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1 ORIGINAL ARTICLE

Exopolysaccharides from *Lactobacillus acidophilus* modulates the antioxidant status of 1,2–dimethyl hydrazine-induced colon cancer rat model

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Abstract

The aim of the current study is to ascertain the anticancer activity of exopolysaccharides (EPS) from probiotic *Lactobacillus acidophilus* in the 1, 2–dimethyl hydrazine (DMH)-induced colon cancer rat model and to determine the antioxidant status. Rats were divided into five groups of six animals each. Group I served as control, group II served as cancer control (DMH AQ1 alone administered), group III as standard drug control (5-FU along with DMH) and group IV and V received EPS in two doses (200 mg/kg body weight and 400 mg/kg body weight along with DMH). EPS administration was found to reduce the number of polyps formed (Group IV—8.25 ± 1.258 and Group V—8.50 ± 1.732 vs Group II—14.50 ± 2.380) and to increase the levels of antioxidant enzymes viz. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and antioxidants like vitamin C (Vit. C), reduced glutathione (GSH) which was found to be reduced in colon cancer control rats. The status of lipid peroxidation (LPO) was also evaluated. All the values which were affected by the supplementation of DMH were brought to near normal levels by the treatment with EPS. The well-preserved histology of colon and the biochemical evaluation also show that EPS could be a potential agent for the prevention and treatment of colon cancer.

Keywords *Lactobacillus acidophilus* · Exopolysaccharides · Antioxidative enzymes · 1,2–dimethyl hydrazine · Colon cancer · Anticancer

Introduction

Several microbes get colonised in the gastrointestinal tract at child birth and become a constant part of the individual's body throughout the life (Rodríguez et al. 2015). This becomes the normal gut microflora and it is composed of many microorganisms especially bacteria. These bacteria colonise and grow under certain conditions at specific sites where it will coexist with the other colonising bacteria and competitively impede the growth of the other pathogenic bacteria (Houghteling and Walker 2015). But, the influence of several conditions like diet, drug intake etc. affect the gut microbiota which also is reflected in the health of the individual. Although several bacteria colonise the human gut most of them fall under the category of either obligate anaerobe or facultative anaerobe (Martels et al. 2017). Lactic acid bacteria (LAB) are a set of beneficial bacteria that produce lactic acid as a product by consuming sugar. They form a set of probiotic bacteria which is defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO 2001).

Probiotic bacteria especially LAB can be found in normal day-to-day used food substances like curd, yogurts, cheese, milk and infant formula (Davis and Milner 2009). LAB is composed of both aerobic and anaerobic bacteria (Martels et al. 2017; Zotta et al. 2017). Many bacteria in the genus *Lactobacillus* that utilise oxygen for growth fall under LAB. Some of them include *L. acidophilus*, *L. lactis*, *L. casei*, etc. (Zotta et al. 2017). Iannitti and

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Palmieri (2010), has reviewed various effects of probiotics including that they are effective against diverse medical conditions like antibiotic-induced diarrhoea, lowering of cholesterol, lactose intolerance, gastroenteritis, genitourinary tract infection, constipation and immunomodulation. Further studies have also suggested that administration of probiotics may play an effective role in the prevention of colorectal cancer (CRC), which is one of leading causes of mortality in humans. These studies have gained importance as this strategy might be helpful to prevent the onset of CRC (Drago 2019).

Exopolysaccharide (EPS) from several probiotic bacteria has been shown to anti-cancer potential under in vitro conditions. EPS from *L. helveticus* on HT-29 cells (Xiao et al. 2020), EPS from four strains of *Lactobacillus* against HT-29 cells (Di et al. 2018), EPS from *L. acidophilus* against CaCo-2 cells (El-Deeb et al. 2018) and EPS from Lactobacillus kefiri against HT-29 cells (Riaz Rajoka et al. 2019) are to name a few. Even after many studies, the precise mechanism behind the preventive action of probiotics on CRC remains to be elucidated. Several mechanisms have been put forward to elucidate the ~~mechanisms~~ which include alteration in the number and species of the intestinal microflora, binding/inactivation of carcinogens, competitive elimination of pathogenic microbiota, immunomodulation, reducing the survival of cancer cells by modulating the cell proliferation and apoptosis, breaking down of undigested food by fermentation. The other strategy of administration of probiotics along with prebiotics has also been reported to increase the efficiency of the anti-cancer mechanisms. Many probiotic species have also been shown to carry out its metabolic activities by acidification of pH, which of course is not considered as a separate mechanism (Perdigón et al. 2001; Le Leu et al. 2010; dos Reis et al. 2017; Drago 2019).

