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Original Research Paper

Screening of Some Market and Home-Made Pickles for Their Genotoxicity

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ABSTRACT

In the present paper 25 different types of market, home and laboratory made mango (*Mangifera indica*) pickle samples were tested for their possible genotoxicity owing to widespread adulteration in market pickles with chemical preservatives, colouring agents and low quality ingredients. Mutagenicity and antimutagenicity tests were performed using Ames' test and using standard chemical mutagens (aminopyrene, sodium azide and nitrosoguanidine). The 66.7% of packed market pickles showed positive antimutagenicity test; and 33.3% positive antimutangenicity test. 100% loosely sold pickles showed mutagenicity test positive and antimutagenicity test negative. Among home-made pickles, 60% showed antimutagenicity test positive, and 40% mutagenicity test positive. The laboratory-made pickles showed negative mutagenicity test and positive antimutagenicity test in all the pickles. The primary findings about the quality of pickles consumed by vast population showed that they are of poor chemical quality and alarming at their potential genotoxicity. On the contrary, the laboratory-made pickles prepared without any chemical preservatives and chemical colourants, and with selective quality ingredients and selective microbial culture mix showed that all the samples have antimutagenicity test positive and mutagenicity test negative.

INTRODUCTION

Pickles are food items commonly used in the daily diet all over the world, though the amount used at a time in the meals or with food dishes is small but considerable. The physicochemical and organoleptic qualities and safety in their consumption definitely matters. In the mango pickle making, after washing, cleaning, surface drying and cutting of raw mango fruits, the pieces are mixed with mango pickle mix which contains common salt, asafoetida, chilly and turmeric powder, mustard dal, edible oil. In addition, chemical preservatives and attractive colourants are also added. The low grade raw materials and ingredients are generally masked by preservatives and attractive colourants. This lowers the quality of products and also poses possible threat or danger of genotoxicity effects on regular consumers. Hence, testing of pickle samples for mutagenicity and antimutagenicity becomes a dire need.

In the present work, an attempt has been made to screen the market and home-made pickles available in packets or loose for their genotoxicity by Ames' test and its comparison with pickles made in the laboratory.

MATERIALS AND METHODS

Collection of market pickle samples: Unbranded mango pickle samples of 100g packets were directly collected, while loosely sold pickle samples were collected in about 100g in sterile glass con-

tainers, brought to the laboratory, and stored at 4°C in a refrigerator till further use.

Preparation of mango pickles in the laboratory: The selective raw mango fruits in 250g quantity were washed with sterile distilled water and cut into pieces of about 1.0 × 1.0 × 1.0 inch size and mixed in a China clay pot with laboratory made pickle mix containing chilly powder, turmeric powder, common salt, asafoetida, mustard dal, edible oil and selective microbial seed mix inoculum. After 15 days of maturing, the pickle was used for further testing.

Standard chemical mutagen solutions (1mg/mL): Three chemicals were used as chemical mutagens, nitrosoguanidine, aminopyrene and sodium azide.

Mutagenicity and antimutagenicity testing by Ames' test: (Ames 1971, OECD 1983, Maron & Ames, 1983, Zeiger & Mortelman 1999, Ames & Durstion, 1973, Kruawan & Kangsadalampai 2006).

Test bacteria: *E. coli* vit B_{12} single reversible auxotrophic mutant and *Salmonella typhimurium* histidine auxotroph

Rat liver homogenate: Rat liver was washed and minced in sterile and ice cold 0.15N KCl, homogenized and centrifuged at 9000 rpm for 10 min aseptically and supernatant was used as rat liver homogenate.

Mineral salt agar medium: M-9 medium was prepared using 6.0 g anhydrous Na_2HPO_4, 3.0 g anhydrous KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl in 1 litre of distilled water and agar (15.0 g for plates or 8.0 g for soft agar) and autoclaved. After autoclaving, before plating the medium was added with 20 mL sterile 20% glucose solution, 10 mL sterile 0.1mM $MgSO_4$ and 10 mL sterile 0.01 mM $CaCl_2$.

Mutagenicity testing: Sterile filter paper discs were impregnated in sterile distilled water (negative control); filter sterilized solution of standard mutagens (positive control) and homogenized pickle extract (test) were placed at the centre on the surface of soft agar layers (2mL soft agar at 45°C seeded with 0.1 mL washed test bacterium freshly grown and 0.5 mL sterile rat liver homogenate) on the mineral salt agar layers in the plates and the plates were incubated at 37°C for 48 hrs. The experiment was done in triplicates using two test bacterial cultures. Development of colonies around filter paper discs were counted. Development of colonies and their increased number in case of test and standard mutagen plates as compared to control plates was taken as positive mutagenicity result.

