LACK OF CARCINOGENICITY OF COMMERCIAL SAFFLOWER YELLOW IN FISCHER 344 RATS

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Abstract: The carcinogenicity of commercial safflower yellow was examined in Fischer 344 rats of both sexes. Commercial safflower yellow was mixed in basal diet at levels of 0, 2.5 or 5.0%, and groups of 50 male and 50 female rats were administered each of these diets *ad libitum* for 108 wk. At final sacrifice no statistically significant differences between treated and control rats regarding mean body weight, or mortality, which was very low, were observed in either male or female. Many tumors developed in all groups including the controls and the organ distribution of neoplasms and their histological characteristics did not differ significantly from those reported to occur spontaneously in this strain of rats. With the exception of C-cell adenomas of the thyroid gland which were increased in males receiving the 5.0% dose, no significant compound-related increases in tumor incidence over the corresponding control group were observed. Furthermore, such C-cell adenomas of the thyroid gland are frequent spontaneous tumors in F344 rats and the observed level was within the reported treatment-independent range. Moreover, no equivalent trend could be distinguished in the females. From these findings, it is concluded that commercial safflower yellow does not induce tumors in F344 rats, when administered orally at doses of up to 5.0% mixed in basal diet for as long as 108 wk. (J Toxicol Pathol 1: 149~155, 1988)

Key words: Safflower yellow, Carcinogenicity, Fischer 344 rats

Introduction

Safflower yellow, the yellow coloring matter extracted from the flowers of *Carthamus tinctorius* L.¹, is used as a coloring agent in various food stuffs. For example, selected candies, wines, soft drinks and jellies contain 0.01-0.02% safflower yellow². The main pigment components are safflomin-A and safflomin-B as demonstrated by repeated column chromatography, and the chemical structures of these two have been reported³. Safflower yellow A and safflower yellow B are further pigment components whose structures have

been analysed⁴ and the proposed chemical structures for safflomin-A, safflomin-B, safflower yellow A and safflower yellow B are illustrated in Fig. 1⁵. It has been suggested that safflomin-A is the most important of the main components of safflower yellow⁵.

Acute toxicity studies of safflower yellow in mice have revealed an LD50 of over 20 g/kg by oral administration², so acute toxicity is very low. However, the effects of prolonged exposure of rats to safflower yellow have not been examined, and the present report concerns the results of a long term carcinogenicity and toxicity study of safflower yellow administered orally to F344 rats.

Materials and methods

Chemicals

Commercial safflower yellow (LOT. 590110) purchased from Sanei Chemical Co., Tokyo,

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Fig. 1. Proposed structures of compounds isolated from safflower yellow

Japan, was used in the present experiments. It was mixed in the basal diet, (Clea CE-2, Tokyo, Japan), at concentrations of 0 (Control), 2.5%, and 5.0%. Basal diet containing 2.5% and 5.0% of commercial safflower yellow contained 0.021% and 0.042% of safflorin-A⁵, respectively.

Animals

Totals of 150 male and 150 female Fischer 344 (F344) rats aged 5 wk were purchased from Charles River Co. Japan Inc., Atsugi, and maintained on basal diet and tap-water until they were 6 wk old when the study was started. They were housed in wire cages (three males or three females/cage) and kept in an air-conditioned room at $23 \pm 2^\circ\text{C}$ with a relative humidity of 40%.

Experimental design

The rats were randomly divided into experimental groups, each consisting of 50 males and 50 females. Since safflower yellow in the basal diet proved very stable, the test diet only was renewed once weekly. Dose levels for this long-term carcinogenicity and toxicity test were chosen on the basis of preliminary subchronic (12-wk) test results regarding six dose levels in the 0–10.0% range. Groups of 50 males and 50 females F344 rats, were administered safflower yellow for 108 wk at concentration of 0 (Control), 2.5, and 5.0% in

