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RESEARCH ARTICLE

POSTNATAL DEVELOPMENTAL TOXICITY OF *CROCUS SATIVUS* (SAFFRON) IN WISTAR RATS

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ABSTRACT

The postnatal developmental toxicity of *Crocus sativus* (saffron) was evaluated in a mammalian species taking Wistar rat as the model. Saffron administered as an oral gavage from implantation (day 5 post coitus) through lactation up to lactation day (LD) 20 at the doses of 50, 250 and 1000 mg/kg/day did not elicit any effects on maternal/lactation body weight gains, food intake and fertility. The mean number of pups born and weight of male and female pups and pup's survivability were unaffected. The results obtained conclude that saffron did not induce any maternal toxicity or any toxicity on the developing fetus/pups including its survivability and hence saffron is considered to have no postnatal developmental toxicity potential.

INTRODUCTION

Crocus sativus is a herbaceous perennial cormous plant belonging to family Iridaceae and is commonly referred to as saffron. The flower styles basically form commercial saffron used by most people either for medicinal or culinary purposes. Saffron contains more than 150 volatile and aroma yielding compounds, among these the important pharmacologically active principles are – Crocin and its derivatives which are responsible for colour, Picrocrocin responsible for the bitter taste and Safranal responsible for the odour/aroma (Rio's *et al*. 1996). Saffron and its constituents were widely evaluated for their pharmacological activities and are found to have anti-inflammatory, anti-diabetic, anti-spasmodic, anti-seizure, hypolipaemic effects including anti-cancer effects (Abdullaev *et al*., 2004). It is noted a review of information on toxicology and safety of saffron is not consistent. Crocetin, a carotenoid isolated from saffron has been found to be a teratogen (Martin *et al*., 2002). At low doses, saffron causes the stimulation of the pregnant uterus and in larger amounts can cause contraction and spasm leading to abortion and possible toxic symptoms. As the toxicity information is not consistent, the present study was conducted to detect adverse effects of orally administered saffron on the pregnant/lactating Wistar rats and on development of the conceptus and the offspring following exposure of female rat from implantation through weaning.

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MATERIALS AND METHODS

Saffron

The material for the study, the saffron (dried stigma of flower) was obtained from Indian Saffron Industry, Bagander, Pampore, Kashmir – 192121, India. The material was authenticated at Central Food Technological Research Institute, Mysore-570020, India by spectrophotometric method as per International Organization for Standardization (ISO) method (5453, Part II, 1996). The results of the analysis indicated that the three main pharmacologically active 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.

Animals and methodology

Wistar rats, obtained from Department of Safety Assessment, Advinus Therapeutics Limited, Peenya Industrial Area, Bengaluru – 560058, India were used in the experiment. 24 presumed pregnant rats confirmed mated by vaginal smear examination with weight ranging from 185 to 238 grams and 11 to 12 weeks old were used. The rats were housed

individually in standard polysulfone rat cages in a barrier facility with standard laboratory condition of 12 – 15 filtered fresh air changes, temperature range of 20 to 23 °C, and 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 project was approved by the Institutional Animal Ethics Committee (Proposal No. 023, dated 21 March, 2012). The 24 presumed pregnant rats were randomly distributed to 4 groups of 6 animals each. The animals in Group I received only the vehicle (Milli-Q water) at 10 mL/kg body weight through oral gavage. The animals in Group II, Group III and Group IV received saffron suspended in Milli-Q water at the doses of 50 mg/kg/day, 250 mg/kg/day and 1000 mg/kg/day, respectively at 10 mL/kg body weight through oral gavage. The vehicle/test item was administered to the respective dose group rats from implantation (day 5 post coitus) through lactation up to lactation day (LD) 20. The rats were weighed on specified intervals of gestation and lactation and food intake was also measured at similar intervals as body weight. Daily records of activity with reference to appearance and behavior were maintained. All the presumed pregnant dams in each group littered expect for one dam at 250 mg/kg/day dose group which was found non-pregnant. At parturition, litter size (number of pups born), sex of pups, external deformities, weight of individual pup were recorded for each dam. On LD 4, standardization of the litter size to 8 pups was made and the pups were subsequently weighed on Days 7, 14 and 21 of lactation period till weaning sacrifice. Based on this data, pup survival index were calculated on Lactation Days 1, 4, 7, 14 and 21.The dams were euthanized under isoflurane anesthesia after completion of the 21 day post-partum period and at necropsy, the maternal viscera were examined macroscopically for gross lesions, liver and kidneys were collected in 10% buffered neutral formalin for microscopic evaluation. Further at necropsy, blood was also collected for biochemical investigation using Roche/Hitachi 902 (Hitachi High-Technologies Corporation, Tokyo, Japan) Automatic Analyzer. In addition, at necropsy, the ovaries were removed and placed in a pre-labeled container and the corpora lutea were counted under a dissecting microscope. Also the number and distribution of implantation sites were counted in the uterus. The Fertility index for dams was also calculated.