It is known that colon cancer arises from the epithelial cells which are found to line the bowel. These modified cells grow at a pace much higher than the normal somatic cells (Guz et al. 2008). But, chronic exposure of unwanted materials might result in the production of various reactive oxygen species (ROS), DNA damage, and imbalance in the redox enzymes which when accumulated results in tumour development (Escamilla et al. 2012). One of the potential approaches to prevent colon cancer is to decrease the level of ROS especially H_2O_2 (hydrogen peroxide). H_2O_2 is reported to be involved in various stages of tumour development like the increased proliferation and tumour progression. This also affects the spreading and metastasis of colon cancer cells. These processes can be regulated by the anti-oxidative enzymes (e.g. catalase) produced by bacteria present in the bowel. This can be used to reduce the risk of development of colon cancer and also reduce the spread and growth of colon cancer. Probiotic bacteria called *L. lactis* has been reported to control colon cancer through the aforementioned mode in DMH-induced experimental models (De Moreno De LeBlanc et al. 2008).

Exopolysaccharide is one of the natural products which is currently being studied for various applications and health benefits. EPS is a polymer of sugar which is produced by microorganisms in the extracellular environment or loosely bound with the cell surface (Han et al. 2014). Various lactic acid bacteria (LAB) have been reported to produce EPS (Lynch et al. 2018). *L. acidophilus, one* among the LAB present in the human intestine also produces EPS. *L. acidophilus* is also a GRAS (Generally considered as safe) organism. Previously we have shown that the purified EPS from *L. acidophilus* has antioxidant and anticancer properties in vitro (Deepak et al. 2016a, b). In this study, we are reporting the anti-cancer properties of EPS in the DMH-induced rat model. EPS were found to reduce the number of polyps formed and the levels of antioxidant enzymes were also upregulated when compared with cancer control.

Materials and methods

Selection, grouping and acclimatisation of laboratory animal

Male Sprague–Dawley rats having age of 21 days and 100 gm body weight were used throughout the study. All rats were kept in cages with wire mesh on top and kept at a temperature of 22 °C (± 2 °C) under 12 h light/12 h dark cycle in the animal house with relative humidity around 50%. Rats were fed with standard commercial pellet diet and water ad libitum freely throughout the study. The animals were quarantined for 15 days prior to study and were maintained in a hygienic environment in the animal house (OECD Guidelines 2002). The study was carried out after obtaining permission from the Institutional Animal Ethical Committee (KMCRET/PhD/15/2014–15) and OECD guidelines 420 were adhered in this study.

Induction of tumour

1, 2–dimethyl hydrazine (DMH) was obtained from sigma chemicals, and all other chemicals used in the study are of analytical grade. For the induction of tumour DMH was dissolved in 1 mM EDTA just prior to use and the pH was adjusted to 6.5 with 1 mM sodium bicarbonate to ensure the stability of the chemicals. Animals were given a weekly subcutaneous (s.c.) injection of DMH in the groin at a dose of 20 mg/kg body weight for a period of 15 weeks (Gurley et al. 2015). The animals were divided into five groups of six animals each. Group I was kept as control, which received 1 ml of normal saline for the entire period of study. Group II received DMH at a dose of 20 mg/kg body weight

