ABSTRACT: In human, colon cancer is the major health problem worldwide. It arises by a well-defined series of histopathological changes (adenoma-carcinoma sequence), which are paralleled by mutations and deletions of tumor suppressor genes leading to chromosomal aberrations. Chromosomal aberrations are considered to be good somatic biomarkers of colon cancer and occur with the greatest frequency in lymphocytes. The present work was undertaken with objective to study the protective effect of probiotic dahi on generation of chromosomal aberrations (numerical and structural) in lymphocytes of 1, 2-dimethylhydrazine induced colon carcinogenesis in rats. The rats were divided into four groups 1) Normal diet control group; 2) DMH control; 3) Normal dahi (ND) group; 4) Probiotic dahi (PD) group. Rats were injected with DMH and sacrificed at 50th week of experiment, metaphasic chromosomes were prepared from blood lymphocytes and incidences of chromosomal aberrations were studied. In normal diet group, DMH control group, ND group and in PD group incidence of chromosomal aberrations were 2.0, 15.33, 12.0 and 4.66% respectively. Administration of probiotic dahi to rats significantly decreased the incidence of chromosomal aberrations in lymphocytes as compared to DMH control rats and rats fed with normal dahi (P<0.05). The findings of present study suggest that probiotic dahi exerts its antigenotoxic effects by preventing the chromosomal aberrations.

KEY WORDS: Chromosomal aberrations (CAs); 1, 2-Dimethylhydrazine (DMH); Genotoxicity; Normal dahi; Probiotic dahi

INTRODUCTION

The consumption of functional foods can be used as valuable strategy to control the physiological function of the body in beneficial manner. Functional foods are also known as nutraceuticals, designer foods, medicinal foods, therapeutic foods etc. The development of such foods has been the growing application of probiotic microorganisms that reside in the intestinal tract and are frequently associated with the health promoting attributes. The lactobacilli are important inhabitants of the intestinal tract of human and animals and are involved in number of health beneficial roles. Several studies have demonstrated the potential health benefits of lactobacilli and bifidobacterium against carcinogenesis. Dietary supplementation with Lactobacillus acidophilus has been reported to delay the development of colon cancer in rats exposed to 1, 2-dimethylhydrazine (Goldin and Gorbach, 1980). 1,2-dimethylhydrazine (DMH) and azoxymethane (AOM) are commonly used to induce colon cancer in experimental rats. It requires activation through a series of oxidative transformation in liver by microsomal oxidases and further oxidation and cytochrome P450 IIE1 metabolism forms the ultimate carcinogen which forms the adduct with DNA and cause the mutations (Drukrey, 1969). Carcinogen induced mutations lead to cause the chromosomal aberrations, which are considered as the important somatic markers in carcinogenic studies. Kanna et al., 2004 reported 11.6%, 41.6% and 45.6% structural chromosomal aberration after 2nd, 4th and 6th weeks of DMH injection while numerical aberrations were 6.4%, 28% and 30.4% after 2nd, 4th and 6th weeks, respectively.

A probiotic dahi was prepared in our laboratory by incorporating the probiotic cultures Lactobacillus acidophilus and Lactobacillus casei. Singh (2007) studied the anticarcinogenic effect of this dahi through number of molecular, histological, fecal and biochemical parameters and reported a strong tumor inhibitory and hepatoprotective activities. However the mechanism of this anticarcinogenecity was not known significantly.

The present work was designed to determine the effect of probiotic dahi on molecular alteration during DMH induced...
MATERIAL AND METHODS

Chemicals and bacterial strains

The probiotic strains Lactobacillus acidophilus, Lactobacillus casei spp. casei and dahi culture DRC-1 (Lactococcus lactis spp. biovar. diacetylactis NCDC-60) were procured from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal, India. Probiotic dahi was prepared by inoculating the Probiotic cultures and dahi culture in milk procured from the Experimental Dairy Unit, NDRI, Karnal. The milk was boiled at 90 °C for 30 minutes with constant stirring and then allowed to cool up to 30 °C. The culture was inoculated and kept for 12 hours on 37 °C. The gross chemical composition of probiotic dahi, viz. moisture (78.23%), total solid (21.08%), protein (3.94%), fat (2.52%), lactose (3.73%), pH value (4.98) and titratable acidity (1.01%) were within the range typically of normal dahi or yoghurt group. In addition normal dahi was prepared using DRC-1 (Lactococcus lactis spp. biovar. diacetylactis) culture and incubated at 30 °C for 12 hr.