the basal diet *ad libitum*. Free access to tap water was maintained throughout. The animals were observed daily and clinical signs and deaths were recorded. Body weights and diet consumption were measured once a week for the first 13 wk of the study and thereafter once every 4 wk. Blood samples were taken from all surviving rats of both sexes from each dosage group at wk 108 for biochemical studies and haematology. Animals which died spontaneously or were killed when becoming moribund during the study or sacrificed at the end of the study were autopsied completely and examined macro- and microscopically for the development of neoplastic and non-neoplastic lesions. The autopsy included examination of the brain, spinal cord, salivary, pituitary and thyroid (including parathyroid) glands, thymus, lungs (including the trachea), heart, oral cavity and tongue, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, liver, pancreas, spleen, adrenal glands, lymph nodes, skin, subcutis, preputial/clitoral gland, ear duct, eyes and Harderian glands, musculature, sternum, femur, and nasal cavity. All organs, tissues, and lesions were fixed with 10% buffered formalin, processed for wax embedding and sectioned. The sections were routinely stained with haematoxylin and eosin,

other specific stains being used when necessary.

The data were evaluated by the Wilcoxon-test or χ^2 -test.

Results

Growth curves, mortality, and diet consumption

The growth curves for rats in each group are shown in Fig. 2. The mean body weights were similar in all groups throughout the experimental period with no effect of safflower yellow being apparent. At the end of the administration period, the numbers of surviving male animals were 42 (84.0%) in the 5.0% group, 41 (82.0%) in the 2.5% group and 45 (90.0%) in the control

group, and for females, 43 (86.0%) in all three groups. Mean survival time was 107 weeks for male in all three groups and 106 weeks for female in all three groups (Table 1), no intergroup differences being observed.

There were also no differences between male or female groups with regard to mean food consumption in animals treated with safflower yellow or receiving basal diet alone (Table 1).

Blood analysis

The results of hematological analysis and laboratory findings regarding serum parameters are shown in Table 2. A decrease in white blood cells (WBC) and glutamic oxaloacetic trans-

Fig. 2. Growth curves of male and female F344 rats treated with safflower yellow.

Table 1. Total Consumption Data and Mean Survival Time of F344 Rats Treated with Safflower Yellow

Conc. (%)	Sex	No. of rats		total consumption of safflower yellow (g/100 g · B.W./rat)	No. of rats surviving until the end of the study	mean survival time and range (wk)
		Initial	Effective			
5.0	male	50	50	83.9	42	107(87-108)
2.0	male	50	50	42.3	41	107(97-108)
0	male	50	50	0	45	107(90-108)
5.0	female	50	50	98.8	43	106(69-108)
2.5	female	50	50	50.5	43	106(83-108)
0	female	50	49	0	43	106(88-108)

Conc.: concentration of commercial safflower yellow

B.W.: body weight

Table 2. Haematological and Serum-Biochemistry Data for F344 Rats Treated with Safflower Yellow

Conc. (%)	Sex	WBC ($10^3/\text{mm}^3$)	RBC ($10^6/\text{mm}^3$)	Hb (g/dl)	Ht (%)	BS (ng/dl)	TP (g/dl)	ALP (K-A)
5.0	male	43 $\pm$ 30*	1264 $\pm$ 1461	17.8 $\pm$ 2.9	57.1 $\pm$ 9.1	190 $\pm$ 58*	6.4 $\pm$ 0.5	12.6 $\pm$ 4.7
2.5	male	114 $\pm$ 450	1119 $\pm$ 235	18.8 $\pm$ 3.3	61.0 $\pm$ 9.6	197 $\pm$ 56*	6.6 $\pm$ 0.3	11.6 $\pm$ 5.1
0	male	164 $\pm$ 38	1085 $\pm$ 259	18.1 $\pm$ 3.9	58.8 $\pm$ 11.4	164 $\pm$ 38	6.4 $\pm$ 0.5	12.1 $\pm$ 6.0
5.0	female	64 $\pm$ 185	987 $\pm$ 165	18.3 $\pm$ 1.5	54.2 $\pm$ 11.0	182 $\pm$ 49	7.1 $\pm$ 0.5	9.8 $\pm$ 4.3
2.5	female	71 $\pm$ 229	993 $\pm$ 176	18.8 $\pm$ 1.7	56.8 $\pm$ 5.0	198 $\pm$ 71	6.8 $\pm$ 0.4	9.9 $\pm$ 2.9
0	female	102 $\pm$ 286	971 $\pm$ 195	17.4 $\pm$ 3.3	55.2 $\pm$ 9.0	174 $\pm$ 64	6.8 $\pm$ 0.6	9.4 $\pm$ 4.3

