impaired ability to metabolize on first-pass.[2]

Medicinal plants, like industrial medications, may cause some irreversible tissue damages due to their unwanted side effects. Studying the toxic and side effects of medicinal plants by conducting experimental tests on laboratory animals would have effective role in identifying and diagnosis of harmful effects of these drugs in human.

On the other hand, identifying the injuries in different body tissues and organs following the use of medicinal plants will be an appropriate strategy to ensure the safety of taking these drugs.[3]

Studies show that women are considerably interested in using medicinal plants and take herbal medicines frequently for treating problems such as dysmenorrhea, menopausal symptoms, menstrual disorders, behavioral disorders, osteoporosis prevention and also pregnancy problems. Most pregnant women initiate self-treatment assuming that treatment with herbs and medicinal plants is not harmful and has no complications for mother and fetus.[4] Therefore, it is necessary to evaluate the effect of different doses of such medicines on different animal models to prevent its use in case of possible harmful effects on different tissues; otherwise, the background for human studies should be provided.[4]

Due to various therapeutic properties and abundant use in conventional medicine, *Carthamus tinctorius L.* (Safflower) flowers have had extensive use since long time ago as a medicinal herb. Besides its use in the food industry, this herb have been considered to relieve the sting pain, refine the lungs and clear the throat and also cure colic.[3,4] Safflower oil is used as a laxative, antiseptic and wound healer and also for reducing the cholesterol level, relieving intestinal cramps, relieving rheumatism, treatment of atherosclerosis, giving body strength and regulating menstrual periods.[4]

Based on empirical studies conducted on laboratory animals, it has been revealed that safflower can be a factor in changing male reproductive potential. Moreover, it may affect the testicular endocrine function.[5] Research has shown that safflower extract can reduce platelet aggregation induced by Adenosine Diphosphate (ADP) and blood coagulation in mice under laboratory condition.[6,7]

In spite of having antioxidant activity[8] safflower may lead to chromosomal aberrations in the bone marrow of mice. In addition, it increases the number of nucleated cells and polychromatic erythrocytes.[9]

The effect of safflower aquatic extract has been studied in neurological and ophthalmic disorders and complications such as defect in the formation of eyelids, cataract and lento-corneal adhesion in mouse embryos, that the ophthalmic and genetic abnormalities are attributed to neural crest impairment and also their effect on alpha-receptors which causes uterine arterial contraction.[10,11] Furthermore, it has been stated that the compounds present in the safflower flowers such as: 8-diols, erythro-alkane-6 and triterpene alcohol derivatives cause mutation through interfering with DNA and RNA activity.[10]

Given the above-mentioned issues, studying the unwanted and toxic effects of this plant as a herbal medicine is very important and the present study has addressed the pathological effect of different doses of safflower extract on liver, kidney, brain and hematologic parameters in the newborns of lactating mice exposed to safflower during the lactation period, in order to determine the side effects induced by this plant during the lactation period.[12-14]

METHODOLOGY

Preparing safflower extract: Safflower used in this study was obtained from the Research Farm of Khurasgan Islamic Azad University (Isfahan province). First, the dried flowers were pulverized using mechanical mill in Medical Plants Research Center of Shahrekord University of Medical Sciences. Then extraction was done with 70% methanolic solvent using maceration method. The extraction procedure was repeated three times and each time for 24 hours. The collected extracts were completely dried by a rotary evaporator under vacuum condition and temperatures below 45°C. Dried extracts were stored in the refrigerator till the time of use.[15]

Determining the acute toxicity of safflower extract: To determine the acute toxicity (LD_{50}), 21 Balb/C mice weighing 25-35g and aging approximately 12 weeks, were divided into three groups of 7 and

received 300, 200, 100mg/kg doses of safflower extract solved in 15ml/kg distilled water in the form of intraperitoneal injections. Then, the mice were observed for 8 hours in two-hour intervals and finally their behaviors, food intake, neurological symptoms, stool and urine output and death status were observed at the end of the 24th hour. The mortality rate after 24 hours was measured and LD_{50} was determined using Litchfield and Wilcoxon method by PCS software.

