

A two generation reproductive toxicity study with curcumin, turmeric yellow, in Wistar rats

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Abstract

The reproductive toxicity of curcumin, turmeric yellow, in Wistar rats was studied in order to generate additional relevant toxicity information for the use of curcumin in humans by oral administration. The two generation reproduction study was designed and conducted in accordance with OECD Guideline No. 416 [OECD, 1983. Guidelines for Testing of Chemicals, Guideline No. 416. Two Generation Reproduction Toxicity Study, adopted on 26th May 1983] and in compliance with Good Laboratory Practices (OECD, 1997 Principles of Good Laboratory Practice for the Testing of Chemicals. OECD, C(97)186/Final). The curcumin, mixed in the experimental diet at the concentrations of 1500, 3000 and 10,000 ppm was fed to three groups of rats, i.e., low, mid and high dose groups, and studied for two successive generations. A concurrent control group received experimental diet without the curcumin mixture. There were no treatment related adverse toxicological effects in the parental animals. No gross or microscopic changes were observed in any of the organs. None of the reproductive parameters were affected and there were no effects on the offspring other than a small reduction in pre-weaning body weight gain of the F2 pups at the highest dose level. It was concluded that the no observed adverse effect level (NOAEL) for reproductive toxicity of curcumin, fed in the diet for two successive generations to rats in this study was 10,000 ppm, which is equivalent to 847 and 959 mg/kg bodyweight (bw) per day for male rats and 1043 and 1076 for females for F0 and F1 generations, respectively. This study was the final toxicology study on curcumin reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the 61st Meeting, 2003. The JECFA group considered that the small body weight reduction in the F2 pups of the highest dose group prevented this from being regarded as a no adverse effect level, and so allocated an ADI for curcumin of 0–3 mg/kg bw based on the intake of 250–320 mg/kg bw in the mid-dose group as the NOEL.

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1. Introduction

Curcumin is the main colouring principle of turmeric a rhizome (*Curcuma longa*) used as a spice in India (Padmaja and Raju, 2004). Curcumin is generally found in turmeric at an average level of 3% (Jain et al., 1987). The potential

toxicity of extracts of turmeric have been tested in different studies and reviewed by the JECFA at various times (JECFA, 1995).

Fertility, reproduction and multigeneration studies in rats and mice using a number of different preparations of turmeric with curcumin contents varying from around 20% to 80% and using doses of curcumin up to 2000 mg/kg in rats and 8400 mg/kg in mice were studied. These studies have not shown any adverse effects on fertility or pregnancy, although effects on body weight gain were seen

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at the higher dose levels. Although no standard teratology studies have been performed, no malformations have been observed in the offspring in the reproduction and the multi-generation studies (Bhavanishankar and Murthy, 1987).

The objective of this two generation reproduction toxicity testing was to provide information on the effects of a purified curcumin preparation on male and female reproductive performance, including gonadal functions, oestrous cycles, mating, conception, parturition, lactation, weaning and on the growth and development of the offspring. It also provided information about potential developmental toxic effects of curcumin such as neonatal morbidity, mortality and structural and functional development. This study provides a rational basis for human health risk assessment.

2. Materials and methods

Curcumin, turmeric yellow (1,7 bis (4 hydroxy-3 methoxy phenyl) 1, 6 heptadiene 3, 5 dione) was manufactured and supplied by Spices Research Foundation, VI/277, Anavathil, Cochin 682002, Kerala, India. It was a free flowing yellowish orange coloured powder with a minimum purity of 95%. The curcumin powder was mixed with dry powdered diet without the use of any solvent or vehicles and the homogeneity and stability of the curcumin in the resulting diet mixtures were confirmed using HPLC with a UV detector.

Young adult Hsd Cpb:WU rats of Wistar strain, weighing between 111–140 g (males) and 101–130 g (females) at the start of the study, bred and maintained at Rallis Research Centre (Currently, Advinus Therapeutics Pvt. Ltd.), Bangalore were used for this experiment. The rats were maintained under standard laboratory conditions in an air conditioned room and housed in sterilized standard polypropylene rat cages, three in a cage, at temperature 22 ± 3 °C and relative humidity 30–70% with air changes of 12–15 cycles per hour. The animal diet, rat maintenance R-Z, supplied by Ssniff Spezialdiäten GmbH, Germany and the filtered water were given *ad libitum*. The animals were acclimatized for five days prior to experimental use.