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151 subcutaneously, once a week for the first 15 weeks. Group III received DMH 20 mg/kg body weight subcutaneously, once a week for the first 15 weeks (pre-neoplastic stage) + 5 Flurouracil 20 mg/kg body weight intraperitoneally for 4 weeks + DMH 20 mg/kg body weight subcutaneously, once a week for 4 weeks (neoplastic stage). Group IV received DMH 20 mg/kg body weight subcutaneously, once a week for the first 15 weeks (pre-neoplastic stage) + EPS 200 mg/kg body weight orally daily + DMH 20 mg/kg body weight subcutaneously, once a week for 4 weeks (neoplastic stage) and Group V received DMH 20 mg/kg body weight subcutaneously, once a week for the first 15 weeks (pre-neoplastic stage) + EPS 400 mg/kg body weight orally daily + DMH 20 mg/kg body weight subcutaneously, once a week for 4 weeks (neoplastic stage).

166 The rats were sacrificed at two different times. The first sacrifice was conducted 1 week after 15 consecutive weeks of injection (pre-neoplastic stage). At the end of the 15th week, one animal each from all four groups were sacrificed to confirm the development of tumour. After the confirmation of tumour in animals, the animals were further treated with standard and test compounds for the next 4 weeks along with DMH. The final sacrifice was done on the 19th week of experiment (neoplastic stage). At the end of the 19th week, all the animals from each group were anaesthetised and blood was collected by retro-orbital bleeding and used for analysis as explained elsewhere. Then the animals were sacrificed, tumours excised out and parameters like tumour incidence, tumour volume, tumour burden and tumour weight were studied (Baskar et al. 2012).

181 Evaluation of haematological parameters

182 At the end of the study, the animals were anaesthetised by ketamine hydrochloride and the blood was collected from retro-orbital sinus using a capillary tube and transferred into a centrifugation tube which contains either EDTA for haematological parameters or without EDTA for serum biochemical parameters. The blood was allowed to clot at room temperature and serum was separated by centrifugation at 10,000 rpm for 10 min.

190 After collecting the blood, the animals were sacrificed by cervical dislocation and their colon was excised immediately and washed in ice-cold normal saline, followed by 0.15 M Tris–HCl (pH 7.4) blotted dry and weighed. A colon homogenate was prepared by collecting the colon, minced it and mixed with 3 volumes (w/v) of buffer and centrifuged at 1200 × *g*. The obtained supernatant is used for further studies. A 10% w/v of homogenate was prepared in 0.15 M Tris–HCl buffer and processed for the estimation of lipid peroxidation. A part of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for estimation of glutathione. The rest of the homogenate was centrifuged 201 at 10,000 rpm for 10 min at 4 °C. The supernatant thus obtained was used for estimation of CAT (Sinha 1972), SOD (Kakkar et al. 1984), GPx (Rotruck et al. 1973), GSH (ELLMAN 1959) and Lipid peroxidation activities (Ohkawa et al. 1979).

207 The blood and other body fluids were removed with the help of blotting paper and then washed in normal saline and transferred to ice-cold containers with 10% formalin solution (Histopathological studies). Some tissues were cleaned with normal saline and again wiped and were used for other parameters like evaluation of in vivo antioxidants and enzyme assays. 213

Histopathological analysis 214

215 Thin pieces of around 3–5 mm thickness were collected from the colon showing gross morbid changes along with normal tissues. The tissues were kept in 10% formalin as fixative for 24–48 h at room temperature for preparation of paraffin and sectioning. The sections were deparaffinised using xylol for 5–10 min, and then absolute alcohol was used to remove xylol. The sections were then cleaned in tap water and again stained with haematoxylin for 3–4 min and again cleaned with tap water. This was then counterstained with 0.5% eosin until the section became light pink in colour. AQ2 The sections were mounted in Canada balsam and observed under 40 × magnification using a light microscope (Slaoui and Fiette 2011). 227

Statistical analysis 228

229 Data are presented as mean ± standard deviation. Treatment effects between groups were analysed by Kruskal–Wallis test and comparison between two samples was performed by Mann–Whitney *U* test. *P* values < 0.05 were considered statistically significant. 233

