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EVALUATION OF TOXICITY OF *CROCUS SATIVUS* (SAFFRON) DURING EMBRYOGENESIS IN WISTAR RATS

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ABSTRACT

Crocus sativus (saffron) is widely used as a food additive and as active components in many traditional medicine and modern pharmacology. Saffron is generally considered as a safe food additive and medicinal agent. However many studies carried out using pregnant mice have shown that saffron causes intrauterine growth retardation and induces embryonic malformations. Hence the present study was aimed to evaluate if a similar effect is seen in pregnant albino rats. In our experiment no deleterious effects were noted in the pregnant dams or on the fetuses up to the level of 500 mg/kg body weight dose.

KEYWORDS: **Saffron, Malformations, Fetus, Albino rats, Food additive**

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1. INTRODUCTION

Saffron, a spice is the dried and dark red stigma of *Crocus sativus Linn* flowers, a member of the large Iridaceae family. The value of saffron is determined by the existence of three main pharmacologically active components: Crocin and its derivatives which were responsible for colour; Picrocrocin responsible for the bitter taste and Safranal, a trepene essential oil obtained through hydrolysis of picrocrocin is responsible for the odour/aroma[14]. In modern pharmacological studies, saffron or its active constituents have demonstrated many therapeutic roles like: in treatment of cancer [1]; Anticonvulsant properties [8]; Anti-inflammatory and antinociceptive properties [6]; Radical scavenger effects [11];Hypolipemic effects[5]; Anti diabetic effects[3]; Anti-genotoxic effects[10]. In contrary to beneficial effect, information related to saffron toxicity is also reported.A high concentration of crocetin (a carotenoid which gives the characteristic golden yellow-orange colour to saffron) was found to be teratogenic in frogs, *Xenopus laevis*[12].Further it is demonstrated that aqueous extract of saffron significantly increased the miscarriage rate in BALB/c mice[19]. Also it is indicated that crocin and safranal, the two pharmacologically active components of saffron can induce embryonic malformations when administered in BALB/c pregnant mice[16].Oral administration of saffron at 2.5 and 100 mg/kg body weight caused intrauterine growth retardation and congenital malformations in mouse embryos[4].Hosseini-Biouki et al. showed that saffron can induce premature birth in Syrian mice as the result of increased motility of uterine smooth muscles especially in third week of pregnancy compared to controls[7].Although generally saffron was considered to be safe, the objective of the present investigation was to determine whether a similar effect is observed in albino rats (Wistar strain of rats used in this investigation) which is a common model used widely in evaluating embryo toxicity of any test chemicals/food additives.

2. MATERIALS AND METHODS

2.1. Saffron

Saffron (dried stigma of flower) was obtained from Indian Saffron Industry, Bagander, Pampore, Kashmir – 192121, India and was authenticated at Central Food Technological Research Institute, Mysore-570020, India as per International Organization for Standardization (ISO) 5453, Part II, 1996.The results indicated that the three main components present in the material on dry basis were: Picrocrocin – 72.7 %, Safranal – 51.6 % and colouring strength – 142.5 %. No added artificial colour was present in the material.