Conc. (%)	Sex	GOT (K-U)	GPT (K-U)	LDH (W-U)	CPK (IU/l)	Chol (mg/dl)	TG (mg/dl)	BUN (mg/dl)	Cr (mg/dl)
5.0	male	288 $\pm$ 121*	67 $\pm$ 50*	4017 $\pm$ 1252*	839 $\pm$ 653	139 $\pm$ 63	136 $\pm$ 87	34 $\pm$ 17*	1.0 $\pm$ 0.3
2.5	male	253 $\pm$ 97*	65 $\pm$ 31*	3426 $\pm$ 835	633 $\pm$ 146	135 $\pm$ 31	125 $\pm$ 94	29 $\pm$ 7	1.0 $\pm$ 0.1
0	male	330 $\pm$ 95	81 $\pm$ 212	2972 $\pm$ 2878	641 $\pm$ 186	112 $\pm$ 24	136 $\pm$ 42	28 $\pm$ 7	0.9 $\pm$ 0.1
5.0	female	244 $\pm$ 104	59 $\pm$ 25	2873 $\pm$ 1716*	561 $\pm$ 188	131 $\pm$ 24	145 $\pm$ 66	24 $\pm$ 8	0.9 $\pm$ 0.3
2.5	female	242 $\pm$ 104	60 $\pm$ 27	3327 $\pm$ 758	511 $\pm$ 149	140 $\pm$ 22	131 $\pm$ 52	26 $\pm$ 7	0.9 $\pm$ 0.2
0	female	291 $\pm$ 274	70 $\pm$ 78	3745 $\pm$ 1683	584 $\pm$ 196	133 $\pm$ 37	145 $\pm$ 76	26 $\pm$ 7	0.8 $\pm$ 0.2

WBC = White blood cells RBC = Red blood cells Hb = Haemoglobin Ht = Haematocrit
 BS = Blood sugar TP = Total protein ALP = Alkaline phosphatase GOT = Glutamic oxaloacetic transaminase GPT = Glutamic pyruvic transaminase LDH = Lactate dehydrogenase CPK = Creatine phosphokinase Chol = Cholesterol TG = Triglycerides BUN = Blood urea nitrogen Cr = Plasma creatinine

* : Significantly different from control value by the Wilcoxon's t-test ($P < 0.05$)

aminase (GOT), and increased glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and blood sugar (BS) were observed in males and an increase in LDH in females, all of these changes being statistically significant ($P < 0.05$) (Table 2). There were no significant differences between treated and control rats of either sex in the values for total proteins, alkaline phosphatase, creatine phosphokinase, cholesterol, triglycerides, plasma creatinine, or serum electrolytes.

Final body and organ weights and tumor incidence

In the groups of males given 5.0% and 2.5% safflower yellow, the mean weights of the left lung were lower than those of the controls, while the weights of the right lung in the female group given 2.5% safflower yellow were increased (Table 3). The overall incidences of benign and malignant tumors in all groups are summarized in Table 4. For the males, tumors were found in 96% of the controls and 94% of both the 2.5%- and 5.0%-safflower yellow groups, while the corresponding figures for the females were 59, 74, and 64%.

These high proportions of tumor-bearing rats primarily due to development of tumors of the testis, mammary glands, pituitary gland, thyroid gland, adrenal glands, haematopoietic system and uterus. The incidences of C-cell adenomas of the thyroid gland were significantly increased in the male treated groups, while that of pheochromocytoma of the adrenal gland in the 5.0% male group was decreased. No other statistically significant differences regarding tumor incidences were observed.

Discussion

The design and conduct of this bioassay were according to recent guidelines for carcinogenicity tests and the evaluation of data⁶⁻⁸.