Antimutangenicity testing: To test antimutangenicity effect of pickle in the above mutagenicity testing in the test and standard mutagen positive control set the filter paper discs impregnated with standard mutagen solution prepared in pickle extract were used. Decreasing colony number pattern in this set as compared to positive control set and the test plate sets together was taken as antimutagenicity effect of the pickle material.

RESULTS AND DISCUSSION

The results of mutagenicity and antimutagenicity tests of different pickle samples using *E. coli* and *S. typhimurium* auxotrophic bacteria and three standard mutagens are shown in Tables 1 and 2. It is evident from the Table 1 that out of nine packed market pickle samples, three samples (33.3%) viz. no. 3, 6 and 9 showed mutagenicity test negative, while remaining six samples (66.7%) viz. no. 1, 2, 4, 5, 7 and 8 showed it positive with both *E. coli* and *S. typhimurium* auxotrophic test bacteria after comparing the test set results with positive control set results of known mutagens and with that of negative control sets without any mutagen.

All the eight pickle samples which were loosely sold in market showed mutagenicity test positive with both the test bacterial cultures.

Out of the five home-made pickle samples, two (40%) were positive for mutagenicity and three (60%) were negative with both the test bacteria. All the three laboratory made pickle samples (100%) showed negative mutagenicity.

Herbal extract/plant materials are known for antimutagenicity potential (Kruawan & Kangsdalampai 2006, Lamaison et al. 1991). It is evident from Table 2 that out of the nine packed market pickle samples, three, viz. no. 3, 6 and 9 samples showed antimutagenic activity. In case of samples 1, 2, 4, 5, 7 and 8, colonies appeared on plates with nitrosoguanidine mutagen ranging from average of 43 to 77 in number. With aminopyrene mutagen, colonies ranged from average of 31 to 61 in number and with that of mutagen sodium azide from 37 to 59. In case of pickle numbers 3, 6 and 9 colonies showed average range of 18-33 for nitrosoguanidine, 18-23 for aminopyrene and 14-21 for sodium azide. Comparative account of appearance of colonies of both the test bacteria on plates after exposure to three known mutagens and these mutagens with pickle sample extracts showed that pickle samples 3, 6 and 9 have same antimutagenic activity. All the eight loosely sold pickle samples showed no antimutagenic activity where the colony number of both the test bacteria, obtained with

Table 1: Mutangnicity testing of pickle extracts with *E. coli* and *Salmonella typhimurium* auxotrophs.

Pickle type used in the experiment	Pickle No.	Colony number average of sets in triplicate with *E. coli*						*S. typhimurium*					
		Negative control	Positive control *1	*2	*3	Test	Remark about test	Negative control	Positive control *1	*2	*3	Test	Remarks about test
Packed market pickles	1	5	60	36	30	16	mutagenic	7	83	52	40	15	mutagenic
	2	4	47	29	40	21	mutagenic	2	71	49	47	19	mutagenic
	3	6	49	41	23	8	non- mutagenic	5	84	38	32	8	non- mutagenic
	4	3	71	26	25	21	mutagenic	8	68	47	33	20	mutagenic
	5	2	44	42	33	27	mutagenic	4	59	41	39	27	mutagenic
	6	7	63	38	22	8	non -mutagenic	6	63	29	46	8	non- mutagenic
	7	6	52	28	26	20	mutagenic	3	70	61	48	19	mutagenic
	8	4	46	30	39	15	mutagenic	3	80	40	44	23	mutagenic
	9	8	44	33	35	10	non- mutagenic	9	73	37	28	12	non- mutagenic
Loosely sold market pickles	1	4	64	51	47	21	mutagenic	11	59	45	39	19	mutagenic
	2	9	68	46	33	18	mutagenic	9	71	61	50	32	mutagenic
	3	7	80	42	38	32	mutagenic	7	68	66	46	31	mutagenic
	4	3	75	49	31	16	mutagenic	3	81	49	40	27	mutagenic
	5	2	57	54	48	18	mutagenic	8	75	54	47	26	mutagenic
	6	11	61	57	42	29	mutagenic	12	59	44	47	39	mutagenic
	7	5	73	41	38	21	mutagenic	6	77	63	51	17	mutagenic
	8	11	77	46	38	27	mutagenic	8	74	60	49	23	mutagenic
Home-made pickles	1	8	65	30	26	14	mutagenic	5	61	53	32	16	mutagenic
	2	11	49	44	41	09	non- mutagenic	7	72	64	38	5	non -mutagenic
	3	2	77	42	33	18	mutagenic	8	57	51	47	21	mutagenic
	4	7	68	54	47	8	non- mutagenic	13	69	54	39	10	non- mutagenic
	5	8	62	40	35	7	non- ,utagenic	9	61	54	49	08	non- mutagenic
Laboratory made pickle samples	1	7	76	35	39	8	non- mutagenic	11	63	49	47	13	non- mutagenic
	2	3	63	39	22	4	non- mutagenic	4	68	65	34	3	non- mutagenic
	3	12	55	53	44	11	non- mutagenic	9	76	54	34	11	non- mutagenic

*1 – Nitrosoguanidine, *2 – Aminopyrene, *3 – Sodium azide

Table 2 : Antimutagenicity Testing of Pickle Extracts using *E. coli* and *S. typhimurium* auxotrophs with standard mutagens.