Statistical analysis

Comparisons were made between the saffron exposure groups and the control using Dunnett's method following one way analysis of variance (ANOVA) for parameters related to maternal gestation/lactation body weight, food consumption, number of corpora lutea, number of implantations, post implantation loss (%) and biochemical parameters. The pup's survival index was subjected to Z test for testing the differences in proportion. A probability of 0.05 was accepted as statistically significant for all the applied tests.

Experimental Results

Mortality and clinical signs

No mortality or clinical signs of toxicity were found in the rodent dams throughout gestation (Ramesh Edamula *et al*. 2014) and lactation period except for the slight yellowish coloured feces at the highest dose of 1000 mg/kg/day dose which is considered to be related to the colour of saffron which is administered and are non-adverse in nature. No gross abnormalities were detected in the dams at day 21 post-partum sacrifice.

Maternal body weights and food intake during pregnancy period

There were no overall statistically significant differences in dam's gestation body weights and gains in any of the dosage groups (Table 1). The food intake was also statistically similar in the three treated groups compared with the control group (Table 1).

Maternal body weights and food intake during lactation period

There were no overall statistically significant differences in dam's lactation body weights and gains in any of the dosage groups (Table 2). The food intake was also statistically similar in the three treated groups compared with the control group (Table 2).

Survival data of pups and fertility index

Saffron at the tested doses showed no adverse effects on fertility of dams and survival indices of pups (Table 3). Incidentally lower female fertility index was noted at 250 mg/kg dose as one dam in this group was found non-pregnant. However the mean number of corpora lutea (CL) and implantation (Imp) counts were comparable to the control group across the treated groups.

Body weight of pups during lactation period

No significant differences between treated groups were noted with respect to body weights monitored until post natal day 21 (Table 4). No gender differences were noted in the rate and pattern of body weight gain.

Biochemical investigation

Biochemical investigation revealed that the markers of normal liver function (AST, ALT, ALP, GGT and Total Bilirubin), markers of normal kidney function (BUN, Creatinine and Albumin), electrolyte levels (sodium, potassium, chloride, calcium) and general metabolism (glucose, total cholesterol and total plasma protein) were all within the normal biological variation at all the treated levels when compared to the control (Table 5).

Microscopic Observations

To determine the possible effects of maternal saffron exposure on the normal functioning of the main organ of metabolism, the liver and main organ involved in excretion, the kidneys revealed that saffron exposure did not cause any treatment related microscopic lesions in these organs. The main observation was increased cytoplasmic glycogen accumulation of minimal nature in the liver which is expected in animals which were in lactation stage, and minimal pelvis dilatation of kidneys which is a common observation in Wistar strain of rats (Table 6).

Table 1. Gestation period body weights and food intake

End Point		Treatment			
		Control	50 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. of dams pregnant		6	6	6	6
No. Littered		6	6	5	6
Maternal Body Weight Gains during Pregnancy (g)					
	GD 0 – 5	12.66±6.10	14.84±5.67	12.94±4.62	12.02±3.32
	GD 5 – 20	74.70±12.69	80.57±15.33	74.41±22.85	85.20±7.28
	GD 0 – 20	87.36±18.67	95.41±18.79	87.36±19.06	97.22±9.58
Food Intake(g/rat/day)					
	GD 0 – 5	16.59±3.73	19.32±2.02	18.84±1.90	17.95±1.16
	GD 5 – 20	21.32±3.63	21.60±3.21	22.34±1.71	21.24±1.68
	GD 0 – 20	20.14±3.65	21.03±2.87	21.46±1.65	20.42±1.50