In the present experiment, many different types of tumors were observed in all groups including the controls, and the organ distribution of these tumors and their histological characteristics were similar to those that are known to occur spontaneously in F344 rats⁹⁻¹². Generally there was no significant difference between their incidences in

Table 3. Absolute and Relative Organ Weights in F344 Rats Treated with Safflower Yellow

Conc. (%)	Sex	Final body weights (g)	Brain (g)	Heart (g)	Lung (g)		Liver (g)	Spleen (g)	Kidney (g)		Testis (g)	
					Left	Right			Left	Right	Left	Right
5.0	male	431 ± 27.3	2.08 ± 0.18 (0.48 ± 0.04)	1.24 ± 0.01 (0.29 ± 0.02)	0.56 ± 0.12* (0.13 ± 0.03)	1.05 ± 0.24 (0.24 ± 0.06)	10.86 ± 1.44 (2.52 ± 0.33)	1.16 ± 0.66 (0.27 ± 0.15)	1.35 ± 0.16 (0.31 ± 0.04)	1.39 ± 0.36 (0.32 ± 0.08)	2.81 ± 1.18 (0.65 ± 0.27)	2.43 ± 1.25 (0.56 ± 0.29)
2.5	male	433 ± 27.4	2.00 ± 0.16 (0.45 ± 0.04)	1.27 ± 0.09 (0.29 ± 0.02)	0.62 ± 0.11* (0.14 ± 0.02)	1.06 ± 0.20 (0.23 ± 0.05)	11.14 ± 2.31 (2.51 ± 0.52)	1.74 ± 2.94 (0.39 ± 0.66)	1.32 ± 0.10 (0.30 ± 0.02)	1.32 ± 0.11 (0.30 ± 0.02)	3.53 ± 1.66 (0.80 ± 0.37)	3.15 ± 1.75 (0.71 ± 0.40)
0	male	431 ± 40.2	2.09 ± 0.12 (0.48 ± 0.03)	1.20 ± 0.14 (0.28 ± 0.03)	0.69 ± 0.15 (0.16 ± 0.03)	0.96 ± 0.24 (0.22 ± 0.06)	10.56 ± 1.88 (2.45 ± 0.44)	1.49 ± 2.14 (0.35 ± 0.50)	1.30 ± 0.10 (0.30 ± 0.02)	1.31 ± 0.10 (0.30 ± 0.02)	2.50 ± 1.00 (0.58 ± 0.23)	2.50 ± 1.30 (0.58 ± 0.30)
5.0	female	295 ± 27.6	1.89 ± 0.12 (0.64 ± 0.04)	0.94 ± 0.10 (0.32 ± 0.03)	0.44 ± 0.10 (0.15 ± 0.03)	0.74 ± 0.10 (0.25 ± 0.03)	6.91 ± 1.16 (2.34 ± 0.39)	0.77 ± 1.32 (0.26 ± 0.45)	0.93 ± 0.09 (0.32 ± 0.03)	0.94 ± 0.09 (0.32 ± 0.03)	—	—
2.5	female	304 ± 28.4	1.79 ± 0.20 (0.59 ± 0.07)	0.99 ± 0.09 (0.33 ± 0.03)	0.44 ± 0.08 (0.14 ± 0.03)	0.77 ± 0.12* (0.25 ± 0.04)	7.25 ± 0.83 (2.38 ± 0.27)	0.62 ± 0.24 (0.20 ± 0.08)	0.98 ± 0.11 (0.32 ± 0.04)	0.96 ± 0.09 (0.32 ± 0.03)	—	—
0	female	302 ± 42.7	1.96 ± 0.26 (0.65 ± 0.09)	0.93 ± 0.10 (0.31 ± 0.03)	0.47 ± 0.09 (0.16 ± 0.03)	0.70 ± 0.13 (0.23 ± 0.04)	7.03 ± 1.08 (2.33 ± 0.36)	0.95 ± 1.39 (0.31 ± 0.46)	0.95 ± 0.09 (0.31 ± 0.03)	0.96 ± 0.10 (0.32 ± 0.03)	—	—

Mean ± standard deviation (%): organ weight final body weight

*: Significantly different from control value by the Wilcoxon's t-test ($P < 0.05$)

Table 4. Organ Distribution and Histological Diagnosis of Tumors in F344 Rats Treated with Safflower Yellow