Keeping the animals & pregnancy: This part of experimental study was conducted on 32 Balb/C mice within the weight range of 25 to 35 grams and aged 12 weeks purchased from Pasteur Institute of Tehran. Mice were kept in laboratory animals in Shahrekord Islamic Azad University which met light, potable water, moisture, food and floor covering standards.[16]

Each two female mice were placed in a cage with a male mouse from 7 pm until 7 am of the following day for mating. After observing the vaginal plug and positive vaginal smear (for pregnancy diagnosis) pregnant female mice were randomly divided into three experimental and a control groups.

Test Design: After determining the acute toxic dose (LD_{50}), the treatment groups received 5, 10 and 20% of the LD_{50} from the delivery day until the end of lactation period and the control group received the same volume of distilled water in the form of intraperitoneal injection. A day after the last injection, cardiac blood sampling was performed in order to conduct hematological studies.

Hematological tests included the measurement of Hemoglobin (Hb) rate, Hematocrit (HCT%) percentage, the number of Red Blood Cells (RBCs), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and the number of White Blood Cells (WBCs). Hematocrit was measured using ultracentrifuge (Pars Azma, Iran) in Hematocrit tubes at 10000rpm.

The hemoglobin rate was also measured using cyanomethemoglobin method. WBC and RBC count was done by cell count device (Sysmex model kx21, made in Japan).[17] After blood sampling, the mice were simply killed by cervical dislocation and their liver, kidney and brain were completely removed and fixed in buffered formalin 10% and sent to the pathology laboratory after autopsy.

Sections of 5-micron diameter were prepared after tissue preparation stages and obtaining paraffin blocks and then were transferred to slides. Slides were stained by Hematoxylin & Eosin method and were mounted at the end.[18] After confirming the normal distribution of the data and since there were more than two groups and a single variable in each comparison, data analysis was carried out by one-way ANOVA and in case of significant difference between the experimental and control groups, Scheffe's test was used at a significance level of $p<0.05$.

RESULTS

Effect of extract on hematological parameters of newborn mice: The obtained results did not show any significant change in the amount of Hemoglobin, Hematocrit, RBCs and WBCs (parameter values remained almost unchanged) (Table-I).

Histopathological findings: Microscopic study of the liver, kidney and brain tissue sections of newborn mice in the control group did not show any structural and pathological change. Histopathology of liver in the experimental group with injection of 10mg/kg safflower extract revealed structural changes such as increased hematopoietic sites and dilated hepatic sinusoids.

Table-I: Effect of taking different doses of safflower extract during lactation period on hematological factors of newborn mice.

Groups *Variables*	*Control*	*10kg/mg*	*20kg/mg*	*40kg/mg*
Number of Red Blood Cells (RBCs) (Million/mm3)	6.55±1.424	7.56±0.929	8.07±0.838	7.18±1.163
Hemoglobin (Hb) (g/dl)	13.11±2.776	15.00±1.732	15.97±1.501	14.25±2.0354
Number of White Blood Cells (WBCs) (Million/mm3)	3.38±1.0399	4.76±0.419	3.54±1.238	3.91±1.137
Hematocrit (HCT) (%)	39.57±8.643	45.67±5.859	48.43±4.756	42.88±6.379
Mean Corpuscular Hemoglobin (MCH) (pm)	20.10±1.94	19.84±1.88	19.78±1.79	19.79±1.74
Mean Corpuscular Volume (MCV) (Femtolitre)	60±0.69	60.40±0.63	60.00±0.56	59.00±0.5554
Mean Corpuscular Hemoglobin Concentration (MCHC) (%)	33.10±0.32	32.84±0.29	32.97±0.31	33.23±0.31

The data are presented as mean±SD.
$p>0.05$ in comparison of all three concentrations with the control group for all variables.

