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Research Article
Piperine Presents Chemo-preventive Property Against 1, 2-Dimethyl Hydrazine Induced Colon Cancer in Mice: Biochemical and Physiological Evidences

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Abstract

Background and Objective: Colorectal carcinoma or colon cancer results from various interactions, one such interaction can originate due to a persistent inflammation. The aim of this study was to evaluate the chemo-preventive effect of piperine on 1, 2-dimethylhydrazine (DMH) induced colorectal cancer in mice. **Materials and Methods:** Male Swiss albino mice were divided into five groups (n = 6). Group I served as control group. Group II served as diseased group and received with DMH (20 mg kg^{-1}) subcutaneously. Group III and IV were the treated groups, received DMH subcutaneously and piperine (25 and 50 mg kg^{-1}) orally, respectively. Group V served as drug control received piperine only (50 mg kg^{-1}). Body weights were daily recorded and at the end of the treatment period, the animals were sacrificed under anaesthesia. The colons were harvested and quantified for Aberrant Crypt Foci (ACF) various biochemical estimations (Malondialdehyde (MDA), Glutathione (GSH), Superoxide dismutase (SOD) and nitrite levels) and histological examination. **Results:** The current results provided evidences that DMH induced colon cancer in mice significantly decreased body weight, GSH, SOD, catalase levels and increased the incidence of ACF, aberrant crypts and crypts multiplicity along with increased levels of malondialdehyde (MDA) and nitrite levels. Histo-pathological evaluation of colon of the DMH treated mice significantly developed dysplasia, inflammation and focal congestion in sub-mucosa and muscularis layers. Concurrent treatment with piperine significantly reversed all the above effects in a dose dependent manner. **Conclusion:** This present study suggests that piperine significantly protects against DMH induced colorectal cancer by virtue of its antioxidants properties.

Key words: 1, 2-dimethylhydrazine, piperine, oxidative stress, aberrant crypt foci, colon cancer

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colon cancer or colorectal cancer is ranked third among the occurrence and fourth in the cause of cancer deaths worldwide. In the year 2016, American Cancer Society has reported 95,270 estimated cases and 49,190 of estimated deaths due to colon cancer[1,2]. The aetiology behind colon cancer is still undetermined, it is a multifactorial disorder arising due to hereditary, environmental or dietary agents. Despite many advanced in the understanding of this particular disorder, therapies including surgery, radiation and chemotherapy drugs are limited to treating the advanced stage of colon cancer. The development of colon cancer is a multi step process and it begins as a benign polyp, a growth of tissue that starts in the lining and grows into the centre of the colon. The benign polyp can develop into an advanced adenoma with a high-grade dysplasia which eventually progresses to an invasive cancer[3,4].

Environment toxin, 1, 2-dimethylhydrazine or DMH, is a specific colon procarcinogen pollutant[5]. The active intermediates of DMH such as, azoxymethane and methyl-azoxymethanol are formed in the liver and are transported into the colon through bile and blood. The active metabolite, Methyl-azoxymethanol, is decomposed and forms methyldiazonium ions. These methyldiazonium ions methylate cellular components. This pollutant produces free radicals that cause oxidative changes at the subcellular level, resulting in DNA damage organs such as the liver and colon. Previous reports have concluded that DNA damage due to reactive oxygen species (ROS) and the presence of oxidative DNA adducts can contribute in the formation of tumor or tumorigenic process[6,7].

In vivo studies exhibited colonic tumors when administered with DMH, the isolated colon physiologies were similar to that of human colonic neoplasms. Therefore, this particular model may prove to be adequate for mimicking colorectal cancer for further research purposes[6-9].

To screen for potentially new chemo-preventive agents markers such as aberrant crypt foci or ACF are used. These markers can also be used to evaluate the protective effects of compounds at the initial stages of the carcinogenic process[6,10]. The ACF comprises of the following anatomical markings such as, abnormal luminal openings, thick epithelia, also these crypts have report to be elevated when observed under a microscopic[11]. Drugs like Resveratrol[11], β-sitosterol[12], Kaempferol[13], Farnesol[14] and Ginger[15] showed protective effect against colon cancer by virtue of their anti-oxidant and anti-tumor properties. Hence, anti-oxidants with significant anti-tumour activity may show a promising effect in colon cancer.

