EFFECT OF FEEDING CHAPATIES PRESERVED WITH SORBIC ACID ON GROWTH AND REPRODUCTION OF ALBINO RATS

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Sorbic acid, used in concentration of 0·3% in chapaties and found satisfactory as a preservative, has been investigated for its safety of use by long term feeding studies with albino rats. The data obtained with regard to growth rate, food consumption, organ to body weight ratio and performance in reproduction show that the preserved foodstuffs containing sorbic acid in the concentration of 0·3% are safe for consumption.

Sorbic acid is in extensive use as a preservative for a variety of foods such as cheese, bread, beverages[1-3] etc. Rao et al[4,5] reported the use of sorbic acid for preservation of chapaties and parottas for at least six months. They recommended a concentration of 0·48 per cent on the basis of atta. They, however, reported that there is a loss of sorbic acid during baking, leading to a final concentration of 0·3 per cent and to a further loss during storage for six months. It is likely that the added sorbic acid has undergone decomposition or interacted with other food constituents in the chapaties or parottas. Work is already in progress in this laboratory to study the fate of sorbic acid in these products on storage. Recent reports[6] indicate the possibility of conversion of sorbic acid into para-sorbic acid, a lactone which might exert deleterious effects.

In no food products sorbic acid is permitted in concentrations of more than 0·1 per cent though the safety of its consumption in concentrations as high as 4—5 per cent is known [7,8]. Since the concentration of sorbic acid in chapaties is 0·3 per cent, a study was undertaken to investigate the effect of long term ingestion of such chapaties. In this paper the methods[9] recommended for testing the chronic toxicity of intentional food additives were employed to obtain comparative data on albino rats fed on

(*i*) Chapaties preserved and stored for six months,

(*ii*) Freshly-made chapaties with and without preservative,

to determine such factors as growth rate, organs to body weight ratio and performance in reproduction.

MATERIALS AND METHODS

Test Animals

Thirty male and ten female albino rats of the strain maintained at this laboratory were selected for testing. 21-28 days old weaning rats of weight range 30-40 gm were grouped in randomised block design based on initial weight as per the procedure of Snedecor[10]. They were housed in individual cages with wire screen bottoms. The rats were divided into three groups consisting of 10 males each. Rats of group I were kept on freshly-made chapaties having no sorbic acid and served as control. Rats of group II were kept on freshly-made chapaties containing sorbic acid. Rats of group III had in addition 10 females and they were all kept on preserved chapaties stored for 6 months.

Diet

The chapaties were prepared and stored as per the method followed by Rao et al[5]. The diet was of normal composition[11] and the nutritive value was as follows :

Protein 20%—6-7 per cent from chapaties and the remainder from the added skimmed milk powder.

Fat 9%—Groundnut oil.

Fat soluble vitamins 1%—Shark liver oil.

Other vitamins 1%—Vitaminised starch.

Salt mixture 2% and water *ad libitum*.

Growth Rate

The body weight of the animals and their intake of food were measured weekly for 13 weeks.

Organ to Body Weight Ratio

After a period of growth of at least 13 weeks, 6 males from each group were examined for gross abnormalities in heart, liver, spleen, adrenals, kidneys, thyroid and gonads. In addition, weights of the above organs and of stomach, ileum and caecum were determined. Samples of liver were examined for fat and protein content. Paraffin wax sections of the samples were preserved in 10% buffered formalin and were stained with haematoxylin and eosin for microscopic examination.

Mating

After 13 weeks, the animals of the third group were regrouped in breeding cages with two females to one male and the appropriate diets were continued. They were allowed to mate for 14 days after which the females were separated and housed in individual cages to litter as usual. At the time of birth, the number in litter and its total weight was recorded. After the littering, the young ones were allowed to grow with their mother, and on the 21st day of littering the weaning weight of all the young ones was recorded.

RESULTS AND DISCUSSION

No deaths or abnormalities in the behaviour or appearance of the animals was observed during the study. There was no significant difference between test and control animals in the rate of body weight gain during 13 weeks. No difference in food consumption between test and control groups was detected. It was observed that the rate of weight gain in the males in all the three groups was almost the same and the final weights at the end of 13 weeks were nearly the same, viz 250 gm, 269 gm and 256 gm respectively (See Table 1). The body weights at the end of 0, 4, 8 and 13 weeks are given in Table 1 and it will be seen that the growth rate during the entire period was normal. This shows that the rats maintained their normal intake of food and growth-rate pattern. It will also be observed that the food intake pattern at various stages of growth as well as the mean for the rats for the entire period, appears to be similar. The final weights were also expected to be higher than the usual, since the protein content of the diets was maintained at 20%, as against 17% level in the stock diet. The high level of proteins was included to correspond with the high protein diets supplied to the Service personnel normally. Also it was not the intention to study the effects that may become evident at low intake of calories or proteins but to observe those that were due to the ingestion of chapaties preserved and stored, and manifest even at high intake of protiens.