Four groups of 30 rats per sex per group were administered diets with curcumin at concentrations of 0, 1500, 3000 or 10,000 ppm. The dose levels were selected based on a 28-day oral toxicity study on curcumin conducted at the test facility, in which increased liver weight and decreased body weight were observed at curcumin concentrations of 10,000 and 15,000 ppm. In the two generation study, the male and female rats were treated for 70 days which covered at least one spermatogenic cycle in males and several oestrous cycles in females prior to the first mating, and was continued in the males until sacrifice, and in the females until weaning of the offspring. For mating, female rats were placed with a single male overnight from the same group in a 1:1 ratio and the following morning the vaginal smears were examined for the presence of sperm. The presence of spermatozoa in the vaginal smear was considered as presumed day zero of pregnancy. Each female pregnant rat was caged separately. To avoid sibling mating paired males and females were chosen from different litters.

Body weight and food consumption of males and females were measured weekly and more frequently during gestation and lactation. Clinical signs were observed daily. Treatment was continued throughout the period of gestation and lactation until weaning. On day one postpartum (pp), the pups were examined for litter size, sex, litter weight by sex, viability and gross malformations. On day 4 pp, litters were culled to 8 pups with equal numbers of each sex where possible. Pups were weighed on days 1, 4, 7, 14 and 21 pp. At weaning on day 21 pp, one male and one female pup per litter were selected to continue as the parents of the F1 generation. F1 generation animals were treated for 70 days prior to mating, and the treatment was continued throughout the F1 generation until the weaning of the F2 litters following the same procedures as used for the first generation. Various reproductive measures (i.e., fertility indices, litter sizes, postimplantation loss, and survival indices) were calculated. The data of bodyweight, bodyweight gains, food consumption, mean number of

implantations and mean litter size were compared by Bartlett's test for homogeneity of intra group variances. When the variances were heterogeneous, the data was transformed using appropriate transformation. The data with homogeneous intra group variances were subjected to one-way analysis of variance (ANOVA—Snedecor and Cochran, 1987). Following ANOVA, when 'F' was found significant, Dunnett's pairwise comparison (Scheffé, 1953) of means of treated groups with the control group mean was done individually.

Results were statistically analysed by Dunnett's 't' test and Z-test. All parental animals (F0 and F1) and weanlings not selected from the F1 generation, and all F2 pups were subjected to complete necropsy at terminal sacrifice. Parental males were killed at around 26 weeks of age. Females failing to produce a viable litter by day 25 of gestation were terminated and examined for the number of corpora lutea, implantations and resorptions. The primary and secondary sex organs, liver, kidney, pituitary and adrenal glands were removed at necropsy from all parental animals, male and female.

The tissues processed by routine paraffin embedding and 5 μ sections were stained with hematoxylin and eosin (H and E) and subjected to histopathological examination.

3. Results

3.1. Clinical signs, mortality and weight gain

There were no treatment related clinical signs of toxicity, ophthalmological changes or mortality during the study. During the pre-mating period, there were no treatment related effects in group mean body weights and net body weight gains (Table 1) or food consumption (data not presented) between treated and control animals of either generation. There were some isolated, statistically significant decreases in mean pre-mating body weights of female rats in the F0 generation and increase in mean pre-mating body weights and net body weight gains of male rats in the F1 generation observed at the 3000 ppm dose level.

Similarly, there were no differences in gestational or postpartum body weights (Table 2) or food consumption (data not presented) in either generation. In the F0 generation, maternal body weight gain during the lactation period was significantly higher in the 3000 and 10,000 ppm dose groups when compared with controls.

3.2. Curcumin intakes

Curcumin intake values were calculated from mean food intake measures. Mean intake levels for the F0 males during the 70 days pre-mating period were 0, 126.4, 253.9 and 847.4 mg/kg bw per day, and for the F1 males were 0, 144.0, 289.9 and 958.5 mg/kg bw per day, for the control, low, mid and high dose groups respectively. The mean curcumin intake levels for the females, calculated from the mean food intake during different phases for F0 generation were: Pre-mating – 0, 138.20, 275.7 and 909.9, Gestation – 0, 109.0, 221.1 and 724.0, Lactation – 0, 282.4, 565.1 and 1913.2 mg/kg bw per day and for the F1 generation were: Pre-mating – 0, 154.1, 308.1 and 1017.1, Gestation – 0, 109.3, 212.3 and 743.1, Lactation – 0, 267.2, 518.6 and 1717.9 mg/kg bw per day for the control and treated groups, respectively.

