Indian J. Anim. Res., **41** (1) : 1 - 8, 2007

STUDIES ON THE GENOTOXIC EFFECTS OF SUCRALOSE IN LABORATORY MICE

Ashwani Sharma, Sunil Panwar, A.K. Singh and K.K. Jakhar*

National Dairy Research Institute,
Karnal - 132 001 Haryana, India

ABSTRACT

Forty female (Swiss albino) mice were divided into 4 groups of ten animals each. Mice of groups I, II and III were fed sucralose 500, 1000 and 1500 ppm, respectively in feed. Group IV was fed sucralose free diet. Five animals were sacrificed from each group on 30th and 60th day of study and metaphase spreads prepared for evaluation of mitotic index and chromosomal aberrations. Liver and kidney samples of mice were also collected on 30th and 60th day of experiment for histopathological study. Among the chromosomal aberrations observed, polyploidy and monosomy were most common. The differences in mitotic indices and chromosomal aberrations between the 4 groups at different intervals were found to be statistically non-significant. Histopathological changes were observed in the liver and kidney of all treated groups. It is concluded from the present study that sucralose consumption up to 1500 ppm for two months did not produce any genotoxicity but it is hepatotoxic and nephrotoxic. However, its effect at higher doses and longer duration needs to be studies.

INTRODUCTION

In India, a large quantity of milk produced is diverted for the manufacturing of sweet milk products. Disease related to lifestyle and dietary habits of human population, account for most of the mortality and morbidity in developed countries. Obesity is a growing concern and has been associated with many other manifestations like diabetes, hypertension and cardiovascular diseases. A large segment of Indian population (30 million) is suffering from diabetes, the single most important metabolic disorder that may effect nearly every organ and system of body. The consumers today have become more health conscious and also the high calorie food is loosing favour fast. Hence, there is an urgent need to formulate food products with the view to suit most of the requirements of the vulnerable groups.

Replacement of sugar with non-nutritive artificial sweeteners has gained momentum in Indian food industry. A number of such sweeteners having low calories, namely aspartame, saccharine, acesulphame-k, sucralose have been introduced in Indian market. On one hand, many food processing companies have requested to be granted permission for the application of these sweeteners in processed food products, whereas, on the other hand, some of these sweeteners are carcinogenic and have toxic effects. Hence, the safety and regulatory aspects of these compounds are yet to be established.

Sucralose is biodegradable, white, odourless, zero calorie compound and 600 times sweeter than sucrose. It has excellent chemical and biological stability under various processing conditions that make it an ideal sugar replacer in dairy products. Sucralose is a chlorinated sugar and like chlorinated pesticides, there is every possibility that it may have toxic effects (Sasaki and Tsuda, 2002). However, no conclusive studies have been conducted on potential genotoxicity of sucralose.

Genetic hazards of a vast array of substances are now becoming evident and numerous sensitive methods for detecting and measuring genotoxic effects have been developed. The chromosomal studies assume special significance as they deal with the

* Veterinary Diagnostic Lab, CCS HAU, Hisar.

primary genetic material (Calberg, 1985; Henkel, 1999; John *et al*., 2000). Thus, chromosome studies provide an objective method which permits direct visual analysis of the damage to the chromosomes. The site and nature of chromosomal aberration can be precisely identified. Keeping the above facts in view, the present investigation was undertaken.

MATERIAL AND METHODS

The present study was conducted on Swiss Albino female mice of 3 to 4 months age obtained from CCS Haryana Agricultural University, Hisar and maintained at Small Animal House of National Dairy Research Institute, Karnal (Haryana) under comfortable and ideal management system. The nutrient requirements were met through regular feed (Table 1) and water provided *ad libitum*.

Forty female Swiss albino mice (weighing 20-30 kg) were randomly distributed into 4 groups of 10 animals each. Three different levels of sucralose mixed in feed and pellets prepared were fed to the 3 treatment groups (500 ppm, 1000 ppm, 1500 ppm) (Table 2). These levels conform to the level of sucralose used in common food products. The sucralose used was of food grade, supplied free by Virchow Laboratories Ltd., Hyderabad. Mice of the fourth group were used as control and fed on without sucralose. Five mice from each group were sacrificed by cervical dislocation on the 30th and 60th day of experiment for the harvest of metaphase chromosomes. Liver and kidney samples were also collected from mice of each group on the 30th and 60th day for histopathological study.