GD: Gestation Days ; Values Mean ± SD

Table 2. Lactation period body weights and food intake

End Point		Treatment			
		Control	50 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. of dams pregnant		6	6	6	6
No. Littered		6	6	5	6
Maternal Body Weight Gains during lactation (g)					
	LD 1 – 4	6.00±10.82	9.41±6.78	12.91±5.64	12.11±9.76
	LD 4 – 7	3.85±3.56	9.21±8.03	12.20±7.89	7.94±5.99
	LD 7 – 11	2.09±5.18	3.42±8.77	2.65±6.82	0.10±4.15
	LD 11 – 14	2.75±7.69	6.13±8.41	5.03±7.77	4.11±8.73
	LD 14 – 18	1.36±6.68	-1.55±3.98	-0.07±5.53	5.10±6.68
	LD 18 – 21	1.11±5.12	-0.77±5.42	3.79±5.72	-1.91±6.90
	LD 1 – 21	17.16±16.86	25.85±11.02	36.50±12.08	27.45±13.46
Food Intake(g/rat/day)					
	LD 1 – 4	17.60±3.69	22.63±6.58	19.28±3.90	19.64±4.55
	LD 4 – 7	31.35±3.54	37.31±5.90	36.53±6.18	34.99±5.23
	LD 7 – 11	39.69±3.84	45.11±3.49	43.46±4.68	41.99±2.30
	LD 11 – 14	47.32±6.20	53.02±6.44	49.51±5.75	45.87±8.78
	LD 14 – 18	49.37±7.88	54.80±3.67	54.60±8.43	54.05±5.73
	LD 18 – 21	57.15±4.19	61.24±6.20	61.43±3.51	60.32±6.33
	LD 1 – 21	39.72±3.81	44.99±4.33	43.41±3.62	42.20±4.53

LD: Lactation Days ; Values Mean ± SD
Either F-test (ANOVA) or the Dunnett's t-test is not statistically significant

Table 3. Fertility and survival data of pups

Parameters	Treatment			
	Control	50 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. of dams pregnant	6	6	6	6
No. Littered	6	6	5	6
Mean gestation length (Mean±SD)	22.5±0.5 5	22.3±0.52	22.8±0.84	22.0±0.63
Mean No. of Corpora Lutea[a]	15.3	17.7	15.0	15.7
Mean No. of Implantations[b]	10.7	14.7	12.8	13.3
Female fertility index % [c]	100.0	100.0	83.3*	100.0
Post Implantation Loss %[d]	6.27	2.17	7.80	4.32
Total No. of pups born	60	86	58	76
Mean litter size[e]	10.0	14.3	11.6	12.7
Pup mortality (number)				
1.PND 0	0	0	0	0
2.PND 1 - 4	0	1	0	5
3.PND 5 – 7	0	0	0	0
4.PND 8 - 14	0	0	0	0
5.PND 15 - 21	0	0	1	0
Live birth index %[f]	100	100	100	100
No. of pups alive after standardization on PND 4	45	48	39	48
No. of pups alive on PND 21 survival index %	45	48	38	48
1.PND 4 [g]	100	98.8	100	93.4
2.PND 7[h]	100	100	100	100
3.PND 14[h]	100	100	100	100
4.PND 21[h]	100	100	97.4	100

*: Significantly different from control group, P≤0.05 PND: Post Natal Day
[a]: Total no. of CL/Total no. of pregnant animals; b: Total no. of Imp/Total no. of pregnant animals
[c]: No. of pregnant females (confirmed at necropsy)/No. of females used for mating X 100
[d]: No. of Imp – No. of live fetuses/No. of Imp X 100
e: Total no. of pups on day 1/No of females littered
[f]: No. of viable pups born (at first observation)/Total no. of pups born (at first observation) X 100
[g]: No. of viable pups on PND 4/No. of viable pups born X 100
[h]: No. of viable pups on respective PND/ No. of viable pups retained on PND 4 X 100

Table 4. Body weight of pups

Parameter		Treatment			
		Control	50 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
Mean weight of male pups (g)					
	PND 1	6.04±0.72	5.42±0.72	5.67±0.44	5.66±0.68
	PND 4	8.96±1.98	7.20±1.20	7.62±1.41	7.75±0.85
	PND 7	12.06±1.66	11.19±1.63	11.35±2.11	11.68±1.00
	PND 14	23.20±3.75	22.39±3.07	23.05±1.82	21.14±2.63
	PND 21	33.66±6.50	33.68±3.94	34.85±2.72	31.60±4.89
Mean weight of female pups (g)					
	PND 1	5.80±0.77	5.16±0.81	5.51±0.44	5.39±0.56
	PND 4	8.83±1.34	7.10±1.41	7.57±1.37	7.56±0.60
	PND 7	11.61±2.06	11.16±2.07	11.40±1.91	11.50±0.81
	PND 14	22.59±4.15	22.36±3.47	23.02±1.80	21.00±2.73
	PND 21	33.24±6.98	33.89±4.20	34.41±2.88	32.47±4.62

Values: Mean±SD PND: Post Natal Day
Either F-test (ANOVA) or the Dunnett's t-test is not statistically significant