Animals

Male albino rats of Wistar Strain (6-8 weeks) were obtained from Small Animal House of NDRI. The experimental protocol was got approved from the Institute Ethics Committee of Animal Experiments (IEAE). The rats were housed in a temperature-regulated experimental room under conventional conditions maintained at 21 ± 1 °C, with 40-60% humidity, and an inverse artificial light-dark cycle of 12 h (light period from 0800 to 2000 h).

Grouping of animals and DMH administration

The rats were distributed into four groups 1) Normal diet control- rats were fed with normal diet; 2) DMH control-rats were fed with normal diet along with DMH injections; 3) Normal dahi (ND) group-normal dahi was given along with normal diet and DMH injections were given; 4) Probiotic dahi (PD) group-probiotic dahi was fed to rats along with normal diet and DMH injections were given. A standard diet containing 45% starch, 25% Bengal gram, 10% casein, 5% sucrose, 10% soybean oil, 1% vitamin mix, 4% mineral mixture, 0.2% choline chloride and 0.35% methionine was given to experimental rats along with supplement diet viz. dahi and milk. Vitamin and mineral mixtures were prepared and blended according to AOAC, 1984. Probiotic dahi and normal dahi was given as 30% of total diet to Probiotic dahi and normal dahi group, respectively. The rats in normal diet group and DMH control group received milk instead of dahi.

Subcutaneous injections of DMH (DMH dissolved in normal saline containing 1.5g/l of EDTA, pH 6.4, at a dose rate of 20mg/kg body weight) was given to animals at 5th week of experiment once weekly for 15 weeks. The rats in the normal diet group were injected with an equal volume of normal saline for 15 weeks.

Analysis of cytogenetical profile in lymphocytes

The in vivo experiment was terminated at 50th week by sacrificing all rats and blood was collected from heart in heparinized vacutainer tubes. Chromosome preparations were obtained by the procedure of short-term whole blood culture technique (Yadav, 1981) with some modifications. The brief description of the technique is as whole blood was cultured in sterile 30 ml culture tube containing 5 ml Hams F-12 medium supplemented with 15% fetal calf serum, 0.1% Phytoheamagglutinin (PHA), 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% tryptose phosphate. The pH of the medium was adjusted to 7.2 with 4.4% sodium bicarbonate solution. Prior to addition of fetal calf serum, media was filtered through Millipore membrane (0.22μm). Blood cells were inoculated to culture medium at a concentration of 5 × 10^7 viable cells/ml. Culture tubes were incubated for 72 hours (3 cell cycles) at 37 °C in a rocking platform incubator. Eight hours before the end of the experiment 100 μl of 0.12% bromodeoxyuridine (BrdU) was added as protocol described by Ramagnano and Richer (1984) for chromosomal banding followed by addition of two drops of colchicin (0.5μg/ml culture media) 30 minutes just before the completion of 72 hours to obtain metaphasic cells. On completion of incubation period of 72 hours, the contents of culture tubes were transferred into centrifuge tubes and cell pellets were obtained by centrifugation at 1500 rpm for 25 min at room temperature. Cell pellets were resuspended in 10 ml hypotonic solution (0.075 M KCl, pre-warmed at 37 °C) and incubated in water bath at 37 °C for 9 min. The cells were fixed by adding 5 ml of chilled fixative (methanol: acetic acid, 3:1) and mixed gently with the help of Pasteur pipette and centrifuged at 1000 rpm for 25 min to obtain cell pellet. The pellet was washed with 10 ml chilled fixative until (3 times) white pellet was obtained. Pellet was resuspended in 5 ml fixative and stored at -20 °C. Chromosome was prepared by dropping metaphasic cells on grease free slides at a height of 1 meter. Slides were allowed to dry in air under Laminar Airflow Biosafety Cabinet. Chromosomes were stained by both conventional Giemsa staining (Sumner, 1980) and banding techniques (Yadav and Balakrishnan, 1985). One hundred fifty well-spread metaphase plates with morphologically straight armed chromosomes were scored from each treatment group, and aberrations; chromatid break, chromatid gap, dicentric chromosomes, aneuploidy, euploidy were observed under microscope (1000X).