Liver and kidney samples of mice from each group sacrificed on the 30th day and 60th day post-treatment were collected in 10 per cent formal saline solution (40% formaldehyde - 200 ml, sodium chloride - 17 g, distilled water - 1800 ml). Fixed tissues were processed for paraffin embedding using ascending grades of alcohol as dehydrating agent and cedar wood oil as cleaning agent. Sections of 4 to 5 µm thickness were cut and stained by routine haematoxylin and eosin

Table 1. Composition of diet for mice per 100.40 kg (as supplied by NDRI Small Animal House)

Ingredients	Quantity (kg)
Wheat crushed	15
Bengal gram, crushed	58
Groundnut cake, crushed	10
Refined oil	4
Skim milk powder	5
Casein	4
Salt mixture	4
Vitamin mixture	0.20
Choline chloride mixture	0.20
Total	**100.40**

Table 2. Treatment schedule for *in vivo* chromosomal aberration test

Group	Concentration of sucralose in feed (ppm)	Number of treated mice	Number of mice sacrificed	
			Day 30	Day 60
I	500	10	5	5
II	1000	10	5	5
III	1500	10	5	5
IV (Control)	-	10	5	5

(H and E) staining (Luna, 1968). These were examined for histopathological changes and photomicrographs of selected sections were taken.

For the evaluation of genotoxic effects of consumption of sucralose, the *in vivo* chromosomal aberration test was conducted in which somatic metaphase chromosomes were prepared from bone marrow cells of mice after treatment. A modified protocol of Ford and Hamerton (1956) was used to study the bone marrow chromosomes. The mitotic index of each experimental group was calculated as per the method of Minot (1968).

Photomicrographs of selected, well stained metaphases with good spatial distribution were taken with Lyca camera on 35 mm Fuji colour 200 ASA film. Karyotypes were prepared from photographs of metaphase plates having distinct morphology of the chromosome and good spatial distribution without overlapping of the chromosomes.

Two-way analysis of variance (ANOVA) was carried out to test the significant differences in mitotic indices and chromosomal aberrations of mice fed different levels sucraslose for different durations.

RESULTS AND DISCUSSION

Effects on liver: On 30th day of experiment, lesions were seen in liver of all the treated groups. In group I Lesions observed were Oedema, marked to severe congestion of central vein and portal vessels; degenerative changes; necrotic changes (nuclei and vacuolization of cytoplasm) and initiation of lymphoid cell aggregation around central vein and near portal triad area. (Plate 1) In group II, Lesions were similar to group I. However, fatty changes were more marked. Also bile pigment accumulation in hepatocytes was noticeable and marked lymphoid aggregation in portal triad area and perivascular tissue was observed. In group III, changes were similar to group II. However, there was focal to massive hemorrhage and no bile pigment accumulation. In group IV (control), no lesion of any pathological significance found.

On 60th day of study Group I showed Similar changes as in 30th day. However, additional changes in form of mild Van-Kpfferson hyperplasia were observed. The nuclei were too big with chromatin clumps and vacuoles showing necrosis. There was mild bile pigment accumulation in canaliculi. Hydropic changes were more and fatty changes less in group II (Plate 2). Vacuolar nuclear necrosis was more prominent. In group III, lesions were similar as in 30th day. However, in one animal, there was marked hyaline degeneration of hepatocytes in parenchyma and around portal triad area. In Group IV (control) no changes of any pathological significance found.

Effect on kidney: On 30th day of exposure in Group I, the lesions observed were congestion, mild leucocytic infiltration in perivascular tissue, increased interstitial tissue because of fibroblast proliferation and mononuclear cell infiltration leading to atrophy of tubules and focal necrosis as indicated by initiation of focal lymphoid cell aggregation to typical lymphoid cell aggregation in parenchyma. In most samples, perirenal fat necrosis was also observed.

Group II showed mild to moderate intertubular congestion and focal areas of necrosis and lymphoid cell aggregation in cortex, perivascular region and medullary region. Peri-renal fat necroses could also be seen. In Group III there was severe congestion, diffuse glomerulo-nephritris and leucocytic infiltration in parenchyma (Plate 3). Similar changes as in group II were seen but lymphoid aggregation were more prominent in parenchyma, perivascular and perirenal fat tissues.No lesions of any pathological significance observed in group IV (control).