Results 234

Effect of EPS on the formation of polyps and colon weight 235 236

237 Table 1 shows the effect of EPS on DMH-induced rats. When the rats were treated with DMH (Group II), there is formation of polyps in the colon. The number of polyps in the colon of EPS-treated rats (Group IV and V) is significantly lower than the group II rats. When 10 cm of colon was weighed, the rats which were supplemented with DMH alone exhibited 20% reduction in weight which was found to be regained with EPS treatment. 244

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Table 1 Effect of EPS on polyp formation and the weight of colon

S. no	Parameter	Normal control (Group I)	Only DMH (Group II)	Standard 5-FU (Group III)	EPS 200 mg/kg b.w (Group IV)	EPS 400 mg/kg b.w (Group V)
1	Tumour burden	0.00 ± 0.000	14.50 ± 2.380	12.50 ± 1.000	8.25 ± 1.258*	8.50 ± 1.732*
2	Tumour incidence	0.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3	Colon weight	1.35 ± 0.152	1.09 ± 0.161*	1.33 ± 0.167	1.44 ± 0.213	1.37 ± 0.131

*The number of polyps were more in cancer control rats (Group II) which is reduced when treated with EPS (Group IV and V) which is also the case for weight of the colon

*$p < 0.05$

Effect of EPS on the haematological parameters

The effect of EPS on the haematopoietic system of rats is given in Table 2. From Table 2, it is evident that, in cancer control rats there is a decrease in the RBC content when compared with the normal rats. In the animals treated with EPS at 200 mg/kg b.w and 400 mg/kg b.w. concentrations the number of RBCs was found to be increased. Also there is an increase in the WBC count in cancer control rats (Group II), when compared to normal control. In animals treated with EPS at concentrations of 200 mg/kg b.w and 400 mg/kg b.w, the WBC content was found to be reduced. There was a significant reduction in the haemoglobin content in the cancer control group, as compared to the normal groups. The same was observed in the animals treated with EPS at concentrations of 200 mg/kg b.w. and 400 mg/kg b.w where the treatment increased the haemoglobin content which is also found to be proportional to the RBC content. Later when the lymphocytes were studied, cancer control rats had reduced lymphocyte number when compared with the control which is increased with the treatment of 200 mg/kg b.w. and 400 mg/kg b.w concentrations of EPS. In all the cases the effect of EPS was found to be similar to the standard drug 5-FU.

Effect of EPS on colon antioxidants

The effect of EPS, on the levels of both the enzymatic and non-enzymatic antioxidants, was checked in rats with DMH-induced colon cancer. The enzymatic antioxidants include SOD, CAT, GPx and the non-enzymatic include Vitamin C and GSH. The results are summarised in Table 3. When the rats were subjected to DMH treatment, the levels of both the enzymatic and non-enzymatic antioxidants were reduced than that of the control rats. But when they were treated with 200 mg/kg b.w. and 400 mg/kg b.w concentrations of EPS (Group IV and V) an increase in the antioxidant levels reaching the normal values could be observed. Besides, the level of LPO was also checked. LPO was also found to be reduced in the cancer control (Group II) when compared with the normal control (Group I). Similar to antioxidants the levels of LPO also increased and found to be reaching normal values when treated with 200 mg/kg b.w. and 400 mg/kg b.w concentrations of EPS (Group IV and V). These results suggest that the EPS may induce anti-colon cancer activity by activating various antioxidative enzymes and antioxidants.

Macroscopic examination

After the study, the animals were sacrificed by euthanasia, and the colon was surgically removed and macroscopic observations were made. Figure 1a–e shows the colons

Table 2 EPS induced changes in hematological parameters in DMH-treated rats

S. no	Parameter	Normal control (Group I)	Only DMH (Group II)	Standard 5-FU (Group III)	EPS 200 mg/kg b.w (Group IV)	EPS 400 mg/kg b.w (Group V)
1	RBC (10^{12} cells/l)	4.05 ± 0.58	2.10 ± 0.10**	3.87 ± 0.27	3.19 ± 0.25*	3.46 ± 0.45
2	WBC (cells/10 mm)	10.60 ± 0.47	6.33 ± 0.27**	10.30 ± 0.38	8.66 ± 0.25	9.70 ± 0.86
3	Hb (g/100 ml)	14.57 ± 0.81	8.20 ± 0.86**	12.90 ± 0.53	10.80 ± 0.62*	12.43 ± 0.53
4	Lymphocytes (No/100 WBC)	85.00 ± 2.36	43.33 ± 1.36**	77.67 ± 2.25	60.67 ± 1.86*	67.00 ± 0.89*