Piperine or chemically known as the trans-isomer of 1-piperoyl piperidine is a pungent substance with a nitrogenous group. It is an alkaloid obtained from the sources, *Piper nigrum* and *Piper longum* of the family Piperaceae. It exhibits the following reported properties, significant anti-oxidant[16,17], anti-inflammatory[18], anti-tumor[18,19], anti proliferative[20] and anti-mutagenic[21] activities. Piperine showed a protective effect against skin cancer[22], lung cancer[22], melanoma[23,24] and various other types of carcinogenesis[17].

In the above mentioned properties of piperine, there have been currently no reports regarding the chemo-protective role of Piperine against colorectal cancer produced by the environmental toxin, i.e., DMH in mice. In this study, piperine will be tested against DMH induced colon cancer in mice and studied qualitatively through morphological analysis and quantitative via estimation of various biochemical parameters.

MATERIALS AND METHODS

Chemicals: Piperine procured from the Department of Pharmacognosy, G. Pulla Reddy College of Pharmacy. Thiobarbituric acid, 1, 2-dimethylhydrazine, Griess reagent, Ellman's Reagent, Trichloroacetic acid and Vanadium trichloride were purchased from Sigma Aldrich, Bangalore. All other reagents procured obtained were of high analytical grade. This study was conducted in 2015 with duration of 6 months.

Animals: Male Swiss albino mice (20-22 g) were procured from national institute of nutrition (NIN), Hyderabad in 2015. The animals were acclimatized, randomized and placed individually into transparent polypropylene cages. The animals had access to food and water *ad libitum* at 12: 12 h dark/light cycle. All the experimental procedures were carried in accordance with the Committee for the purpose of control and supervision of experiments on animals (320/CPCSCEA). This study was approved by the Institutional Animal Ethical committee (GPRCP/IAEC/2/12/3/PCL/AE-2A-mice-M/F-36), G. Pulla Reddy College of pharmacy, Hyderabad.

Induction of colorectal cancer in mice: To assess the therapeutic activity of piperine against colon cancer, DMH was dissolved in saline (0.9% w/v NaCl) and dosed subcutaneously (20 mg kg^{-1}) twice a week for a period of 2 weeks.

Experimental design: Thirty Swiss albino mice were divided into five groups (n = 6). Group I received vehicle, group II received DMH (20 mg kg^{-1}, s.c). Group III received DMH and Piperine (25 mg kg^{-1}) orally for eight weeks. Group IV were administered with DMH and Piperine (50 mg kg^{-1}). Group V received vehicle for the 1st week and Piperine (50 mg kg^{-1}) alone for the next 7 weeks to assess any toxicity induced by piperine. Clinical parameter such as, body weights were measured at a weekly interval during the treatment period and at the end of which (8 weeks), all the groups were sacrificed under anesthesia. The animals underwent necropsy and colons were harvested to observe for ACF, estimate various biochemical parameters (MDA, GSH, Catalase, SOD and Nitrite levels) and also histological evaluation.

Evaluation parameters

Aberrant crypt foci detection: Aberrant crypt foci (ACF) were counted by Bird's method[6]. Isolated colons were flushed with phosphate buffered saline (PBS) and opened longitudinally and fixed between two pieces of filter paper pre-coated with 10% neutral buffered formalin for 24 h. The colonic tissues that were fixed in formalin were removed and stained with 0.2% methylene blue for 5 min then briefly rinsed with distilled water. The sections were later observed under 40X magnification. The ACFs were differentiated by their slit-like opening, deeply stained, peri-cryptal zone and slight elevation compared to normal crypts.

Biochemical analysis: Colon tissue MDA, SOD, CAT, GSH and Nitrite levels were analyzed using according to the methods described by Ohkawa *et al.*[25], Fridovich *et al.*[26], Aebi *et al.*[27], Ellman's method[28] and Griess Reaction[29,30], respectively.