Statistical differences between treatment groups were evaluated by applying Z- test. Differences were considered significant at P <0.05
FIGURE 1. (A) Normal metaphase chromosomes of rat, (B) Metaphase plate showing double monosomy (loss of two chromosome within set) with dicentric chromosome (bold arrow) and chromatid break (light arrow), (C) Metaphase plate showing chromatid gap (bold arrow) and chromatid break (light arrow), (D) R-Banding of Metaphase plate showing (arrow) monosomy (loss of one chromosome within set) and chromatid break. (E) Metaphase plate showing polyploidy, and (F) Metaphase plate showing pulverization of chromosomes in DMH control rats.
RESULTS

The effect of probiotic dahi in protection of genotoxicity induced by colon carcinogen DMH was mainly conducted to record the structural and numerical aberrations of chromosomes in four experimental groups. The various chromosomal aberrations recorded were chromatid break, chromatid gap, dicentric chromosome, monosomy, trisomy and polyploidy. Figure 1A shows normal metaphasic chromosomes of Wistar rat. The diploid chromosome number in control rat from normal group was 42 (42, XY). Figures 1B and 1C show effects of DMH in terms of genotoxicity viz. monosomy, chromatid gap and chromatid break. Figure 1D represents R-banding of metaphasic chromosomes showing the monosomy and chromatid breaks. Metaphasic plates also showed hypoaneuploidy (monosomy, Figure 1B) and allopolyploidy (Figure 1E). Metaphasic plates of DMH control rats showed pulverization (spontaneous breakage of DNA during metaphasic stage) of chromosomes (Figure 1F). Incidences of various types of chromosomal abnormalities in all groups have been summarized in Table 1. In normal diet group, DMH control group, ND group and in PD group incidence of chromosomal aberrations were 2.0, 15.33, 12.0 and 4.66% respectively. The incidence of different types of aberrations in normal group, DMH control group, ND group and PD group was found to be chromatid break- 0.0% 5.33%, 4.0%, 0.66%; chromatid gap- 0.66%, 4.66%, 3.33% 0.66%; dicentric chromosome- 0.0%, 2.0%, 2.66%, 0.66%; monosomy- 0.0%, 1.33%, 0.66%, 0.66%; trisomy- 0.0%, 1.33%, 0.66%, 0.66% and polyploidy- 1.33%, 0.66%, 0.66%, 1.33% respectively. Among all the groups DMH control group showed the highest incidence of chromosomal aberrations which was significantly higher (P<0.01) than rats fed with probiotic dahi. Rats fed with probiotic dahi showed significantly lower (P<0.05) incidence of chromosomal aberrations than rats fed with normal dahi. Rats in ND group showed no significant difference in incidence of chromosomal aberration as compared to DMH control group.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NUMBER OF METAPHASE STUDIED</th>
<th>NO. OF ABERRANT CHROMOSOMES</th>
<th>STRUCTURAL ABERRATIONS</th>
<th>NUMERICAL ABERRATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CB</td>
<td>CG</td>
<td>DC</td>
</tr>
<tr>
<td>ND</td>
<td>150</td>
<td>03 (2.0)*</td>
<td>NIL</td>
<td>01b (0.66)</td>
</tr>
<tr>
<td>DMH-CONTROL</td>
<td>150</td>
<td>23 (15.33)</td>
<td>08a (5.33)</td>
<td>07a (4.66)</td>
</tr>
<tr>
<td>PD</td>
<td>150</td>
<td>07 (4.66)</td>
<td>01b (0.66)</td>
<td>01b (0.66)</td>
</tr>
</tbody>
</table>

( )* Numbers in bracket indicate incidence of chromosomal aberration in percentage

The data was analyzed statistically by applying z-test

The values in columns bearing different superscript are significantly different (P<0.05)

