Bull. Environ. Contam. Toxicol. (1992) 49:306–311

Environmental Contamination and Toxicology

Effect of Food Flavor Cinnamaldehyde on Liver Microsomal Cytochrome P-450 in Rats

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About 3000 agents are currently approved for use as food additives. Three types of food additives have, in particular, given rise to concern about possible carcinogenic hazards - food colors, Sweeteners and antioxidants. Cinnamaldehyde, a food flavoring agent is commonly encountered in several Indian food products. It is used in beverages, ice creams, candy, baked foods, gelatins, puddings, chewing gums, tooth paste, condiments and meat preparations at concentrations ranging from 7.7 ppm (ice creams) to 700 ppm (sweets) according to the surveys conducted by the flavoring extract Manufacturer's Association (Hall and Oser 1965). Cinnamaldehyde is an unsaturated aromatic aldehyde in use, since the 1940's as an imitation group of Cherry flavor in foods (Opdyke 1975). It was positive in the Ames test at relatively low dose level and also induce chromosomal abberations (Ishidate et al 1984). With regard to the developmental toxicity, only the Cinnamic acid, a parental compound was tested and found to be not embryotoxic in the rat (Zaitsev et al 1975). Abramovici and Rachmuth-Roizman (1983) reported that the Cinnamaldehyde has teratogenic effect on the chick embryo. Mutagenic activity of Cinnamaldehyde has been shown in several procaryotic and eukaryotic systems including hamster fibroblasts (Ishidate et al 1984). Cinnamic acid and its derivatives are chemically related to a number of toxic compounds such as styrene and coumarines known for their toxic properties (Hoskins 1984). When Cinnamaldehyde was fed to male and female rats at a low concentration (10,000 ppm in diet) over a 16 week period slight swelling of the squamous portion of the stomach lining was observed (Hagan et al 1967). Cinnamaldehyde in food is under a cloud because it is suspected to be carcinogenic and the safety of this compound has been evaluated by the Joint FAO/WHO expert

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committee on food additive (1984) and it was concluded that additional invivo studies were necessary.

In the present investigation long term feeding experiments were carried out with different doses for 24 weeks and the results are discussed. The highest dose level 2.5 mg/kg body weight was about 0.1% of the oral LD_{50}(2.22 g/kg body weight) as reported by (Jenner et al 1964).

MATERIALS AND METHODS

Food grade cinnamaldehyde was purchased from BASIL & COMPANY, Madras and used in our studies. Thirty weaning albino male rats derived from the Wistar strain were obtained from Central Leather Research Institute, Guindy, Madras and divided into three groups (I, II and III) with uniformity in average weight. Two groups of rats were given the high (235 ug/day) or low (133 ug/day) doses of the cinnamaldehyde mixed in food for 24 weeks. A group matched controls were observed simultaneously. At the end of the test period, the animals were sacrificed and biochemical analysis were performed with the liver.

The Microsomes were prepared from rat liver by a modification of the method of Mitoma et al(1956) and cytochrome P-450 content was estimated by the method of Omura and Sato (1964). The protein concentrations were determined by the method of Lowry et al (1951).

RESULTS AND DISCUSSION

Mortality was observed only in the high dosed experimental group which was fed 2.5 mg/kg/day of the food flavor cinnamaldehyde. 60% of the high dose group and 100% of the low dose and control groups lived to the end of the experimental period. No clinical signs attributable to the treatment were observed except epilation [falling of hair] from the abdomenal region of the rats of high dose group. A significant decrease in body weight gain was also noticed in experimental animals (Table I). Very recently Mantovani et al (1989) reported that when cinnamaldehyde was administered by gavage to rats on days 7 to 17 of pregnancy significant lowering of weight gain of the rats was observed at the higher dose levels.

Table-I shows the effect of cinnamaldehyde treatment on the liver. Cinnamaldehyde strongly stimulated the liver. The liver of high dose rats weighed 6.43 $\pm$ 0.24 g/100g body weight while the controls ranged around

Table 1. Terminal body weight, relative weight of liver and protein content in liver of rats fed 0-2.5 mg of food flavor cinnamaldehyde/kg body weight/day for 24 weeks.

