STUDIES ON WHOLESOMENESS EVALUATION OF RADURIZED INDIAN MACKEREL (RASTRELLIGER KANAGURTA)

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INTRODUCTION

Pasteurization doses of gamma radiation have been shown to extend the useful shelf-life of a variety of fishery products (1,2). Preservation of mackerel (Rastrelliger kanagurta) up to 23-28 days when held in ice (0-2°C) by a radurization process, employing 150 krad gamma radiation dose has been reported earlier (3). Besides, development of suitable radiation processing methods, a part of our effort has gone into the wholesomeness evaluation of some of the products subjected to radiation treatment (5). The present study relates to the safety and wholesomeness evaluation of radurised Indian mackerel, incorporated into the diet of Wistar rats and also on the mutagenicity evaluation in male mice and rats.

The radurization process for mackerel has been outlined elsewhere (3). The treated fish were stored for about 18 days at 0°C to 2°C which were deboned and dried at 60°C to about 8% moisture. The mackerel powder was packed in polythene bags and stored at -10 to -15°C. The mackerel powder was incorporated in the diet at 35% level and the diets were made nutritionally adequate with other ingredients such as starch, sugar, oil, salt mixture etc. as described elsewhere (4). The protein content of the diet was 26%. The diets thus prepared were fed to inbred Wistar strain albino rats and Swiss mice of both sexes reared in the animal house of this Research Centre. The detailed grouping of the animals used in these studies have been described elsewhere (4).

WHOLESOMENESS OF RADURISED MACKEREL

Food intake and growth: Body weight gains of males and females fed the irradiated mackerel diet were comparable with those fed on unirradiated mackerel diet, throughout the study. The group fed on stock ration exhibited relatively lower body weights than the mackerel fed groups. Daily food consumption, determined for first 28 days, showed comparable values among the different groups. The mean food efficiency ratios were similar in groups fed the irradiated and the unirradiated mackerel diets, but was relatively low for the stock ration group.

Reproductive performance: Reproduction studies did not reveal any major differences in the fertility index, litter size and growth of pups during lactation among the three groups. Maternal body weight during gestation and lactation periods, as well as the survival of pups during lactation were comparable between the groups fed on irradiated and unirradiated mackerel diets. However, as compared with the stock ration group the total progeny losses were high in both the groups fed on the mackerel diets, due to high cannibalism exhibited by these animals. No differences in the loss of progeny were noticed between the groups fed on the irradiated and the unirradiated mackerel diets. A comprehensive evaluation of reproductive capacity did not show any adverse effect due to feeding of irradiated mackerel diet.

Haematology and clinical chemistry: There were no significant intergroup differences in the haematological profile of animals and the values for various parameters were within the normal limits. Likewise, detailed clinical chemistry data and other biochemical investigations involving various serum and liver enzymes and other constituents did not show any significant changes between the group fed on diets containing the unirradiated or the irradiated mackerel. The mean serum albumin value for males fed on unirradiated mackerel diet was lower than the males fed on the irradiated mackerel diet, no such effect was noticed in females. The content of alpha, beta and gamma-globulin in serum was not significantly different between the groups.

With the exception of relative liver weight of the males no major changes in either the absolute or the relative organ weights were observed between groups fed on irradiated or unirradiated diets for 90 days. The gross pathological changes that were evident were minor and were equally distributed among different groups.

MUTAGENICITY EVALUATION

Micronucleus test: After the completion of a total of 21 weeks of feeding, animals fed on irradiated or unirradiated mackerel or stock ration showed no significant differences either in the frequency of micronuclei or the ratio of poly to normochromatic cells whereas the animals treated with hycanthone showed a significant increase in the incidence of micronuclei and also affected the polychromatic to normochromatic cells (Table I).