Table 1
Body weight gain (g) in male and female rats treated orally with curcumin prior to mating

Doses (ppm)		F0 Generation						F1 Generation					
		Males			Females			Males			Females		
		\$	W10	Net weight	\$	W10	Net weight	\$	W12	Net weight	\$	W12	Net weight
0	Mean	132	381	250	112	220	250	58	381	323	57	227	170
	SD	8.1	25.7	24.9	5.7	13.3	15.4	7.1	25.8	24.8	6.6	11.4	13.4
	N	30	30	30	30	30	30	30	30	30	30	30	30
1500	Mean	132	388	256	113	213	247	54	389	335	52	229	176
	SD	7.1	25.8	27.2	6.1	10.6	12.4	6.9	30.4	28.5	6.6	13.1	13.0
	N	30	30	30	30	30	30	30	30	30	30	30	30
3000	Mean	132	389	257	112	211 ^a	246	58	405 ^a	347 ^a	55	226	171
	SD	8.0	27.0	25.6	6.3	14.8	16.0	8.6	34.6	32.1	8.6	19.2	14.7
	N	30	30	30	30	30	30	30	30	30	30	30	30
10,000	Mean	134	389	255	113	218	254	58	393	335	55	229	174
	SD	6.6	30.4	29.0	6.4	16.6	19.4	6.7	34.6	34.3	5.6	16.8	17.4
	N	30	30	30	30	30	30	30	30	30	30	30	30

W: week.

\$: initial body weight.

Net weight: net body weight gain.

N: No. of observations.

^a Significant at $P \leq 0.05$ level.

The whole pre-mating, gestation and lactation phases for the F0 generation were 0, 156.8, 313.9 and 1043.2 mg/kg bw per day, and for the F1 generation were 0, 163.5, 322.7 and 1076.3 mg/kg bw per day, for the control and treated groups, respectively.

3.3. Reproductive parameters

3.3.1. Survival data

Among the offspring of both generations, there were no differences in mean litter size and mean viable litter size at birth, live birth index, survival indices on days 4, 7, 14 and 21 (Tables 3 and 4). There was a small but significant increase in pup weight observed with the 3000 ppm dose on postpartum day 1 and at the 10,000 ppm dose on postpartum days 1, 4 and 7 in F1 generation (Table 3). Reduction in pup weight was observed at the 3000 ppm dose on postpartum day 7 and with the 10,000 ppm dose on postpartum days 7, 14 and 21 in the F2 generation. The live birth index was significantly higher in the 3000 and 10,000 ppm dose levels in the F1 generation and lower in the 3000 ppm dose level in the F2 generation, but these differences were small and not of any biological significance.

3.3.2. Fertility data

No statistically significant differences were observed between control and treated parental animals of both generations for male and female fertility indices, fecundity index, parturition percentage, post implantation loss and percentage of live pups born (Table 5). Mean numbers of implantations were lower in all the treated groups than control in the F0 generation, but this was not dose related, and all the data were within the historical control range.

3.4. Pathological investigations

No treatment related gross or histopathological changes were observed in any of the animals.

4. Discussion

In previous studies in which rats were fed turmeric (500 mg/kg bw) and an alcohol extract of turmeric (60 mg/kg bw) for three generations, no adverse effects on reproductive capacity were observed (Bhavanishankar and Murthy, 1987). In the present study with curcumin, the main colouring principle of turmeric, the physical appearance, behaviour and reproductive performance of rats in the treated groups of both generations were normal. These observations are in accordance with earlier findings (Bhavanishankar and Murthy, 1987).

In the current study, curcumin had no adverse effect on group mean body weights and net body weight gains or food consumption during the pre-mating phase, gestation or postpartum in either generation. Decreased mean body weights of female rats in the F0 generation and increased mean body weights and net body weight gains of male rats in the F1 generation at the 3000 ppm dose level were not considered to be treatment related as there was a lack of dose response relationship. Similarly, statistically significant increased bodyweight gains during the lactation period in the F0 generation at the 3000 and 10,000 ppm dose levels are not regarded as of biological importance.