On 60th day of study group I showed changes similar as on 30th day. But there was increase in interstitial tissue and so atrophy and obliteration of glomeruli and tubules. In group

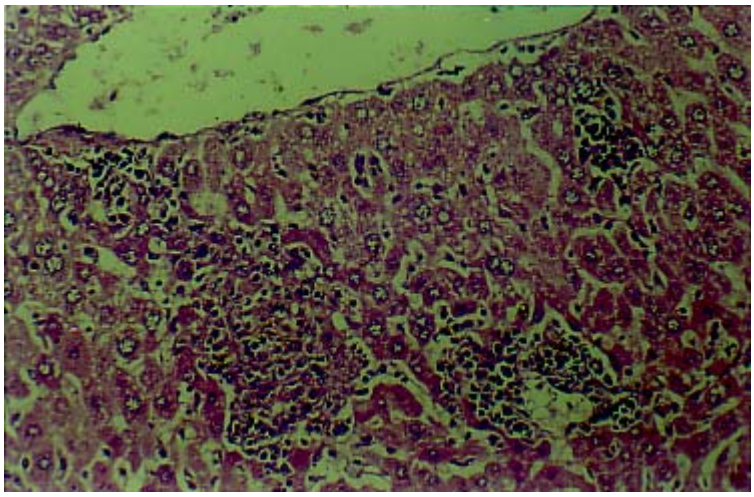

Plate 1. Group I (500 ppm 30 Days exposure) Liver showing initiation of lymphoid follicle formation in liver parenchyma subsequent to necrosis of hepatocytes (H and E x 33)

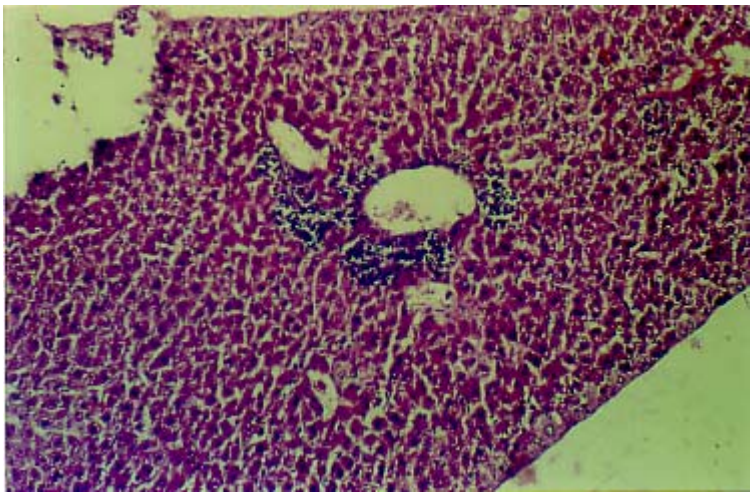

Plate 2. Group II (1000 ppm 60 Days exposure) Liver showing well developed multiple lymphoid follicles in liver parenchyma and peri-vascular tissue (H and E x 33)

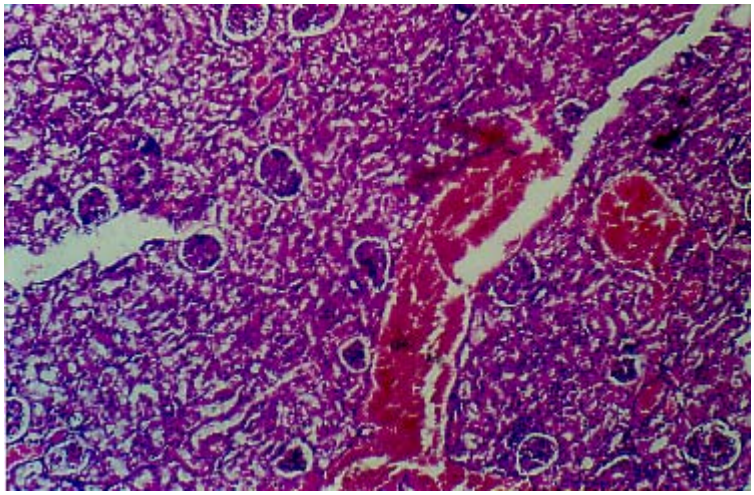

Plate 3. Group III (1500 ppm 30 Days exposure) Kidney showing severe congestion, diffuse glomerulo nephritis and leukocytic infiltration in parenchyma (H and E x 33)