Histo-pathological evaluation: The colons were harvest and flushed with saline, sliced open longitudinally along the main axis and washed again with saline. These colonic sections were fixated in 10% buffered formalin for a minimum time of 24 h. Later these sections were dehydrated in ascending grades of ethanol and embedded in paraffin wax. Blocks with section embedded were prepared and sliced into sections of 5 µm thickness. Xylene and ethanol was used to remove the paraffin from the colonic tissue sections. The sections were later washed with PBS and permeabilized with permeabilization solution i.e., 0.1 M citrate and 0.1% Triton X-100. After permeabilizing, these sections were stained with hematoxylin and eosin and observed under light microscope at 40X magnifications to investigate the histo-architecture of colonic mucosa.

Transmission electron microscopic analysis: Transmission electron microscopy (TEM) employs an electron beam that istransmitted through ultra-thin specimens. After harvesting isolated crypts and intact tissues were fixed immediately with 3% gluteraldehyde and processed in a routine fashion for electron microscope analysis according to Gaudio *et al.*[31]. The TEM images were analysed for the integrity of the isolated crypts preparations and in the intact tissue[31].

Statistical analysis: The data was collected in triplicates and the results are expressed as Mean±SEM. Statistical Analysis was done using one-way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5. The differences were considered to be statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Body weight: The DMH induced colon cancer significantly decreased body weights of the diseased group when compared to normal group. Treatment with piperine significantly improved excessive loss in body weight when compared to disease control mice. This effect wasproven as dose dependent, however, administration of piperine in normal mice did not exhibit any change in body weight when compared to normal group (Table 1).

Aberrant crypt foci: The number of aberrant crypts, ACF and crypt multiplicity in the mice colons in different groups wereobserved and analyzed (Table 2A). It was concluded that there were no signs of ACF in the harvested colon of the animals belonging to the control group. Whereas the groups administered with DMH caused an average of ~36 ACF with ~75 total aberrant crypts and ~2.1 crypt multiplicity (Table 2B). Piperine, (25 and 50 mg kg^{-1}) significantly ($p<0.05$) reduced the number of ACF and its multiplicity in a dose-dependent manner.

Table 1: Initial and final body weights and body-weight gain in control and DMH administered mice concurrently treated with vehicle and piperine

	Body weight (g)		
Groups	Initial	Final	Weight gain
Normal control	24.67±1.211	31.00±2.828	6.330±1.211
Disease control	25.33±1.211	28.67±0.8165	4.167±0.752$^{\gamma}$
Piperine(25 mg kg^{-1})	25.00±1.673	29.83±1.169	4.833±0.983
Piperine(50 mg kg^{-1})	24.33±1.633	30.50±1.643	6.167±0.408
Piperine control	25.00±1.414	31.50±1.517	6.500±1.049

Body weights were recorded at a weekly interval. The data is expressed as Mean±SD. $^{\gamma}p<0.01$vs. normal control

Table 2: Effect of piperine (25 and 50 mg kg^{-1}) on mice administered with DMH. After eight weeks of treatment, the animals were sacrificed, colons were harvested and observed for crypt multiplicity (A) and ACF formation (B)

A	Number of ACF with			
Groups	1 crypt	2 crypts	3 crypts	≥4 crypts
Disease control	16.2±1.643	9.4±1.673	6.4±1.14	4.4±0.547
Piperine (25 mg kg^{-1})	14.0±2.0	8.0±1.0	5.0±1.225	2.8 ± 0.837^{b}
Piperine (50 mg kg^{-1})	11.8 ± 1.48^{c}	6.8 ± 0.836^{d}	2.4 ± 0.547^{a}	1.6 ± 0.547^{a}

B			
Groups	Aberrant crypts (AC)	Aberrant crypt foci (ACF)	Crypt multiplicity (AC/ACF)
Disease control	74.8±6.611	36.4±3.209	2.053±0.054
Piperine (25 mg kg^{-1})	58.4 ± 6.025^{c}	29.8 ± 2.168^{b}	1.963 ± 0.0856
Piperine (50 mg kg^{-1})	41.0 ± 6.285^{a}	22.6 ± 2.302^{a}	1.809 ± 0.103^{c}

The data is expressed as Mean±SD, $^{a}p<0.0001$, $^{b}p<0.001$, $^{c}p<0.01$, $^{d}p<0.05$ vs. disease control

Table 3: Harvested colons were estimated for biochemical parameters such as, MDA, nitrite, SOD, GSH and catalase in normal and DMH induced colon cancer mice concurrently treated with piperine