Curcumin administration has not shown any effects on male or female mating performance, fertility, postimplantation loss, parturition, mean litter size and mean viable litter size at birth, survival indices on postpartum days 4, 7, 14 and 21, or on histopathological changes in either

Table 2
Maternal gestation and lactation body weights (g)

Days of pregnancy and lactation period	F0 Generation				F1 Generation			
	0	1500	3000	10,000	0	1500	3000	10,000
0P								
Mean	224	218	218	223	237	235	236	233
SD	14.4	12.4	18.3	19.2	12.0	14.3	12.0	17.9
N	28	25	24	29	29	29	30	28
5P								
Mean	237	229	230	237	251	251	251	249
SD	12.4	12.2	19.3	20.0	12.2	14.7	21.3	18.5
N	28	25	24	29	29	29	30	28
10P								
Mean	248	246	241	249	263	262	263	260
SD	16.3	12.7	16.4	22.4	12.3	15.8	21.4	19.4
N	28	25	24	29	29	29	30	28
15P								
Mean	268	257	258	264	279	280	279	277
SD	16.8	12.3	16.3	24.4	15.2	16.9	21.1	22.7
N	28	25	24	29	29	29	30	28
20P								
Mean	310	306	307	313	329	332	332	329
SD	23.9	15.9	17.4	28.3	18.9	23.8	22.4	25.5
N	28	25	24	29	29	29	30	28
Weight gain 0–20P								
Mean	93.5	88.2	89.3	89.7	92.2	96.7	96.3	95.7
SD	15.7	9.2	14.5	14.2	12.3	13.6	15.1	13.4
N	28	25	24	29	29	29	30	28
1L								
Mean	255	247	243	248	265	262	269	262
SD	19.4	17.0	16.1	22.4	13.0	16.1	23.8	23.5
N	28	25	24	29	29	29	30	28
4L								
Mean	255	248	252	256	274	265	267	267
SD	18.6	15.1	14.6	21.9	13.1	16.2	22.9	20.8
N	28	25	24	29	29	29	30	28
7L								
Mean	270	262	260	267	281	273	276	275
SD	16.9	15.9	14.1	22.7	12.2	16.4	22.1	20.5
N	28	25	24	29	29	29	30	28
14L								
Mean	284	276	277	284	288	284	293	281
SD	17.8	14.2	15.4	24.6	13.4	14.1	21.4	21.7
N	28	25	24	29	29	29	30	28
21L								
Mean	290	283	289	293	296	296	296	295
SD	17.1	18.6	16.7	23.9	13.7	19.5	23.0	22.5
N	28	25	24	29	29	29	30	28
Weight gain 1L–21L								
Mean	34.3	36.1	46.0 ^a	45.2	31.1	27.8	27.5	32.6
SD	16.3	15.9	9.0	11.9	12.0	15.8	12.1	12.5
N	28	25	24	29	29	29	30	28

N: No. of observations.

P: pregnancy.

L: lactation.

Doses (ppm): 0, 1500, 3000, 10,000.

^a Significant at $P \leq 0.05$ level.

Table 3
Body weight (g) of pups and litter size during lactation period

	F0 → F1 Generation				F1 → F2 Generation			
	0	1500	3000	10,000	0	1500	3000	10,000
<i>Day 1</i>								
Body weight				<i>d</i>				
Mean	6.1	6.4	6.6 ^a	6.7 ^a	6.9	6.6	6.5 ^a	6.6
SD	0.4	0.5	0.5	0.5	0.4	0.7	0.6	0.6
Litter size								
Mean	10.6	10.2	9.7	9.8	9.5	10.9	9.9	10.1
SD	2.6	1.5	2.4	2.4	2.5	2.1	2.2	1.9
<i>N</i>	28	25	24	29	29	29	30	28
<i>Day 4</i>								
Body weight								
Mean	9.1	9.3	9.7	10.0 ^a	10.4	9.9	9.7	9.9
SD	0.9	0.9	1.2	1.0	0.8	1.2	1.1	1.1
Litter size								
Mean	7.6	8	7.6	7.8	7.5	8.0	7.7	7.9
SD	0.9	0.2	1.0	0.5	1.4	0.2	0.8	0.6
<i>N</i>	28	25	24	29	29	29	30	28
<i>Day 7</i>								
Body weight								
Mean	14.1	14.2	14.9	15.1 ^a	16.6	15.3	15.0 ^a	15.0 ^a
SD	1.2	1.1	1.6	1.1	1.1	1.6	1.6	1.2
Litter size								
Mean	7.6	8.0	7.5	7.8	7.5	8.0	7.7	7.9
SD	0.9	0.2	1.0	0.5	1.4	0.2	0.8	0.6
<i>N</i>	28	25	24	29	29	29	30	28
<i>Day 14</i>								
Body weight								
Mean	28.5	27.8	28.5	28.7	29.5	29.4	28.8	28.0 ^a
SD	2.3	1.5	2.3	2.0	1.9	2.0	2.7	2.0
Litter size								
Mean	7.6	7.9	7.5	7.8	7.5	7.9	7.6	7.8
SD	0.9	0.4	1.0	0.5	1.4	0.4	0.9	0.7
<i>N</i>	28	25	24	29	29	29	30	28
<i>Day 21</i>								
Body weight								
Mean	43.1	41.5	43.9	44.2	45.4	44.3	44.1	42.5 ^a
SD	4.2	2.9	3.2	2.8	3.2	3.3	4.6	3.1
Litter size								
Mean	7.6	7.9	7.5	7.8	7.4	7.9	7.6	7.8
SD	0.9	0.4	1.0	0.5	1.4	0.4	0.9	0.7
<i>N</i>	28	25	24	29	29	29	30	28

