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INHIBITION OF 1,2 DIMETHYLHYDRAZINE INDUCED GENOTOXICITY IN RATS BY THE ADMINISTRATION OF PROBIOTIC CURD

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ABSTRACT: ***In the present study, anticancer effect of probiotic curd was evaluated by monitoring the DNA damage through comet assay, during the early stages of 1, 2 dimethylhydrazine induced colon carcinogenesis in male rats. Rats were divided in to four groups, first group was pre- initiation group in which curd was given for 4 weeks before the DMH (1, 2 dimethylhydrazine) injection, second one was initiation group in which curd was administered with first DMH injection, third group was DMH control group and Fourth group was normal control group. All animals received subcutaneous injection of DMH dissolved in normal saline at a dose rate of 20mg/kg body weight, once weekly for 15 weeks. Blood was collected one week after the last injection and comet assay in blood was done to assess the DNA damage. A significant reduction in DNA damage (55%) was observed in first group as compare to third group (93.33%). The difference between the pre-initiation group (55%) and the initiation group (48.83%) was not significant. Probiotic curd was significantly effective to reduce the L: W ratio. The present results showed that probiotic curd is able to reduce the DNA damage in colon cancer induced rats.***

KEY WORD: Comet assay, 1,2-Dimethylhydrazine, Genotoxicity, Length: Width (L: W), Probiotic curd

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INTRODUCTION

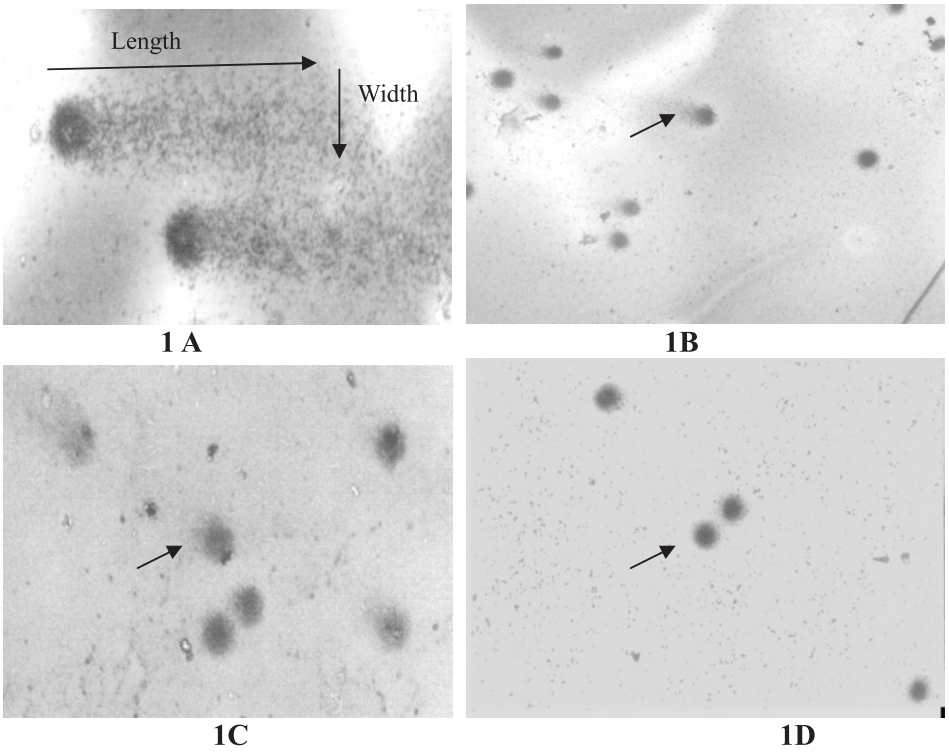
Abundant evidence implies that specific bacterial species used for fermentation of dairy products such as yogurt and selected from healthy gut microflora have powerful anti pathogenic and anti-inflammatory properties (Adolffsson et al., 2004). Epidemiologic and experimental studies suggest that the consumption of fermented milk products and lactic acid bacteria decrease the incidence of certain types of cancer. Probiotic organisms are effective in preventing colon carcinogenesis, which is the main cause of mortality and morbidity in western countries. 1, 2 dimethylhydrazine (DMH) is a common colon carcinogen used in animal studies. It is a pro carcinogen that requires activation through a series of *in vivo* chemical transformation in liver by oxidation through microsomal oxidases to the carcinogen azomethane, which on further oxidation, produces azoxymethane (AOM), also a common intestinal carcinogen. Cytochrome P450IIE1 metabolizes AOM to MAM, which degrades to formaldehyde and methyl diazonium ion. This ion breaks down to nitrogen and the ultimate carcinogen, the methyl carbonium ion, an alkylating species that forms adduct with nucleic acids (Weisburger, 1994).

Comet assay is the common technique to assess the genotoxicity. Comet assay can be used to investigate the genotoxicity of industrial chemicals, biocides, rays and pharmaceuticals. Comet assay can be applied to any tissue, provided that a single cell/nucleus suspension can be obtained. Two short term studies (Pool-zobel et al.,1996; Wollowski et al.,1999) using 1, 2-dimethylhydrazine as a genotoxic agent in rats, showed that various lactic acid bacteria (lactobacillus, bifidobacterium and *Streptococcus thermophilus*) are able to decrease colon DNA damage screened using the comet assay.