Hematological parameters where values which were affected by the treatment of DMH (Group II) is brought back to near normal levels by the action of EPS (Group IV and V)

*$p < 0.05$

**$p < 0.01$)

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Table 3 Status of the antioxidative enzymes in the colon of DMH and EPS-treated rats

S. no	Parameter	Normal control (Group I)	Only DMH (Group II)	Standard 5-FU (Group III)	EPS 200 mg/kg b.w (Group IV)	EPS 400 mg/kg b.w (Group V)
1	SOD units/min/mg	5.55 ± 0.53	2.86 ± 0.24*	6.06 ± 0.20	3.78 ± 0.18*	4.66 ± 0.24
2	Catalase µmole/min/mg	88.33 ± 4.13	52.33 ± 2.33**	89.33 ± 3.77	67.00 ± 2.36*	77.00 ± 2.36
3	LPO nM of MDA/mg	89.96 ± 56.46	64.81 ± 6.51**	101.15 ± 17.76	95.81 ± 6.50	83.31 ± 52.72
4	GPx µmoles/min/mg	43.50 ± 2.25	21.83 ± 1.72**	48.83 ± 1.94	33.50 ± 2.58*	39.83 ± .94
5	GSH µg/mg	31.00 ± 1.26	14.83 ± 1.72**	37.83 ± 1.47	20.83 ± 1.16*	31.33 ± 1.63
6	Vitamin C µg/mg	1.15 ± 0.14	0.33 ± 0.06*	1.13 ± 0.13	0.72 ± 0.06	0.92 ± 0.15

In all the cases the amount of antioxidants/antioxidative enzyme is reduced in the cancer control rats (Group II) which is brought to near normal levels by the treatment with EPS (Group IV and V)

*$p < 0.05$

**$p < 0.01$

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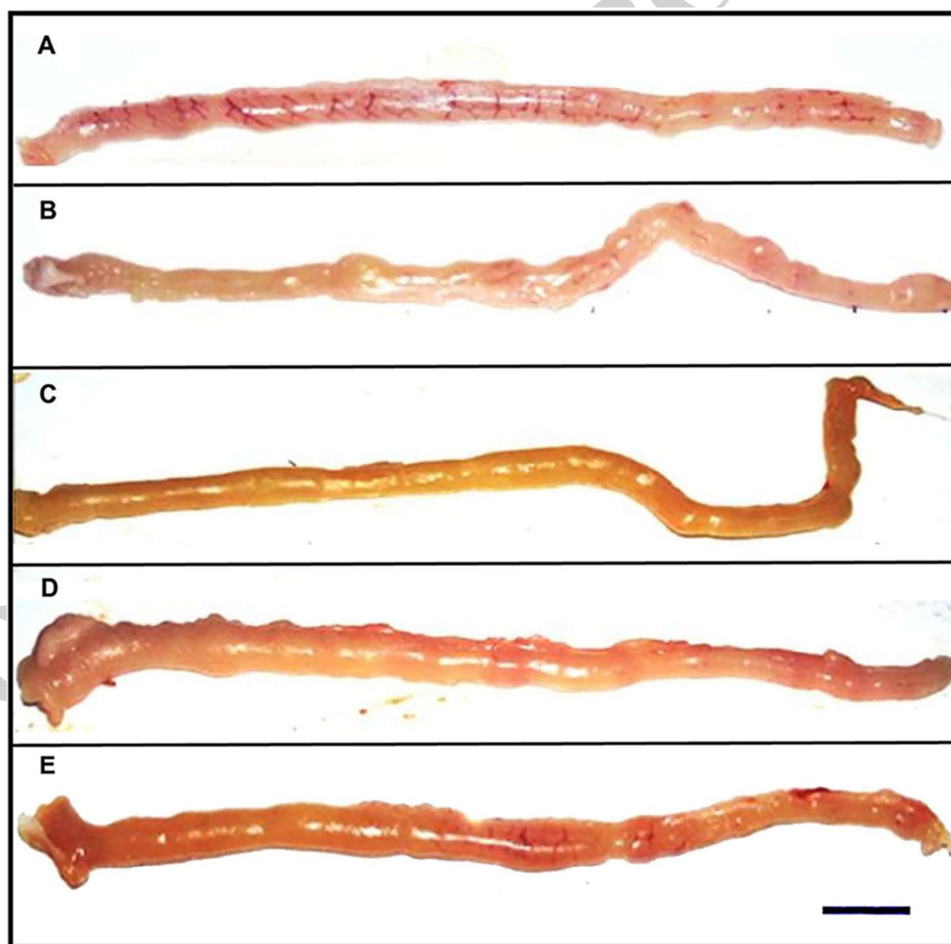