Table I. INCIDENCE OF MICRONUCLEI IN THE BONE MARROW ERYTHROCYTES OF MICE

	Stock diet	Unirradiated mackerel diet	Irradiated mackerel diet	EMS
% Poly-E with MN	0.29 ± 0.03	0.20 ± 0.04	0.30 ± 0.05	*3.62 ± 0.58
% Normo-E with MN	0.14 ± 0.07	0.16 ± 0.03	0.15 ± 0.04	0.30 ± 0.06
Total	0.43	0.36	0.45	* 3.92
P/N ratio	0.93 ± 0.01	0.89 ± 0.02	0.93 ± 0.03	0.56 ± 0.01

Poly-E = Polychromatic erythrocytes, Normo-E = Normochromatic erythrocytes, MN = Micronuclei, P/N ratio = ratio of total polychromatic to normochromatic cells.
*Values are significantly different from corresponding values for remaining groups.

Dominant lethal assay: After 16 weeks of feeding 20 males from each group were given laboratory stock ration and paired with 2 virgin females for a period of one week. The females were replaced weekly and five such weekly pairings were carried out sequentially. The females were killed 10 - 11 days after the separation from the males and examined for dead (deciduomas and dead embryos) and live implantations as reported earlier (6). The number of dead implantations showed no significant difference among the groups fed on stock ration or irradiated or unirradiated mackerel diets at any stage of the test period (Table II). The incidence of females

Table II. DEAD IMPLANTATIONS PER PREGNANCY

Week	Stock diet		Unirradiated mackerel diet		Irradiated mackerel diet		EMS	
	DI %	Freq fem with DI %	DI %	Freq fem with DI %	DI %	Freq fem with DI %	DI %	Freq fem with DI %
1	11.4	53	10.7	50	9.1	45	15.1	77
2	8.5	42	9.7	46	8.9	54	50.0	100
3	10.2	62	11.1	52	11.1	64	16.3	63
4	8.7	41	4.7	37	7.9	42	4.6	31
5	12.8	56	13.2	63	9.2	70	9.4	52

DI = Dead implants
The value marked with asterisk is significantly different from control group.

with dead implantations among the various groups was not significantly different. There was also no increase in the pre-implantation or total lethality. As expected, EMS treated mice showed a highly significant increase in the dead implantation rate and a reduction of the liver implantations during the post-meiotic phase of spermatogenesis.

A dominant lethal study was conducted using the F_1 male rats obtained from the first generation animals of a 90-day feeding study. No adverse effects of feeding radurised mackerel were observed. On the other hand, EMS-treated positive control group showed a highly significant increase in the dead implantation rates and a profound reduction of the live implantations during post-meiotic phase of spermatogenesis.

BONE MARROW METAPHASE ANALYSIS IN WISTAR RATS

Three groups of male and female Wistar rats after 90 days of feeding on the respective diets were given colchicine (1 mg/kg) and killed 2 hr after the injection and bone marrow preparations were made as reported earlier (6). No significant differences were observed among different groups with regard to total abnormal cells, chromatid gap and break, number of polyploid cells remained comparable between animals fed on irradiated or unirradiated mackerel diets. The frequency of chromosome breaks and fragments was low in the unirradiated mackerel group. The incidence of chromosomal breaks was comparable between animals fed on stock ration and irradiated mackerel diet and within normal limits (Table III). The difference between the animals

Table III. BONE MARROW METAPHASE ANALYSIS IN WISTAR RATS FED RADURIZED INDIAN MACKEREL

Group		No. of metaphases scored	Chromatid gap	Chromatid break	Chromosomes break	Fragment	Polyploidy	Total abnormal cells
Stock ration	No.	1741	5	3	2	3	10	23
	%		0.29	0.17	0.12	0.17	0.57	1.32
Unirradiated mackerel diet	No.	1938	6	6	-	1	2	15
	%		0.31	0.31		0.05	0.10	0.77
Irradiated mackerel diet	No.	1816	7	5	5	2	3	22
	%		0.39	0.28	0.28	0.11	0.17	1.21

fed on the unirradiated and the irradiated mackerel diets was also not significant as revealed by the Kolmogorov-Smirnov test of nonparametric analysis. The incidence of polyploidy was comparable between the irradiated and unirradiated mackerel diet.

In conclusion, the feeding of radurised mackerel diets to rats did not show any deleterious effects on growth reproduction and other biochemical parameters. These studies also did not indicate any mutagenic effects in the somatic and germ cells of mice and rats that could be attributed to consumption of radurised mackerel.

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