2.2. Animals and methodology

Wistar rats for the experiment were procured from the Department of Safety Assessment, Advinus Therapeutic Limited, Peenya Industrial Area, Bengaluru – 560058, India. Ten '0' day pregnant rats confirmed mated by vaginal smear examination with weight ranging from 188 to 219 grams and 11 to 12 weeks old were divided into 2 groups of 5 each. These rats were housed in standard laboratory condition of 12 – 15 filtered fresh air changes, temperature range of 20 to 24 °C, relative humidity of 30 to 70 % with 12 hours fluorescent light and 12 hours dark cycle and with free access to food and water. The experimental procedures were approved by the Institutional Animal Ethics Committee (Proposal No. 023, dated 21 March, 2012). The control group comprising of 5 presumed pregnant rats received the vehicle (Milli-Q water), while the remaining 5 rats received saffron suspended in Milli-Q water at the dose of 500 mg/kg/day. The test material/vehicle was administered orally by gavage within 15 to 30 minutes post preparation using disposable plastic syringe attached with a metal feeding/intubation cannula at the dose volume of 10 mL/kg body weight once daily from Day 5 of gestation (day of implantation) until Day 19 of gestation. Animals were weighed twice a week along with the measurement of food intake. All the pregnant female rats were observed daily throughout the experiment for mortality, morbidity, general appearance and behavior. All the presumed pregnant females were euthanized under isoflurane anesthesia on Day 20 of gestation, blood collected from abdominal aorta for clinical chemistry analysis using Roche/Hitachi 902 (Hitachi High-Technologies Corporation, Tokyo, Japan) Automatic Analyzer. Once the maternal viscera were examined macroscopically, the ovaries were removed and the corpora lutea count was take under an illuminated magnifying lens at a magnification of 5X. The gravid uterus was cut open along the ante-mesometrial side which exposed the amniotic sacs. The sacs were ruptured and the number and position of implantation, early or late resorptions and dead or live fetuses were recorded. The umbilical cord of each fetus was cut and fetuses removed in a sequential order as present in the uterus, blotted dry and placed in a tray. The fetuses were then sexed, individually weighed and the crown-rump length measured using a digital vernier caliper. External examination of fetuses for morphological abnormalities under an illuminated magnifying lens at a magnification of 5X/10X was made. All the live fetuses were euthanized under isoflurane anesthesia and 50 % of fetuses were transferred into 70 % ethyl alcohol for visceral/soft tissue evaluation under an illuminated magnifying lens at a magnification of 5X/10X[17] and the remaining 50% fetuses were skinned, eviscerated and processed and stained using alizarin red stain in order to evaluate for skeletal abnormalities[18]. The skeletal specimens were evaluated under a Stereoscopic Zoom microscope with typical magnification levels of 8X to 80X.

3. Statistical analysis

Comparisons were made between the saffron exposure group and the control using students 't' test for parameters related to maternal body weight, corrected maternal body weight, gravid uterine weight, food

consumption, number of corpora lutea, number of implantations, litter size, litter weight and length and fetus number. The incidences of pre and post implantation loss, number of early and late resorptions were analyzed using Kruskal Wallis test. The percentages of skeletal malformations and sex ratio were analyzed using 2X2 contingency table. A probability of 0.05 was accepted as statistically significant for all the applied tests.

4. Experimental Results

4.1. Mortality and clinical signs

In general, no mortality or clinical signs of toxicity were found in the rodent dams throughout the treatment period. In addition, no gross abnormalities were detected in the dams at caesarean section.

4.2. Maternal body weight during pregnancy

Maternal body weights were unaffected by the administration of saffron (Table 1).

Table 1
Maternal body weight on different days of gestation

Group No.	Dose (mg/kg/day)	No. of Dams	Group mean body weight (g) on gestation day							
			0	3	5	8	11	14	17	20
Vehicle Control	0	5	208.60 ±7.03	216.19 ± 9.32	224.70 ± 8.00	233.12 ± 7.49	241.90 ± 9.81	252.02 ± 9.01	270.51 ± 13.53	303.30 ± 19.54
Saffron	500	5	206.45 ± 12.16	216.76 ±15.53	222.57 ± 12.98	228.81 ± 14.70	242.94 ± 19.36	260.22 ± 19.91	276.34 ± 20.24	308.84 ± 25.80

Values: Mean ±SD

4.3. Maternal food intake during pregnancy

The food intake was also statistically similar when compared with the control group (Table 2).

Table 2
Maternal food intake on different days of gestation

Group No.	Dose (mg/kg/day)	No. of Dams	Food intake (g/rat/day) during gestation period						
			0-3	3-5	5-8	8-11	11-14	14-17	17-20
Vehicle Control	0	5	15.47 ±2.05	17.88 ±1.78	18.1 ±2.93	19.84 ±1.69	20.3 ±2.41	21.21 ±1.78	21.37 ±2.01
Saffron	500	5	13.92 ±0.51	19.02 ±1.09	18.77 ±1.61	19.50 ±2.76	21.48 ±2.02	21.71 ±1.79	21.97 ±2.78

Values: Mean ± SD

4.4. Maternal Parameters

The total number of corpora lutea, the total number of implantations and the percentage of pre and post implantation loss were statistically comparable with the control group (Table 3).