In the present study, we assessed the inhibition of DMH induced genotoxicity in rat's blood by feeding probiotic curd containing mixture of probiotic microorganisms. Tumors normally appear in 40 weeks so damage in colon tissue was not observed. Further this was the first study in our knowledge, where colon cancer induced DNA damage in lymphocytes cells was observed and correlated with protection through probiotic microorganisms. Probiotic curd was prepared by inoculating the Probiotic cultures (*Lactobacillus acidophilus* and *Lactobacillus casei*; National Collection of Dairy Culture, National Dairy Research Institute (NDRI), Karnal) and curd culture (*Lactococcus lactis* biovar. *diacetylactis* DRC-1) in milk procured from the experimental dairy, 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. A total of 24 rats were divided into 4 experimental groups consisting of 6 animals in each group: A) Pre initiation group: Probiotic curd was given 4 weeks before the DMH injection; B) Initiation group: Curd was given along with

the 1st injection of DMH C) DMH control group; D) Normal diet control group. A standard synthetic diet (containing 58% starch, 17% casein, 10% sucrose, 10% soybean oil, 1% vitamin mix, 4% mineral mixture, 0.2% choline chloride and 0.35 methionine) along with vitamin and mineral mixture prepared and mixed according to AOAC (1984). Curd was given as 30% of total diet while control group rats received equal amount of milk. At 10 weeks of age, all animals received subcutaneous injection of DMH dissolved in normal saline at a dose rate of 20mg/kg body weight, once weekly for 15 weeks. Blood was collected one week after the last DMH injection. Comet assay in lymphocytes was done by following the protocol of Ahuja and Saran (1999). Silver staining (Cereda et al., 1997; Nain et al., 2001) was done to stain the comet slides. The slides were observed under the inverted microscope (CK40, Olympus) fitted with ocular micrometer. The ratio of DNA length to width (L: W) and damage DNA per group was observed. Further to estimate the damage, the damaged DNA was scored by following the damage scoring no. in which the damage DNA had given some number from 0-4 having no damage to maximum damage. Then the observed DNA was matched with that scoring number and scoring number was assigned to that comet. Two slides were prepared from each animal's blood and hundred comets were observed from each slide.

FIGURE 1. Effect of feeding Probiotic curd on DNA damage A) Most damaged, B and C) Less damaged, D) Undamaged



Various types of DNA damage among groups are shown in Figure 1. The comet assay results are shown in Table 1. Probiotic curd significantly reduced the DNA damage in comparison to DMH control group. There are no significant differences in DNA damage and L: W ratio between Group A and B. The mean length to width ratio of the DNA mass, which indicates the extent of DNA damage, in group C was significantly higher ($p<0.01$) among all groups. In DNA damage scoring (Table 2), the DMH control group has maximum scoring number in the range of 3 and 4 while curd fed rat's DNA are in the range of 1 and 2 (no comet with scoring number 4).

In present study, the Probiotic curd significantly reduces the DNA damage. The probiotic microorganisms present in the probiotic curd were able to reduce the free radicals generated by the DMH metabolism and prevent adduct formation. This may be one of the reasons for less DNA damage in curd treated groups. Tavan et al. (2002) evaluated the effect of some LAB (Lactic acid bacteria) on heterocyclic aromatic amines induced colon carcinogenesis in rats. They divided the sixty rats in four groups with supplementation of tap water, 30% non fermented skimmed milk, with 30% *B. animalis* DN- 173 010 fermented milk and 30% *Streptococcus thermophilus* DN – 001 158 fermented milk. Comet assay and 3D test was done to assess the genotoxicity. They reported the significant decrease in DNA damage only for rats fed with fermented milk.

TABLE 1. Effect of Probiotic curd feeding on 1, 2-DMH induced DNA damage[a].
[a]Each value represents the mean ±Standard Error of Mean of data from 6 animals. DNA Damage percentage; L: W – length/width ratio of comet DNA mass. [b,c,d]Different superscripts are significantly different ($p<0.01$)

GROUP	TREATMENT	NO. OF RATS	CELLS (%) WITH DAMAGED DNA	L: W
A	Pre initiation	6	55.0 ± 1.125^{b}	1.370 ± 0.028^{b}
B	Initiation	6	48.83 ± 0.946^{b}	1.537 ± 0.017^{b}
C	DMH control	6	93.33 ± 0.333^{c}	3.138 ± 0.129^{c}
D	Normal control	6	15.03 ± 0.71^{d}	1.02 ± 0.05^{d}

TABLE 2. Scoring of comets in groups

GROUPS	COMETS (PERCENTAGE) IN SCORING NO.				
	0	1	2	3	4
A	52	40	7	1	0
B	45	46	8	1	0
C	6	26	18	39	11
D	85	14	1	0	0

Hence, in present study it may be concluded that probiotic curd inhibits the DNA damage in DMH induced colon carcinogenesis in rats, which may be one of the protective mechanisms of probiotic curd against colon carcinogenesis.

CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest in this study.

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