d: Significant dose correlation.

N: No. of observations.

Doses (ppm): 0, 1500, 3000, 10,000.

^a Significant at $P \leq 0.05$ level.

generation. These findings are in accordance with earlier findings (Bhavanishankar and Murthy, 1987).

The small increase in pup bodyweight compared with the control group in the F1 treated litters and the decrease in

pup bodyweight in the F2 litters were marginal and not likely to be of biological importance. The changes in the live birth index are also of no biological importance. The statistically significant lower mean number of implantations

Table 4
Survival indices

Parameters	F0 → F1				F1 → F2			
	0	1500	3000	10,000	0	1500	3000	10,000
Mean litter size	11.0	10.3	9.8	9.9	9.6	11.0	10.3	10.3
Mean viable litter size	10.6	10.2	9.7	9.8	9.5	10.9	9.9	10.1
24 hour survival index (%)	100	100	100	100	100	100	100	100
Day 4 survival index (%)	99.3	99.6	99.6	100	100	99.4	99	98.9
Day 7 survival index (%)	100	100	99.5	99.6	100	100	100	100
Day 14 survival index (%)	100	99.5	99.5	99.6	99.5	99.6	99.6	99.1
Day 21 survival index (%)	99.5	99.5	98.4	99.6	98.6	98.7	99.1	98.6
Live birth index (%)	97.1	99.2	99.6 ^a	99.3 ^a	99.3	99.1	96.4 ^a	98.3

Doses (ppm): 0, 1500, 3000, 10,000.

^a Significant at $P \leq 0.05$ level.Table 5
Fertility indices

Parameters	F0 Generation				F1 Generation			
	0	1500	3000	10,000	0	1500	3000	10,000
Male fertility index (%)	93.3	100	96.7	100	93.3	90	96.7	96.7
Female fertility index (%)	100	100	100	100	100	100	100	100
Fecundity index (%)	93.3	83.3	83.3	96.7	96.7	96.7	100	93.3
Mean no. of implantation	12.0	10.8 ^a	9.7 ^a	10.6 ^a	10.6	11.7	10.8	10.8
Parturition (%)	100	100	96	100	100	100	100	100
Post implantation loss (%)	8.4	4.4	6.0	7.1	10.1	6.5	7.7	6.0
Percentage of live pups born	89.0	94.8	93.6	92.2	89.9	93.5	92.3	94.0

Doses (ppm): 0, 1500, 3000, 10,000.

^a Significant at $P \leq 0.05$ level.

in all the treated groups in the F0 generation is not considered to be of biological importance as the mean litter size was unaffected and a dose relationship was not observed.

It was concluded that in this study, there were no adverse toxicological effects on the reproductive capacity of rats that received dietary concentrations of curcumin up to 10,000 ppm for two successive generations. The no observed adverse effect level for reproductive parameters measured in Wistar rats for curcumin of 10,000 ppm in the diet is equivalent to 847.4 and 958.5 mg/kg bw per day for male rats and 1043.4 and 1076.3 mg/kg bw per day for female rats for the F0 and F1 generations, respectively. The original report of this study was reviewed by the JECFA at its 61st meeting in Rome, 2003 and together with the other toxicological studies previously submitted led to the allocation of an ADI for curcumin of 0–3 mg/kg bw. This was based on the conclusion by the JECFA that the small pre-weaning decrease in body weight gain in the F2 pups at the highest dose level prevented this dose from being regarded as a NOEL. They therefore concluded that the mid-dose level should be regarded as the no effect level with an intake of 250–320 mg/kg bw. Application of the standard safety factor of 100 was used to define the ADI of 0–3 mg/kg bw (JECFA, 2004).

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