Table 3
Maternal parameters

End Point	Treatment	
	Control	500 mg/kg/day
No. of Dams	5	5
Gravid Uterine weight (g)	54.14±11.76	61.97±3.56
No. of Corpora Lutea[a]	11.80±1.10	13.40±1.67
No. of Implantations[a]	9.80±1.92	12.00±1.58
No. of resorptions[a]		
1. Early resorptions	0.40±0.55	0.20±0.45
2. Late resorptions	0	0
% Implantation Loss		
1. Early	4.10	1.67
2. Late	0	0
Pre-implantation Loss (%)	16.95	10.45
Post-implantation Loss (%)	4.08	1.67

[a:] Mean±SD

4.5. Litter parameters

The litter parameters at 500 mg/kg/day dose were comparable with the controls (Table 4).

Table 4
Litter parameters

End Point	Treatment	
	Control	500 mg/kg/day
No. of Litters	5	5
Total No. of Fetuses	47	59
Dead Fetuses	0	0
Mean Litter Size	9.4	11.8
Fetal Body weights (g)[a]		
1. Males	3.70±0.36	3.64±0.21
2. Females	3.30±0.14	3.56±0.25
Fetal length (mm)[a]		
1. Males	35.87±1.41	35.76±1.29
2. Females	34.25±0.86	35.16±1.61
Sex Ratio (Male : Female)	1:1.04	1:1.36

[a:] Mean±SD

4.6. Fetal morphological observations

External examination of fetuses did not reveal any morphological abnormalities at 500 mg/kg/day dose. The visceral/soft tissue evaluation also revealed no abnormalities in any of the organs. The skeletal evaluation of the fetuses which were stained with alizarin red stain also showed no major malformations (Table 5) except for some normal variations related to the ossification of some bone components like delayed/incomplete/poor ossification. In addition some minor anomalies like hypoplastic sternum, dumbbell thoracic vertebral centra/rudimentary/wavy ribs were seen which were comparable between the treated and control group.

Table 5
Fetal morphological observations

End Point	Treatment	
	Control	500 mg/kg/day
No. of Litters	5	5
Total No. of Fetuses	47	59
External Examination[a]	47 (5)	59 (5)
Abnormalities[b]	0 (0)	0 (0)
Soft tissue alterations[a]	23 (5)	29 (5)
Abnormalities[b]	0 (0)	0 (0)
Skeletal Examination[a]	24 (5)	30 (5)
Minor anomalies		
Hypoplastic Sternum No. 6[b]	02 (1)	1 (1)
Fetus (Litter) %	8.33 (6.67)	3.33 (16.67)
Wavy Rib No. 3[b]	01 (1)	0 (0)
Fetus (Litter) %	4.17 (6.67)	0 (0)
Rudimentary Rib No. 14[b]	12 (5)	14 (5)
Fetus (Litter) %	50 (47.33)	46.67 (46.76)
Dumb bell thoracic vertebralcentra 4/13[b]	0 (0)	02 (1)
Fetus (Litter) %	0 (0)	6.67 (28.57)
Major Malformations[b]	0 (0)	0 (0)

[a:] Number of fetuses (litters)
[b:] Total Number of fetuses (litters) exhibiting variations/malformations

4.7. Clinical chemistryinvestigation

The blood collected at sacrifice was centrifuged to obtain plasma. Plasma was used for the analysis of markers of normal liver function [Aspartate Amino Transferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP)] and markers of normal kidney function [Blood Urea Nitrogen (BUN) and Albumin (Alb)]. In addition glucose and total protein was also measured. All these parameters were within the normal biological variation at the treated dose level when compared to the control (Table 6).