Fig. 1 Macroscopic examination of colon from rats. **a** represents colon from Group I rats where there is no polyps formation; **b** represents colon from Group II rats where the formed polyps are visible and severe; **c** represents the colon from Group III rats where the number and severity of polyps are reduced due to the 5-FU treatment; **d** represents colon from the Group IV rats where the number and severity of polyps are reduced due to the 200 mg/kg b.w. EPS treatment; **e** represents colon from the Group V rats where the number and severity of polyps are reduced due to the 400 mg/kg b.w. EPS treatment

293 from different groups. Figure 1a shows the colon from nor-
294 mal group, the colon is intact without any polyp formation
295 and exhibit no evidence of malignancy. In Fig. 1b, colon
296 from Group II is presented; the colon contains numerous
297 polyps with malignancy. In group III, there is a visible
298 reduction in the number of polyps formed (Fig. 1c). In
299 group IV and group V (Fig. 1d, e) which are treated with
300 EPS at 200 mg/kg b.w. and 400 mg/kg b.w. concentrations
301 there is a significant reduction in the polyps formation,
302 which is better in the groups treated with standard drug.
303 These results clearly indicate that the EPS has exhibited a
304 potential anticancer activity in the colon.

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Histopathological analysis

The microscopic observation of the colon of control animals revealed normal epithelium and the lamina propria showed scattered lymphocytic infiltration. The muscular layer and serosa showed no significant pathology and there was no evidence of malignancy. In rats that received daily DMH dose (Group II), the colon showed normal epithelium and the lamina propria showed diffuse scattered lymphocytic infiltration and some glands showed intra epithelial lymphocytes; nuclear crowding, stratification and, occasional nucleoli (Fig. 2) were also observed which is consistent with adenoma. In the groups treated with standard drugs, the colon showed normal epithelium and the lamina propria showed mild lymphocytic infiltration. The muscular layer and serosa showed no significant pathology. In the group treated with EPS at 200 mg/kg b.w., the section showed dysplastic changes with individual cells found to be round to oval having moderate eosinophilic cytoplasm and round to oval vesicular nuclei showing stratification and crowding. The lamina propria and both the muscularis and serosa layers showed lymphocytes infiltration. In the group treated with EPS at 400 mg/kg b.w the section studied showed normal epithelium with the lamina propria showing mild lymphocytic infiltration. Both the muscularis and serosa layers showed mild inflammatory infiltrates. There was no evidence of malignancy found in the section studied. From these results it can be concluded that the EPS has offered protective effects on the colon as the malignancy conditions were almost found to be reversed in the group treated with 400 mg/kg b.w.