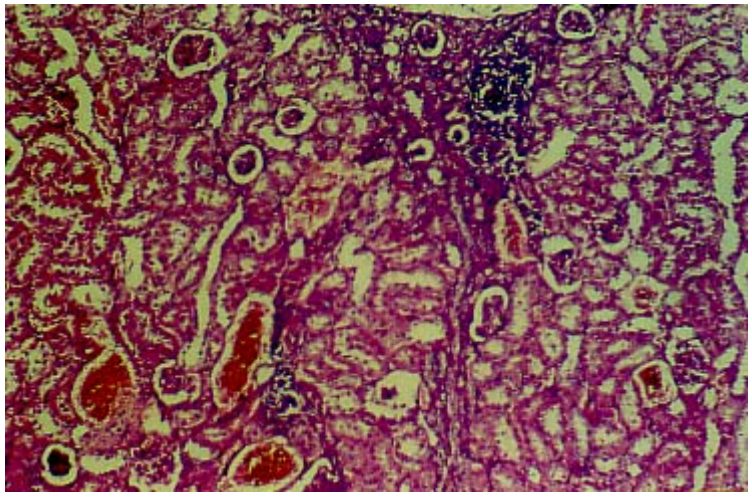

Plate 4. Group III (1500 ppm 60 Days exposure) Kidney showing massive area of coagulative necrosis of tubules and leucotic infiltration in interstitial tissue forming aggregates (H and E x 33)

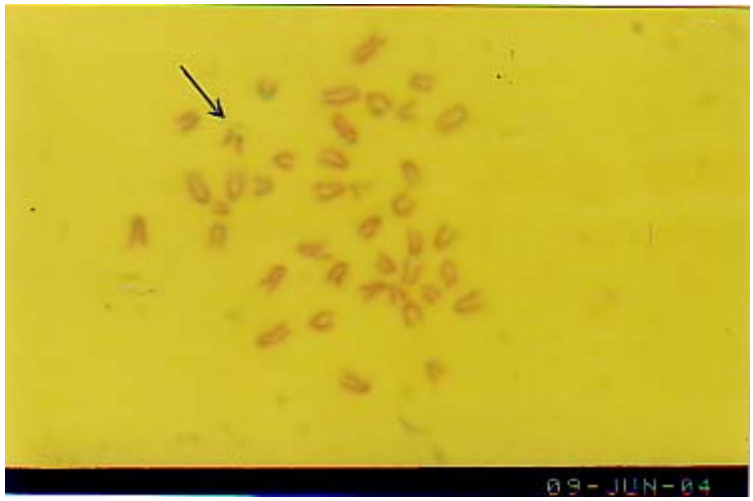

Plate 5. Metaphase plate showing centromeric break (arrow)

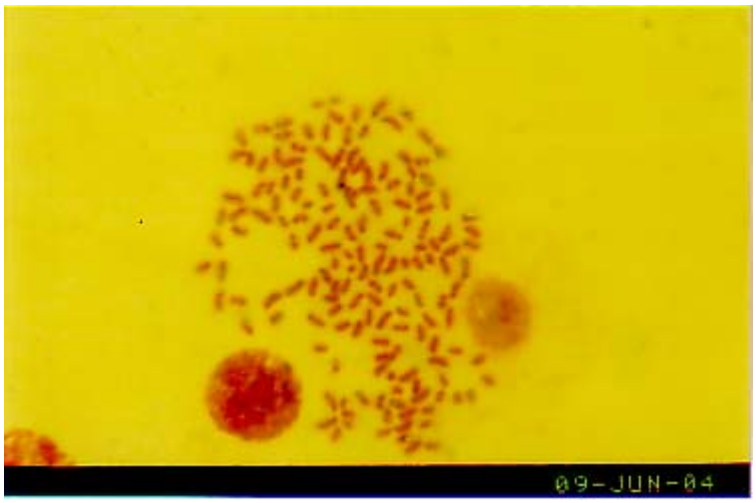

Plate 6. Metaphase plate showing polyploidy

II similar changes as in 30th day were observed and additionally mild hyaline degeneration of tubules was seen. Focal haemorrhages in parenchyma of one animal was also observed. There was atrophy of tubules, obliteration of glomeruli because of proliferation of fibroblasts and mononuclear cells. There was massive area of focal necrosis in parenchyma alongwith lymphoid cell aggregation (Plate 4). In group III the lesions were similar as in group II. However, no focal haemorrhage in parenchyma. No lesions of any pathological significance observed in group IV.

From the above observations, it appears that the sucralose gets accumulated in liver and kidney in sufficient quantities to cause damage to the hepatic and renal tissue. However, the effect on 60th day is not more severe than 30th day. The effect of these changes on the health of animals need to be investigated further. More studies need to be conducted to establish the harmful effect of sucralose on other tissues also.

Effects on bone marrow: The mitotic index (% of blast cells and metaphase cells) was calculated to assess the effect of sucralose on the mitotic activity of bone marrow cells. In group I, the mitotic indices were 2.9 and 2.9. In group II, they were 2.8 and 3. In group III, they were 3.1 and 2.9 and in group IV (control) they were 3 and 3.1 per cent on the 30th and 60th day of experiment, respectively (Table 3).