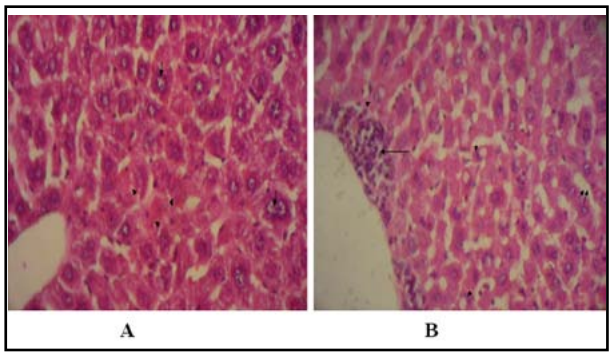

Fig.1: Histopathological study of the effects of safflower extract during lactation period on the liver tissue of newborn laboratory mice aged 25 days.
A: The experimental group treated with 20mg/kg safflower extract, arrowhead: degeneration of hepatocytes without inflammatory reaction (Hepatosis); Arrow: hepatocyte nucleus vesicularization, the group treated with 20mg/kg safflower extract, (H & E × 400).
B: The experimental group treated with 40mg/kg safflower extract (H & E × 100); Arrow: inflammatory cells infiltration around the centrilobular vein, arrowhead: placement of hepatocyte nuclei outside the hepatocyte center (fatty changes); Two arrows: sinusoidal dilation.

With the 20mg/kg dosage of the extract, mice showed pathological changes such as increased hematopoietic sites, hepatocyte nucleus vesicularization, inflammatory cell infiltration (lymphocytes, plasma cells and neutrophils) in the portal space, around the central vein and the dilation of hepatic sinusoidal space (Fig.1). Histopathological lesions of liver in the experimental group receiving 40mg/kg dose of the extract showed more injuries including the increased hematopoietic sites, hepatocyte nucleus vesicularization, inflammatory cell infiltration in the portal space and around the central vein, dilation of hepatic sinusoids and presence of lipid vacuoles within the hepatocytes (fatty change) In male newborns of the experimental group that received 10ml/kg safflower extract, the kidney structure was normal histopathologically and no specific lesion was observed.

Pathological studies on the kidney tissue of experimental group that received 20mg/kg safflower extract showed interstitial nephritis accompanied with mononuclear inflammatory cell infiltration (lymphocytes and plasma cells) around the renal tubules, especially around the renal arteries and the presence of eosinophilic protein-rich fluid within renal tubules (Fig.2). The kidney tissue of the experimental group that received 40mg/kg extract showed interstitial nephritis accompanied with mononuclear inflammatory cell infiltration around the renal tubules (esp. around the arteries), mesangial cell proliferation, and presence of protein-rich fluid within renal tubules and glomeruli disappearance in some areas of the cortical region (Fig.2).

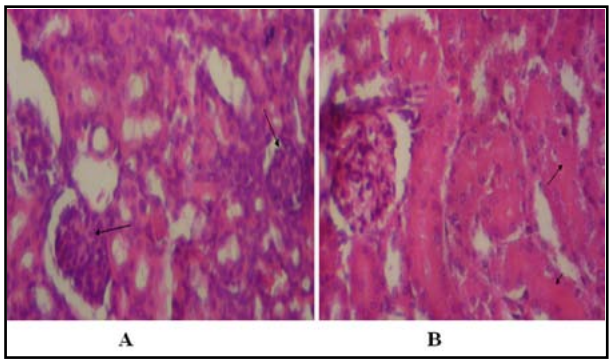

Fig.2: Histopathological study of the safflower extract effects during lactation period on the kidney tissue of newborn laboratory mice aged 25 days.
A: The experimental group treated with 20mg/kg safflower extract; Arrow: protein-rich fluid inside the renal tubules (H & E×400).
B: The experimental group treated with 40mg/kg safflower extract; Arrow: the hypercellularity of mesangial cells and increased glomerular basement membrane thickness (H & E×400).

Brain tissue in the experimental groups that received 10 and 20mg/kg safflower extract showed motor neuron degenerative changes in brain (strongly eosinophilic cytoplasm and compressed dark nucleus outside the light halo around neurons), amygdale region neural degeneration and degeneration of the pyramidal neurons of hippocampal CA1 region.