Discussion

Treatment with EPS, at 200 mg/kg and 400 mg/kg body weight concentrations, has effectively decreased the polyp formation/polyp incidence in DMH-treated rodents. The reduced levels of antioxidative enzymes during carcinogenesis reversed with the administration of EPS. DMH-induced rat colon cancer is one of the suited models to study human colon cancer (Aranganathan et al. 2009). When DMH is administered, the molecule gets converted to azoxymethane and methylazoxymethanol in liver which finally gets metabolised into carbonium ion via, methyldizonium ion in colon, resulting in the methylation of nucleic acids and thus resulting in tumours in colon (Fiala 1977). Administration of EPS reduced the polyps incidence in DMH-treated rats at both the concentration tested. We previously have shown that EPS from *L. acidophilus* showed anticancer potential in vitro against colon cancer cells (Deepak et al. 2016a, b).

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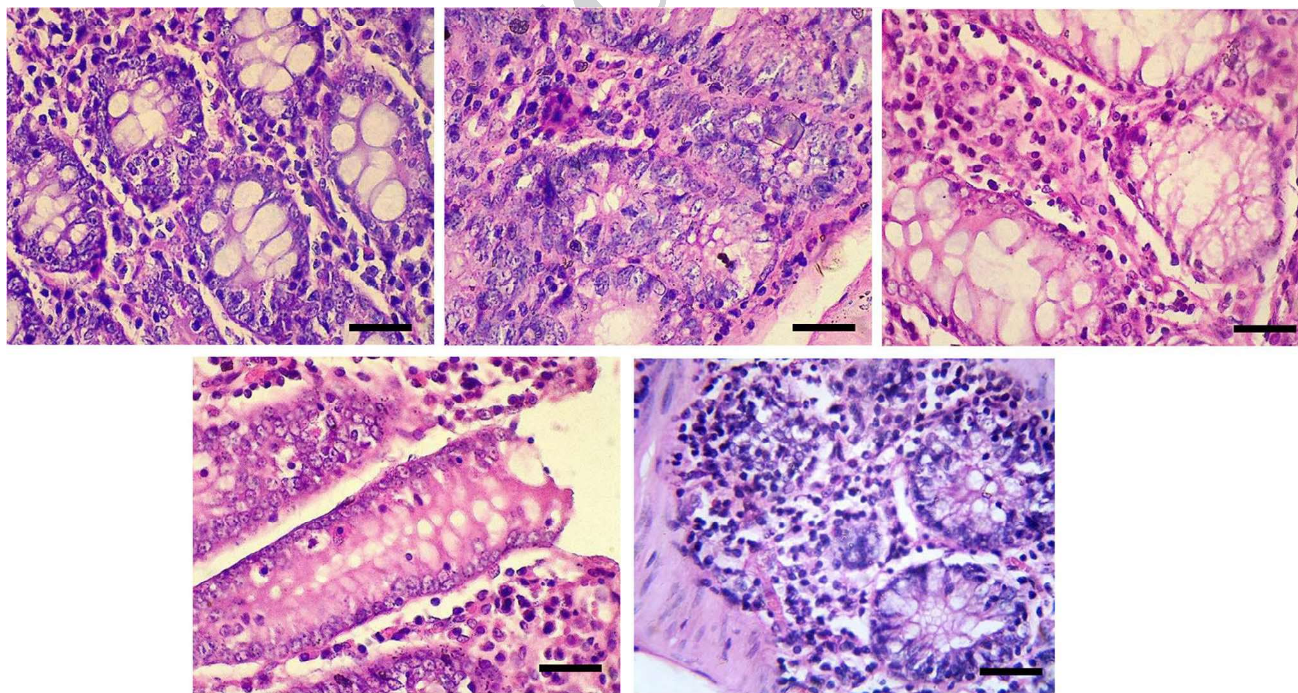

Fig. 2 Histopathological analysis of colon from rats. **a** represents normal mucosa of the Group I rats; **b** represents colon exhibiting nuclear crowding stratification and occasional nucleoli in Group II rats; **c** represents mild lymphocytic infiltration in Group III rats; **d** represents less intraepithelial lymphocytes in Group IV rats; **e** represents mucosa with lamina propria normal in Group V rats. All the images are taken at 40× resolution