Parameter	Control	Experimental	
		Low dose	High dose
Number of animals	10	10	6
Dose level mg/kg/day	0	1.25	2.5
Terminal body weight (in grams)	150±8	138±6	*120±7
Relative weight (% of body weight) Liver	3.88±0.22	4.17±0.26	*6.43±0.24
Protein content of liver (mg/g of wet tissue)	123.07±3.09	163±3.59	*199.9±3.42

* indicates statistically significant $p > 0.05$.

3.88 ± 0.22g/100g body weight. The protein content incresed markedly in the liver of the experimental animals. The percent protein content increase in the high dose experimental rats when compared with controls is 61% in liver. Kato *et al* (1968) stated that when foreign chemicals were administered a parallel increase in relative liver weight and microsomal protein was observed. Earlier studies have shown that cinnamaldehyde interacted with human serum albumin to form a schiff base [Majeti and Suskind 1977] and also caused dehelication of the albumin [Bagdasaryan and Troitskii 1971]. Cinnamaldehyde is a strong sensitizer and many cases of contact dermatitis and allergy were reported in occupationally exposed persons and others who used cinnamaldehyde in fragrances. The allergen is proved to be a schiff base formed by reaction of the aldehyde with protein (Hoskins 1984]. The significant increase of protein level in liver observed in our studies may have a role to play in the increased production of allergen by this mechanism.

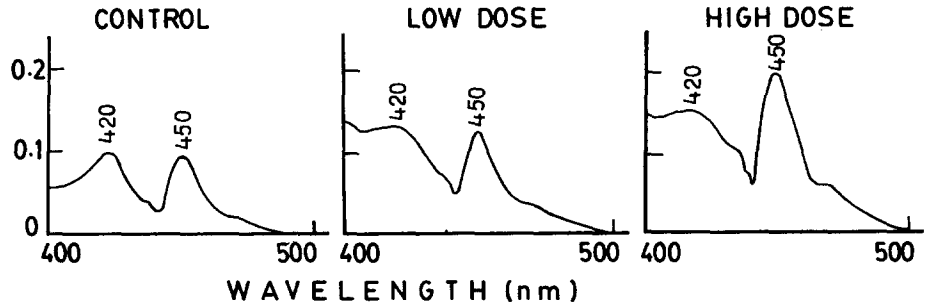

Fig.1 Spectra of liver microsomal cytochrome p-450

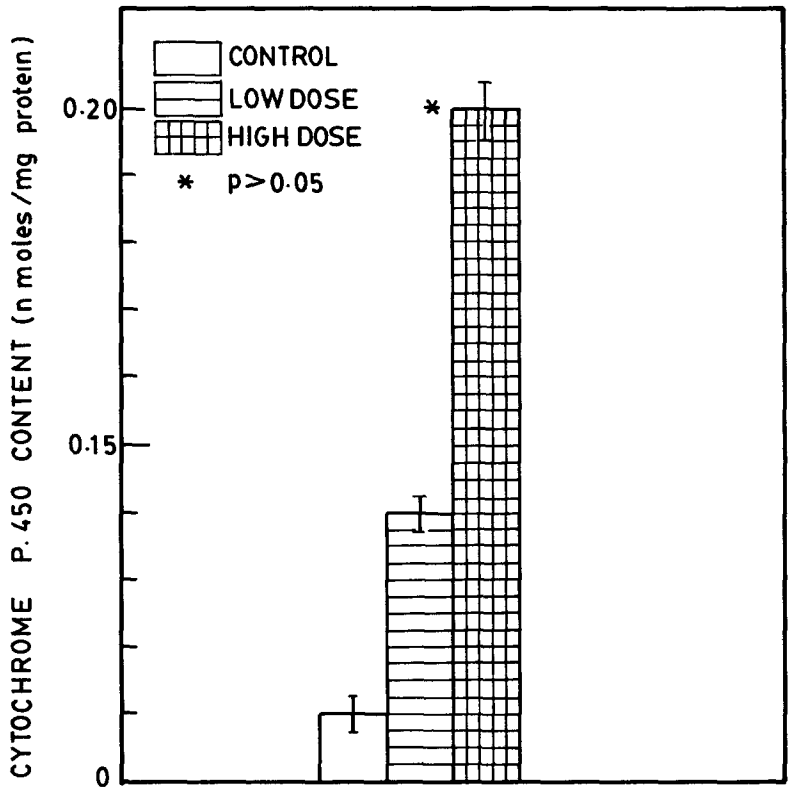

Fig.2 Liver microsomal cytochrome P.450 content in the rats administered cinnamaldehyde in the diet for 24 weeks.