Statistical analysis of the results by two way analysis of variance (ANOVA) showed that there were no significant differences in mitotic indices between the treatment groups fed different levels of sucralose and between the two durations of feeding at both 1 and 5 per cent levels of significance. This indicates that at the levels used in the study, there was no bone marrow toxicity produced by sucralose.

Effects on chromosomes: The study revealed that the normal diploid chromosome number in mice (*Mus musculus*) is 40 (2n = 40, XY) with all the chromosomes being acrocentric. The X chromosome is large acrocentric with size lying between the first and second pair of chromosomes. The Y chomosome is small acrocentric and lies between the 18th and 19th pair in size.

The genotoxic studies were mainly conducted to record the structural and numerical aberrations of chromosomes in experimental groups I, II, III and IV. The incidence of various types of chromosomal abnormalities in the 4 groups have been summarised in Tables 4 and 5. In group I, chromosomal aberrations were 2.00 and 2.67 per cent; in group II they were 1.33 and 2.00 per cent; in group III they were 2.67 and 2.00 per cent and in group IV they were 2.00 and 2.00 per cent of the metahase studied on 30th and 60th day of experiment, respectively. The aberrations recorded were chromatid break, isochromatid break, chromatid gaps, centromeric breaks, polyploidy, nullisomy, monosomy and trisomy (Plates 5 and 6). Polyploidy and monosomy were the most frequently observed chromosomal aberrations. Similar results were observed by Mukhopadhyay *et al.* (2000) and Sasaki *et al.* (2002) in mice fed with aspartame and acesulphame-K.

Statistical analysis of the data by two way analysis of variance showed that the difference in the frequencies of chromosomal aberrations between groups fed different levels of sucralose and between the two durations of feeding were not statistically significant at 1 and 5 per cent levels of significance.

The results of the present study establish that sucralose at the concentration of 500, 1000 and 1500 ppm is not genotoxic when fed for a duration of 60 days. However, its effect at higher doses and longer periods needs to be investigated further.

Table 3. Mitotic index (%) of mice fed sucralose

Group	Cells counted	Period	
		30th day	60th day
I	1000	2.9	2.9
II	1000	2.8	3.0
III	1000	3.1	2.9
IV (Control)	1000	3.0	3.1

Table 4. Various types of chromosomal aberrations observed

Period	Group	No. of metaphase studied	No. of aberrant chromosomes	Structural aberrations					Numerical aberrations			
				CB	ICB	CG	ICG	CMB	Polyploidy	Nullisomy	Monosomy	Trisomy
30th day	I	150	3	–	1	–	–	–	2	–	–	–
	II	150	2	1	–	–	–	–	1	–	–	–
	III	I50	4	–	–	–	–	1	3	–	–	–
	IV	150	3	–	–	–	–	–	2	–	1	–
60th day	I	150	4	–	1	–	–	–	2	–	1	–
	II	150	3	1	–	–	–	1	–	–	1	–
	III	150	3	–	–	–	–	–	2	–	1	–
	IV	150	3	–	1	–	–	–	2	–	–	–

CB = chromatid break; ICB = isochromatid break; CG = chromatid gap;
ICG = isochromatid gap; CMB = centromeric break.

Table 5. Incidence and percentage of chromosomal aberrations in mice bone marrow cells

Group	Period	
	30th day	60th day
I	3 (2.00%)	4 (2.67%)
II	2 (1.33%)	3 (2.00%)
III	4 (2.67%)	3 (2.00%)
IV (Control	3 (2.00%)	3 (2.00%)

REFERENCES

Carlberg, F.W. (1985). *Fd. Chem. Toxic*, **23**(4/5): 499-506.
Ford, C.E. and Hamerton, J.L. (1956). *Stain Technol.*, **31**(6): 247-251.
Henkel, J. (1999). *FDA Consum.*, **33**(6): 12-16.
John, B.A. *et al.* (2000). *Fd. Chem. Toxic*, **38**(2): S107-110.
Luna, L.G. (1968). *Histologic Staining Methods.* 3rd edn. McGraw Hill Book Company, New York.
Minot, C.S. (1968). A Study on Cytomorphosis. Putman's Sons, New York.
Mukhopodhyay, M. *et al.* (2000). *Fd. Chem. Toxic*, **38**: 75-77.
Roberts, A. *et al.* (2000). *Fd. Chem. Toxic*, **38**(2): S31-41.
Sasaki, Y.F. and Tsuda (2002). *Mutation Res.*, **519**(1-2): 103-109.