Table 6
Biochemical investigation

Parameter	Treatment	
	Control	500 mg/kg/day
No. of GD 20 dams →	5	5
Glucose (mmol/L)	5.67±1.00	5.30±0.26
AST (U/L)	59.33±2.52	57.00±5.05
ALT (U/L)	63.67±13.65	61.60±8.02
ALP (U/L)	48.67±8.08	46.20±11.43
BUN (mmol/L)	4.70±1.46	4.29±0.49
Albumin (g/L)	38.90±3.56	38.08±3.28
Total Protein (g/L)	63.93±5.40	61.68±4.68
Values: Mean±SD		

5. DISCUSSION

Saffron is the dried and dark red stigma of *Crocus sativus Linn* flowers.The pharmacologically important active constituents of saffron comprises the volatile agents (safranal), bitter principles (picrocrocin) and the colour component (crocetin and its glycosidic, crocin). In modern pharmacological studies, saffron or its active constituents have demonstrated many therapeutic roles like: in treatment of cancer, anticonvulsant properties, anti-inflammatory and antinociceptive properties, radical scavenging effects, hypolipaemic properties,anti-diabetic effects, anti-genotoxic effects and many more. In contrary to beneficial effect, information related to saffron toxicity are also reported[7,16,19], Fatma Al-Qudsi and Amal Ayedh (2014)] in many embryo toxicity studies carried out in mice (BALB/c, Swiss White Rodeless mice, Syrian mice) with doses ranging from 2.5 mg/kg body weight to 600 mg/kg body weight. Also it is reported that high concentrations of crocetin, a carotenoid component giving a characteristic golden yellow orange colour to saffron was found to be teratogenic in frogs, *Xenopus laevis*[12]. Increased miscarriage rate in female farmers who worked in saffron fields have also been reported[2]. So the objective of the present investigation was to determine whether a similar effect as observed in mice is observed in albino rats (Wistar strain of rats used in this investigation) which is a common model used widely in evaluating embryo toxicity of any test chemicals/food additives. A dose of 500 mg/kg body weight was selected based on the reported toxicity in mice studies. Further as it is reported that saffron can stimulate uterine contractions in pregnant women leading to abortions, oral route was selected to administer the test material as it simulated the exposure pattern of the human population. Saffron was administered orally to pregnant rats during gestation days 5 through 19 which were in-lines with the regulatory toxicity guidelines related to embryo toxicity testing[9,13]. The control rats received Milli-Q water, which was used to suspend the saffron in this investigation. Treatment with saffron at 500 mg/kg/day did not elicit any adverse clinical signs, effects on gestation body weight or food intake. The maternal parameters comprising of gravid uterine weight, corpora lutea and implantation counts, early and late resorptions, pre and post implantation loss were all comparable to the control. The litter parameters comprising of the number of fetuses, weight and length of the fetuses were all comparable to the control. These findings were contrary to the observations reported in mice which states that there were significant decrease in length and weight (intrauterine growth retardation) of fetuses[16]. Fetal external and visceral examination revealed no signs of embryo toxicity at the dose of 500 mg/kg/day. Skeletal examination also revealed no major malformations except for some minor anomalies like hypoplastic sternum, dumbbell centra, rudimentary/wavy ribs which are commonly seen in a fetus from day 20 gestation dam. The observations in skeletal examination in rats were also contrary to the observations reported in mice which states that there were mandible and calvaria malformations[16]. Clinical Chemistry analysis carried out from the blood collected at caesarean section to detect any adverse biochemical effects indicative of abnormal liver and kidney functioning revealed that all the parameters were within the normal biological variation at the dose of 500 mg/kg/day dose when compared to the control.

6. CONCLUSION

This study indicated that saffron did not induce any maternal toxicity and embryo toxicity in Wistar rats when saffron was administered orally daily by gavage during gestation days 5 to 19 at the tested dose of 500 mg/kg/day. These observations were contrary to the observations reported in mice. Probably rats are more resistant than mice and a study with a higher dose needs to be carried out to know whether a similar toxicity as manifested in mice can be demonstrated.

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8. CONFLICT OF INTEREST STATEMENT

Authors declare that there are no conflicts of interest.

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