Further confirmation of the safety of the ingestion of the preserved and stored chapaties was sought by examining for any gross abnormalities in some of the important organs. Table 2 gives data on the absolute and relative organ weights of the animals after 15 weeks. The terminal weight of the organs (average of six) are given for the male animals and the average relative weights given in the second part of the table. Since the data are obtained at one stage of growth only (15 weeks), comparison is made on the basis of relative organ weights. The data available from literature for the relative organ weights of comparable body weights for male rats are given in parenthesis alongwith the data for the rats fed on fresh chapaties[12]. It

TABLE 1

COMPARATIVE BODY WEIGHTS AND FOOD CONSUMPTION OF RATS

Diet	Body weight at week				Body weight gain at 13th week	Food consumption rat/day at week				Mean Food consumption rat/day	Sorbic acid intake per rat at week			
	0 (gm)	4 (gm)	8 (gm)	13 (gm)	(gm)	0 (gm)	4 (gm)	8 (gm)	13 (gm)	(gm)	0 (mg)	4 (mg)	8 (mg)	13 (mg)
I. Freshly prepared chapati without sorbic acid (control) —10 Males	35·5	136·8	237·7	285·4	249·9	5·6	7·8	12·3	14·0	9·92	—	—	—	—
II. Freshly prepared Chapati with sorbic acid—10 Males	36·6	152·7	258·2	306·0	269·4	4·9	7·7	13·0	14·3	9·97	12·2	19·2	32·5	35·7
III. Chapati with sorbic acid (stored)—10 Males	36·5	145·1	243·2	292·5	256·0	4·6	7·7	12·4	14·2	9·72	11·5	19·2	31·0	35·5
IV. Chapati with sorbic acid (stored)—10 Females	36·5	123·1	170·4	162·1	125·5	4·3	6·4	11·9	13·2	8·95	10·7	16·0	29·7	33·0

will be seen that the values for all organs studied are in general agreement. Similar agreement was found with the data collected on animals maintained on stock diet. There is a slight decrease in the relative weight of the liver on incorporation of sorbic acid (3·6 per cent to 3·0 per cent) which further decreases to 2·8 per cent when the animals were fed on preserved chapaties stored for six months. Similar change in liver weight has been reported by Deuel and Alfin Slater[3] in their animals fed on sorbic acid but the diets they used for their studies were fat free. Histological examination of the livers, however, showed no pathological manifestations. The moisture, fat and protein were also found to be normal in all livers (66-67 per cent, 4·3 - 4·7 per cent and 4 - 4·5 per cent respectively on fresh liver basis).

TABLE 2

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS AFTER FEEDING STUDIES

Diet	No. of animals	Absolute organ weights									Terminal weights
		Heart	Adrenals	Gonads	Stomach	Ileum	Caecum	Kidneys	Spleen	Liver	
		(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
Fresh chapati without sorbic acid	6	0·891	0·043	2·58	1·438	5·626	0·836	1·944	0·648	11·586	324
Fresh chapati with sorbic acid	6	0·776	0·060	2·89	1·252	4·847	0·771	2·057	0·596	11·810	330
Stored chapati with sorbic acid	6	0·886	0·066	2·42	1·094	4·740	0·744	1·924	0·594	8·967	314
		Relative organ weights gm/100 gm body weight									
Fresh chapati without sorbic acid	6	0·278 (0·28)	0·013 (0·014)	0·127 (0·75)	0·451 (0·39)	1·737 (1·600)	0·258 (0·23)	0·670 (0·670)	0·230 (0·17)	3·57 (3·05)	
Fresh chapati with sorbic acid	6	0·277	0·018	0·876	0·379	1·774	0·234	0·760	0·164	3·54	
Stored chapati with sorbic acid	6	0·259	0·020	0·628	0·428	1·810	0·237	0·613	0·190	2·847	

The effect of subsistence on the preserved stored chapaties from birth to reproductive stage has been investigated by mating the limited number of animals available and the data are given in the Table 3. The number used is too small to have much significance for interpreting the teratogenic effects. However, the average litter size of the experimental groups is definitely on the higher side as compared to normal litter

TABLE 3

EFFECT OF SUBSISTENCE ON CHAPATIES WITH SORBIC ACID ON THE LITTER SIZE, BIRTH WEIGHT, WEANING WEIGHT OF YOUNG ONES BORN

Sl. No.	Diet	Average initial wt of rats (g)		No. of animals	No. of pregnant	No. of litters (total)	Average No. of litters	Surviving	Average birth wt per rat	Weaning weights of young ones	Ratio of males to females of newly born
		Males	Females						(gm)	(gm)	
1.	Stored chapati with sorbic acid	285	185	10	5	50	10	43	4±1	35±5	1 : 1
2.	Control (Stock Diet)	200	175	10	6	30	5	26	4±1	35±5	1 : 1

size obtained in other studies in our laboratory, and appears similar to the increase in litter size reported recently[13]. Studies with the second generation of rats maintained on sorbic acid with preserved chapaties are being continued with the respective diets to observe any effects that may become evident after prolonged feeding in the second generation. The first generation of rats are also being continued with the diets to see the effects on longevity and abnormal effects on the normal degenerative processes of organs

Preserved chapaties form the major component of the pack rations meant for Service personnel, for their use under operations when no cooking or preparation of the food is possible. It is necessary to ensure that consumption of such rations is not only wholesome and nutritious but that it is free from any deleterious effects that may manifest after continued use. This preliminary report confirms that the sorbic acid added in chapaties and subjected to the heat treatment normal to the making of these items has no deleterious effect either when consumed freshly made or after six months storage.

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