Tumor	Safflower yellow conc. (%) Effective no. of rats	No. of rats affected					
		Male			Female		
		0	2.5	5.0	0	2.5	5.0
		50	50	50	49	50	50
No. of rats demonstrating tumor development		48(96)	47(94)	47(94)	29(59)	37(74)	32(64)
Genital system							
Testis: interstitial cell tumor		48(96)	47(94)	45(90)	—	—	—
Prostate: adenocarcinoma		4(8)	3(6)	4(8)	—	—	—
Uterus: endometrial polyp		—	—	—	4(8)	8(16)	4(8)
adenocarcinoma		—	—	—	0	2(4)	0
endometrial sarcoma		—	—	—	1(2)	2(4)	1(2)
Ovary: granulosa cell tumor		—	—	—	1(2)	1(2)	1(2)
theca cell tumor		—	—	—	0	1(2)	0
Mammary gland: fibroma		6(12)	7(14)	3(6)	1(2)	0	2(4)
fibroadenoma		2(4)	0	2(4)	4(8)	2(4)	3(6)
adenoma		1(2)	0	1(2)	2(4)	3(6)	2(4)
Endocrine system							
Pituitary gland: adenoma		4(8)	5(10)	7(14)	14(28)	11(22)	13(26)
Thyroid gland: C-cell adenoma		2(4)	8(16)	9(18)*	3(6)	2(4)	1(2)
C-cell carcinoma		2(4)	1(2)	2(4)	1(2)	0	3(6)
follicular adenoma		0	1(2)	0	0	0	0
Adrenal gland: pheochromocytoma		6(12)	5(10)	1(2)*	0	1(2)	2(4)
malignant pheochromocytoma		1(2)	0	1(2)	0	0	0
Pancreas: island cell tumor		3(6)	3(6)	1(2)	0	1(2)	2(4)
Digestive system							
Forestomach: papilloma		0	1(2)	0	0	0	0
Small intestine: leiomyosarcoma		0	0	1(2)	0	0	0
Liver: neoplastic nodule		3(6)	0	4(8)	1(2)	1(2)	0
sarcoma		0	1(2)	0	0	0	0
Respiratory system							
Lung: adenoma		1(2)	1(2)	0	0	1(2)	0
adenocarcinoma		0	1(2)	0	0	0	0
Urinary system							
Kidney: renal cell carcinoma		0	0	1(2)	0	0	0
Renal pelvis: transitional cell carcinoma		0	0	1(2)	0	0	0
Bladder: papilloma		0	0	0	0	1(2)	1(2)
Cardiovascular system							
Heart: fibroma		0	0	0	0	0	1(2)
Body cavity							
Peritoneum: mesothelioma		2(4)	1(2)	0	0	0	0
Haematopoietic system							
Leukemia		6(12)	7(14)	8(16)	7(14)	8(16)	3(6)
Integument, musculoskeletal system							
Skin: squamous cell carcinoma		0	0	0	0	0	1(2)
basal cell carcinoma		0	0	0	1(2)	0	0
sebaceous adenoma		1(2)	3(6)	1(2)	0	2(4)	0
preputial clitoral gland tumor		4(8)	3(6)	4(8)	3(6)	3(6)	3(6)
Subcutis: fibroma		1(2)	3(6)	2(4)	1(2)	0	1(2)
liposarcoma		1(2)	1(2)	0	0	0	0
leiomyosarcoma		1(2)	0	1(2)	0	1(2)	0
hemangiosarcoma		0	0	0	0	1(2)	0

(): % * : Significantly different from control value by the χ^2 -test ($P < 0.05$)

safflower yellow-treated and control groups except for the increased incidence of C-cell adenoma of the thyroid gland in males. However, since C-cell adenomas of the thyroid gland commonly occur spontaneously at high incidence in male F344 rats: for example 11.1% in the study of Maekawa *et al.*¹⁰, the observed difference between the 5.0% safflower yellow treated male rats and controls was not considered to be treatment related, but rather fortuitous in nature. Similarly with the observed decrease in adrenal gland pheochromocytomas.

Thus, it was concluded that commercial safflower yellow is not carcinogenic in F344 rats when administered at levels of 2.5% or 5.0% in the diet continuously for 2 years.

The fact that safflower yellow earlier exhibited mutagenicity toward *Salmonella typhimurium* TA100 both with and without S9 mix, and toward TA98 with S9 mix¹³ suggests that this is irrelevant to carcinogenicity or that the responsible agent might be at such a low level in commercial safflower yellow that weak carcinogenicity could not be detected. Future investigation of individual components should clarify this question.

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