Table 5. Biochemical investigation

	Treatment			
	Control	50 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. of Littered dams	6	6	5	6
Parameter				
Glucose (mmol/L)	7.40±0.43	7.43±0.20	8.04±1.12	7.62±0.87
Total Cholesterol (mmol/L)	2.53±0.40	2.11±0.31	2.78±0.35	2.88±0.61
Total Protein (g/L)	65.38±3.04	65.20±4.00	64.30±2.00	66.07±3.26
AST (U/L)	140.50±31.48	136.17±22.52	142.80±41.81	154.17±28.85
ALT (U/L)	151.50±28.98	144.67±22.46	137.80±19.49	131.50±18.62
ALP (U/L)	156.17±57.66	148.33±57.57	157.40±38.27	152.50±48.44
GGT (U/L)	00.00±00.00	00.00±00.00	1.20±2.68	00.00±00.00
Total Bilirubin (µmol/L)	4.57±0.28	4.35±0.64	4.23±0.54	4.33±0.33
BUN (mmol/L)	10.08±0.29	10.57±0.96	10.53±1.42	12.21±1.01*
Creatinine (µmol/L)	38.00±4.60	39.67±5.24	43.20±7.19	44.17±6.31
Albumin (g/L)	41.13±3.19	41.28±2.85	38.96±2.40	40.65±1.91
Calcium (mEq/L)	2.49±0.15	2.62±0.13	2.40±0.06	2.44±0.13
Sodium (mEq/L)	153.83±7.00	151.32±5.16	158.14±7.61	153.50±5.88
Potassium (mEq/L)	4.68±0.18	4.40±0.25	4.41±0.47	4.65±0.63
Chloride (mEq/L)	105.80±5.65	106.83±6.51	101.02±5.02	105.32±8.43
Values: Mean±SD	*: Significantly different from control group, P≤0.05			

Table 6. Microscopic observations

Tissue	Treatment			
	Control	50 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
No. of rats	6	6	5	6
Liver (Incidences)				
1. Increased cytoplasmic glycogen accumulation, minimal	1	3	1	3
2. Chronic Inflammatory Foci, minimal	0	0	1	1
Kidneys (Incidences)				
Pelvis dilatation, minimal	1	0	1	1

DISCUSSION

Commercially saffron constitutes the dried stigma of the flower. The pharmacologically important active constituents of saffron comprise the volatile agents (safranal), bitter principles (picrocrocin) and the colour component (crocetin and its glycoside, crocin). Saffron is widely consumed by pregnant women mainly in the belief to increase fairness in newborn. It has been reported that saffron can stimulate uterine contractions in pregnant women leading to abortions. 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*. As the toxicity information on toxicology and safety of saffron is not consistent, the objective of the present investigation was to detect adverse effects of orally administered saffron on the pregnant/lactating Wistar rats and on development of the conceptus and the offspring following exposure of female rat from implantation through weaning. Rat was selected as the model due its common and wide use in toxicity testing. The oral route was selected to administer the test material as it simulated the exposure pattern of the human population.

The highest dose selected for the study was 1000 mg/kg/day which was the maximum feasible dose and also referred to as the limit dose by regulatory toxicity guidelines related to reproduction toxicity testing [Guidelines ICH S5 (R2)]. 1/4ththe highest dose (250 mg/kg/day) was selected as the mid dose and 1/5th the mid dose (50 mg/kg/day) was selected as the low dose which closely relates to human dose. The control rats received the Milli-Q water, which was used to suspend the saffron at low, mid and high doses.

Treatment with saffron up to the highest dose of 1000 mg/kg/day did not elicit any adverse clinical signs, effects on gestation/lactation body weight or food intake. The mean number and weight of male and female pups measured at different intervals during lactation period, pup's survivability were not affected by saffron exposure. Biochemical investigations carried out from the blood collected from the lactating dams at weaning sacrifice on postpartum day 21to detect any adverse biochemical effects indicative of abnormal liver and kidney functioning, electrolyte imbalances or general metabolism revealed that all the parameters were within the normal biological variation up to the highest dose of 1000 mg/kg/day dose when compared to the control. Also there were no adverse microscopic lesions in the liver and kidneys which were contrary to the results by Daryoush Mohajeri *et al*., 2007 who have reported mild to severe hepatic and renal tissue injuries with associated biochemical changes.

Conclusion

The present findings indicated that exposure of saffron through gestation and lactation up to 1000 mg/kg/day did not pose any potential health risk to the developing rat off springs including survival and also did not induce any maternal toxicity.

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