Histopathological study of the brain in the experimental group that received 40mg/kg safflower extract showed degenerative changes in the neurons of hippocampus DG area and

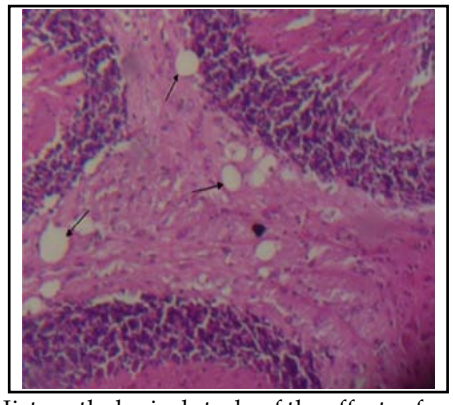

Fig.3: Histopathological study of the effects of safflower extract during lactation period on the cerebellum tissue of newborn laboratory mice aged 25 days; Arrow: spongiosis in the cerebellar white matter (H & E×100).

the pyramidal neurons of CA3 region and the spongiosis of cerebellar white matter and olfactory lobes in addition to the lesions observed in previous mentioned experimental groups (Fig.3).

DISCUSSION

Safflower extract did not cause any significant difference in the number of White Blood Cells, Red Blood Cells, Hemoglobin and Hematocrit of the treatment groups in comparison with the control group. A slight increase in RBCs and Hematocrit in newborns should be due to the mineral component available in Safflower pollens and Safflower yellow flowers.[19]

The histopathological results of this study showed that safflower extract has caused some histopathological changes in kidney, liver parenchyma, motor neurons of the brain, the limbic system and cerebellar white substance of the studied newborns at average doses (20mg/kg) and high doses (40mg/kg). It has also led to mild accumulation of lipid in hepatocytes especially in central lobular areas that may be due to defect in the Lysosomal Acid Lipase (LAL) and sensitivity of these cells to hypoxia.[20] Liver and kidney are responsible for the metabolism and excretion of safflower extract metabolites and the extract has high durability in tissues such as liver and other organs due to its lipophilic nature while entering milk.[20]

Floege et al. have shown that mesangial cell and glomerular extracellular matrix proliferation of the rats is influenced by Platelet Derived Growth Factor (PDGF) and Fibroblast Growth Factor (FGF) and this causes renal failure.[21]

It seems that the presence of myelin vacuoles in cerebellar white substance is due to the inhibitory effect of safflower extract on oligodendrocyte RNA and protein synthesis or by direct effect on the myelin sheath.[22]

The histopathological findings of liver, kidney and brain in the present study confirm the direct toxic effects of safflower extract on newborn mice that received it through milk. Moreover, previous studies show that consumption of safflower extract in small quantities during the gestation period and in high quantities during infancy has toxic effects depending on the extract concentration.[10,11,23-25] Zhifeng et al showed that in poisoning with safflower extract at the doses of 20, 60 and 180mg/kg for 90 days, only the dose of 180mg/kg caused kidney damages.[26]

Although various effects of this medicinal plant have been studied[27], no similar study on the effects of the given extract on body tissues was found for comparison with the present results. On the other hand, the mechanism of effects and the destructive impacts are still not well specified. Future studies are essential in order to study the changes in liver and kidney specific enzymes so that one may comment more decisively on the effects of this herb on the liver and kidney of infant mice.

According to the results of this study and other studies, consumption of this herb as additive or medicinal plant is not recommended during lactation and it should be taken with more caution.

CONCLUSION

The results of this study showed that consumption of safflower extract during lactation period is toxic and it is better to avoid the use of safflower during pregnancy and lactation period. Nevertheless, the effective ingredients in the extract and also molecular and cellular mechanisms of the toxic effects of safflower are not fully known and require extensive studies.

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Authors Contribution:

AN prepared the primary draft. AN, ATJ, HN and MRK wrote some parts of the manuscript. AN, MRK, HN, AB and ATJ edited the manuscript. MRK prepared the final manuscript.