Figure-1 indicates an increase in the activity of the microsomal cytochrome p-450 when the food flavor cinnamaldehyde was added in the diet at a dose of 2.5 mg/kg/day to the rats. The spectra shown in Figure-1 indicates further that the microsomes used were virtually free from hemoglobin and methemoglobin.

In the experimental animals, the cytochrome p-450 content increased sharply 52% and significantly ($p > 0.05$) (Figure-2), when compared to controls. Long term administration of the food flavor cinnamaldehyde leads to changes in cytochrome p-450 in the high dose experimental and subsequently may be responsible for liver nodule formation. Parke and Gray (1978) reported that another food flavor safrole formed stable complex with cytochrome p-450 and produced liver nodule formation and hepatocellularcarcinoma. Histopathological examination of the liver of high dose cinnamaldehyde treated animals revealed parenchymal hypertrophy and hyperplasia (Devaraj and Esthersuseela, unpublished data). This may provide some clues to the presence of neoplastic growth in the liver. The results suggest the possibility that the food flavor cinnamaldehyde administered at higher concentration for a longer period may have a potential for neoplastic growth in the liver. Further histopathological examinations will throw more light on these aspects.

Acknowledgments. The authors gratefully acknowledge the University Grants Commission, New Delhi for the financial assistance (Project NO.F.3-156/90 SRII).

REFERENCES

Abramovici A, Rachmuth - Roizman P (1983) Molecular structure - teratogenicity relationships of some fragrance additives. Toxicology 29: 143

Bagdasaryan SN, Troitskii GV (1971) Study of albumin-aldehyde complexes by the optical rotary dispersion and peptide map methods. Biochemistry 36 : 615 (English translation of Biokhimiya 36 : 732-8)

Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM, Long EL, Nelson AA, Brouwer JB (1967) Food flavorings and compounds of related structure II. Subacute and Chronic toxicity. Fd Cosmet Toxicol 5 : 141-157

Hall RL, Oser BL (1965) Recent Progress in the consideration of flavoring ingredients under the food additives amendment III. GRAS substances. Fd Technol 19 : 151-155

Hoskins A (1984) The occurrence, Metabolism and

Toxicity of Cinnamic acid and related compounds. J Appl Toxicol 4 : 283-292
Ishidate M, Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A (1984) Primary Mutagenicity Screening of Food Additives currently used in Japan. Fd Chem Toxic 22 : 623-636
Jenner PM, Hagan EC, Taylor JM, Cook EL, Fitzhugh OG (1964) Food flavorings and Compounds of related structure. I. Acute oral toxicity. Fd Cosmet Toxicol 2 : 327-343
Kato R, Oshima T, Tomizawa S (1968) Toxicity and Metabolism of drugs in relation to dietary Protein. Jap J Pharmac 18 : 356-366
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein Measurement with the Folin-Phenol reagent. J Biol Chem 193 : 265
Majeti VA, Suskind RR, (1977) Mechanisms of cinnamaldehyde sensitization. Contact Dermat 3 : 16
Mantovani A, stazi AV, Macri C, Ricciardi C, Piccioni A, Badellino E (1989) Pre-natal (Segment II) toxicity study of cinnamicaldehyde in the sprague-dawley rat. Fd Chem Toxic 27 : 781-786
Mitoma C, Posner HS, Reitz HC, Udenfriend S (1956) Enzymatic hydroxylation of aromatic Compounds. Arch Biochem Biophys 61 : 431-441
Omura T, Sato R (1964) Isolation of Microsomes and estimation of cytochrome P 450. J Biol Chem 239 : 2370 - 2381
Opdyke DLJ, (1975) Cinnamyl anthranilate. Fd Cosmet Toxicol 13 : 751 - 752
Parke DV. and Gray TJB (1978) In : Remmer H, Bolt H.M, Bannasch P, Popper H, (ed) primary Liver Tumors. Lancaster: MTP Press, 335
Zaitsev AN, Maganova NB (1975) The embryotoxic action of some food aromatizers. Voprosui Pitaniya 3: 64-68

Received October 30, 1990; accepted February 18, 1992.