DISCUSSION

Experimental colon carcinogenesis is a multistep process involving three distinct stages, initiation, which alters the DNA coding information of normal cells followed by promotion and progression, which ultimately results in phenotypically altered transformed cells (Pitot, 1986). In view of the importance of chromosomal aberrations (CAs) as initiating lesions of multistage carcinogenesis, the present study involved to evaluate CAs to gain mechanistic insight into protective effects of probiotic dahi towards DMH induced genotoxicity and tumorigenicity. Feeding probiotic dahi resulted in reduction of CAs in lymphocytes from DMH treated rats. Chromosomal aberrations are considered to be a good somatic biomarker of cancer, since CAs occurs with greatest frequency in cells that are highly proliferative (Yarmoneko, 1998). Hagmar et al., 1998 reported that increase CAs frequency in lymphocytes was directly related with the risk of cancer in healthy human. An increase in total CAs was observed in rat’s lymphocytes as a result of the DMH treatment. Similar results were observed during preneoplastic period in the liver cells of rat treated with hepatocarcinogenic doses of Diethylnitrosamine (Hitachi et al., 1974 and Bishayee et al., 1997).

A number of genes have been reported to be responsible for repairing DNA breaks and preserving chromosomal integrity (Zhou et al., 2000; Khanna et al., 2001; Pierce et al., 2001). The loss of function of some of these genes can result in widespread chromosomal damage and contribute to tumorigenesis (Myung and Chen, 2001; Weiss et al., 2002). In mammalian cells, double-strand DNA breaks (DSBs) are repaired by homology-directed recombinations (HDR), non-homologous end joining (NHEJ), and single strand annealing (SSA) (Richardson et al., 2000b). Defects in any of these repair mechanisms can result in chromosomal breaks, fusions, and translocations (Richardson and Jasin, 2000a). Asa et al., 2003 reported that the overexpression of c-myc protooncogene during neoplasia disrupts the repair of double-strand DNA breaks resulting a high extent of increase in chromosomal breaks and translocations, so fuel tumorigenesis by inducing chromosomal damage. They also reported an increase in frequency of double strand breaks and chromosomal translocations in c-myc overexpressed cell when compared to normal cells.

Several workers have evaluated the protective effect of lactic acid bacteria against genotoxicity in mice. Goldin et al., 1980 reported that Lactobacillus acidophilus feeding reduced the incidence of DMH
induced colon tumors in rats. Singh, 2007 reported a strong tumor inhibitory and hepatoprotective activities of probiotic dahi. He also reported anticarcinogenic effect of probiotic dahi and found probiotic dahi as an effective chemopreventive for colon tumorigenesis by inhibiting aberrant crypt foci formation and increasing antioxidative enzymes in liver and colon. The results of present study are consistent with the finding of previous studies regarding DNA damage and chromosomal aberrations and support the role of probiotic dahi in protecting DNA. Singh et al., 2007 reported feeding probiotic dahi to rats significantly enhanced the activities of Glutathione - S transferase, Superoxide dismutase (SOD) and catalase in the liver and colon cells. They also reported that the administration of probiotic dahi significantly reduced the level of thiobarbituric acid reactive substances (TBARS) as compared to DMH control rats. Metabolism of DMH in the liver produced azoxymethane (a known carcinogen) ultimately leading to the generation of active electrophiles which form adducts with DNA and cause the mutation and DNA damage. In the present study, probiotic dahi feeding reduced the incidence of CAs. Probiotic dahi may enhance the antioxidant system resulting in the scavenging of free radicals and toxic carcinogenic electrophiles. Decrease in generation of active electrophiles may result in reduction in formation of DNA adducts with ultimate carcinogen, finally decreasing the mutation and DNA damage. Thus probiotic dahi may exert antigenotoxic effects in protecting DNA by preventing the formation of ultimate carcinogen which forms adduct with DNA and causes damage.

CONCLUSION

Comparison of cytogenetical changes during 1, 2-dimethylhydrazine induced carcinogenesis revealed that probiotic dahi is able to inhibit these changes strongly. The results provided experimental evidence indicating that probiotic dahi containing probiotic microorganisms _Lactobacillus acidophilus_ and _Lactobacillus casei_ show antigenotoxic effects in DMH induced colon carcinogenesis in rats.

REFERENCES


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