Pickle Type	Pickle No.	Colony number average of sets in triplicate with *E. coli* *1	*2	*3	Remark*	S. typhimurium *1	*2	*3	Remark *
Packed	1	63	51	37	non antimutagenic	84	63	68	non antimutagenic
market	2	57	44	46	non antimutagenic	77	61	61	non antimutagenic
pickles	3	33	18	14	antimutagenic	50	27	21	antimutagenic
	4	77	31	39	non antimutagenic	71	60	60	non antimutagenic
	5	61	53	38	non antimutagenic	73	73	72	non antimutagenic
	6	29	21	17	antimutagenic	20	21	27	antimutagenic
	7	71	44	49	non antimutagenic	78	81	53	non antimutagenic
	8	43	61	59	non antimutagenic	83	68	49	non antimutagenic
	9	18	23	21	antimutagenic	31	18	18	antimutagenic
Loosely	1	69	58	71	non antimutagenic	64	68	47	non antimutagenic
sold market	2	75	58	53	non antimutagenic	83	70	72	non antimutagenic
pickle samples	3	95	61	71	non antimutagenic	89	76	73	non antimutagenic
	4	81	74	46	non antimutagenic	96	76	53	non antimutagenic
	5	88	69	55	non antimutagenic	79	68	71	non antimutagenic
	6	69	73	57	non antimutagenic	74	51	61	non antimutagenic
	7	94	68	49	non antimutagenic	81	79	65	non antimutagenic
	8	81	65	57	non antimutagenic	91	75	76	non antimutagenic
Home made	1	71	34	38	non antimutagenic	69	61	44	non antimutagenic
pickle samples	2	31	23	24	antimutagenic	34	39	26	antimutagenic
	3	83	49	41	non antimutagenic	61	68	55	non antimutagenic
	4	27	23	21	antimutagenic	44	37	21	antimutagenic
	5	29	25	20	antimutagenic	51	37	23	antimutagenic
Laboratory	1	29	13	23	antimutagenic	36	31	34	antimutagenic
made pickle	2	20	10	13	antimutagenic	41	29	19	antimutagenic
samples	3	16	19	18	antimutagenic	38	24	17	Antimutagenic

*1 – Nitrosoguanidine, *2 – Aminopyrene, *3 – Sodium azide

* Remark about sets of standard mutagen with pickle extract and in comparison with results in Table 1

and without pickle extract, in case of nitrosoguanidine, aminopyrene and sodium azide are almost comparable. Out of the five home-made pickle samples, three (60%), viz. 2, 4 and 5 showed decreased colony number, i.e., 31, 27 and 29 for nitrosoguanidine, 23, 23 and 25 for aminopyrene, and 24, 21 and 20 for sodium azide after mixing of these mutagens with pickle extracts and as compared to only mutagens (without mixing with pickle extracts) indicating that the home made pickle samples 2, 4 and 5 possessed considerable antimutagenic activity. The remaining loosely sold pickles, viz. 1 and 3, showed no decrease in colonies indicating lack of antimutagenic activity. All the three laboratory made pickles, viz. 1, 2 and 3 showed significant decrease in colony numbers of both the test bacteria after exposure to test mutagens along with these pickle extracts indicating significant antimutagenic activity.

Further, it was found that the pickle samples 3, 6 and 9 from packed market pickles, no. 2, 4 and 5 from home-made pickles and all the three laboratory made pickles showed no mutagenic activity but showed antimutagenic activity, i.e. out of total 25 pickle samples tested 9 pickle samples (36%) showed antimutagenic activity while remaining 64% showed mutagenicity but not the antimuta-

genic activity.

There are few reports (Surh et al. 1999, Tseng et al. 1998, Calomme et al. 1996, Lamaison et al. 1991) of detection of genotoxicity through mutagenic potential of food items but reports are scanty with respect to genotoxicity through mutagenicity in case of different pickle samples consumed in India. Hence, there is dire need of assessment of genotoxicity potential of different pickle samples so as to prevent slow poisoning of vast majority of human population in India owing to consumption of such underquality pickles.

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