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352 EPS reduced the tumour incidence in DMH-induced colon
353 cancer rat model.
354 1, 2–dimethyl hydrazine carcinogenesis includes gen-
355 eration of hydroxyl radicals/hydrogen peroxide results in
356 lipid peroxidation and DNA damage. This further leads to
357 excess generation of ROS and, the disruption caused in the
358 oxidant/antioxidant balance could play a pivotal role in the
359 carcinogenesis (Baskar et al. 2012). Various studies by other
360 researchers also suggest that LPO decreases significantly in
361 tumour cells and tissues compared to normal cells and tis-
362 sues (Sreedharan et al. 2009; Darband et al. 2020; Fan et al.
363 2020). In animals treated with EPS there is an increase in
364 LPO, which shows that EPS exhibits antioxidant activity
365 under in vivo condition too.
366 In general the reactive oxygen species are neutralised by
367 the antioxidant/antioxidant enzymes under normal physi-
368 ological conditions. When ROS production supersedes the
369 antioxidant mechanism, it may result in oxidative damage
370 to DNA and the cell resulting in carcinogenesis (Waris and
371 Ahsan 2006). Some of the ROS such as superoxide anions,
372 hydrogen peroxide, hydroxyl radical and singlet oxygen are
373 very harmful to the cells as they damage cellular proteins,
374 lipids and DNA (Nordberg and Arnér 2001; Zińczuk et al.
375 2020). Antioxidant enzymes like GPx, SOD and CAT play a
376 major role in protecting cells against these toxic compounds
377 by either directly eliminating the electrophiles and toxic free
378 radicals or by scavenging superoxide anions or by convert-
379 ing hydrogen peroxide into water and oxygen (Yu 1994;
380 Rajeshkumar and Kuttan 2003). In the present study we
381 have observed a decreased levels of SOD and CAT activi-
382 ties in DMH alone treated animals when compared to con-
383 trol groups. Treatment with EPS has elevated the levels of
384 both SOD and CAT, indicating the protective effect of EPS.
385 The anticancer activity of some of the previously reported
386 chemopreventive drugs are attributed to antioxidant activity.
387 We have previously reported the in vitro antioxidant activity
388 of EPS which could explain the anticancer activity of EPS.
389 A couple of crucial components of antioxidant defence
390 mechanisms are GSH and vitamin C which function as a
391 direct reactive free radical scavenger (Ighodaro and Akinloye
392 2018). In the DMH alone group, there is a decrease in the
393 GSH and vitamin C levels which may be the result of DMH
394 administration. But in groups treated with EPS there was
395 a reversal of the GSH and vitamin C levels suggesting the
396 protective effect of EPS on DMH-induced tumours. One of
397 the major problems encountered in cancer chemotherapy is
398 anaemia and myelosuppression (Gilreath et al. 2014). Treat-
399 ment with EPS has shown to restore the levels of RBC and
400 WBC levels to more or less normal levels indicating the
401 protective effect of EPS on the hematopoietic system. Fur-
402 thermore the histopathological studies revealed that DMH
403 group animals showed lymphocytic infiltration with intra
404 epithelial lymphocytes in some glands. Nuclear crowding,
405 stratification and adenoma were also observed. Whereas, in
406 EPS-treated groups only a mild lymphocytic infiltration was
407 observed and there was no infiltration of glands. This obser-
408 vation and the absence of adenoma further substantiates the
409 chemo protective potential of EPS.

Conclusion 410

411 The present study showed that EPS from probiotic bacteria
412 enhances the antioxidant status of colon in the rat model
413 and the novelty of the work is the inhibition of DMH-
414 induced colon cancer through the modulation of antioxida-
415 tive enzymes. This indicates that dietary supplementation
416 of EPS might potentially prevent the development of colon
417 cancer. But further investigations are warranted to elucidate
418 the complete mechanism of action and compatibility of EPS
419 for the treatment of colon cancer.

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ments. SRKP helped with analysis. SDS, NH and KS designed experi- 425
ments and were involved in manuscript preparation. 426

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Data availability Yes. 428

Declarations 429

Conflict of interest No conflicts of interest. 430

Ethical approval The study was carried out after obtaining permis- 431
sion from the Institutional Animal Ethical Committee (KMCRET/ 432
PhD/15/2014–15). 433

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