Groups	MDA (nmoles mg^{-1} tissue)	Nitrite (μmoles mg^{-1} tissue)	SOD (μmoles g^{-1} tissue)	GSH (μmoles g^{-1} tissue)	Catalase (K mL^{-1})
Normal control	19.02±3.755	8.896±1.269	5.168±0.754	4.83±0.627	0.588±0.093
Disease control	$67.43\pm8.181^{\alpha}$	$31.870\pm3.834^{\alpha}$	$2.632\pm0.672^{\alpha}$	$2.65\pm0.345^{\alpha}$	$0.276\pm0.052^{\alpha}$
Piperine (25 mg kg^{-1})	$29.77\pm3.283^{\gamma,a}$	$15.180\pm2.218^{\beta,a}$	$3.902\pm0.469^{\gamma,c}$	$3.66\pm0.416^{\beta,c}$	$0.403\pm0.044^{\beta}$
Piperine (50 mg kg^{-1})	19.80 ± 1.808^{a}	10.400 ± 1.866^{a}	5.020 ± 0.542^{a}	4.05 ± 0.635^{b}	0.492 ± 0.05^{b}
Piperine control	16.56±2.983	8.737±0.849	5.220±0.707	4.84±0.224	0.589±0.107

The data expressed as Mean±SD, $^{\alpha}p<0.0001$, $^{\beta}p<0.001$, $^{\gamma}p<0.01$ vs. normal control, $^{a}p<0.0001$, $^{b}p<0.001$, $^{c}p<0.01$ vs. disease control

Colonic levels of MDA, Nitrite, SOD, GSH and catalase: As shown in Table 3, induction of colon cancer via DMH administration significantly increased MDA and nitrite levels and decreased SOD, GSH and catalase levels in disease control group when compared to that of the normal control. Concurrent treatment with piperine (50 mg kg^{-1}) significantly restored the mentioned biochemical markers to normal levels. However, no effect was observed in the duration of piperin treatment to mice without DMH.

Histopathologic examinations for crypts: Harvested colons were sectioned and stained with methylene blue. These sections were then observed under a light microscope (40X) and analyzed for the colonic ACF with crypts (Fig. 1). Sections obtained from the control group (Fig. 1a), were absent of any ACF, where DMH treated group depicted many (Fig. 1d). Animals treated with Piperine (50 and 25 mg kg^{-1}) has exhibited one crypt (Fig. 1b) and two crypts (Fig. 1c), respectively.

Histopathologic examinations: Histo-pathological findings of colons harvested from the control group revealed, normal epithelium with regular crypts and goblet cells with no inflammation and dysplasia (Fig. 2a). Whereas, DMH treated mice had shown abnormal anatomical changes such as, presence of inflammatory cells and focal congestion in sub-mucosal and muscularis mucosa and dysplasia (Fig. 2b). The groups treated with piperine (25 and 50 mg kg^{-1}) significantly ameliorated the focal congestion in the muscularis mucosa and sub-mucosal layer along with the inflammation. However, moderate and mild dysplasia are observed in mice treated with Piperine (25 mg kg^{-1}, Fig. 2c and 50 mg kg^{-1}, Fig. 2d).

Transmission electron microscopic (TEM) studies: The sections of the control groupexhibited normal cellular structures with normal function organelles such as, mitochondria, endoplasmic reticulum and nucleus with centrally placed nucleolus (Fig. 3a). The TEM however revealed the opposite in rodents treated with DMH. The sections exhibited, marginated chromatin, degenerated and shrunken mitochondria, endoplasmic reticulum and swollen nucleus with ex-centric nucleolus and there was also evidence of rapidly dividing nucleus and vacuolar degeneration. Thereby, suggesting that DMH treatment causes rapid cellular proliferation with abnormal cellular organelles (Fig. 3b). Treatment with Piperine (25 mg kg^{-1}) had a mild effect towards the intracellular organelles, where condensed chromatin and little vacuolar de generation were still exhibited (Fig. 3c). Whereas, treatment with Piperine (50 mg kg^{-1}) exhibited more therapeutic effect, the cells were uniform with the presence of mild vacuolar degeneration, distinct sub-cellular organelles like endoplasmic reticulum and mitochondrial matrix (Fig. 3d). Therefore, from the TEM analysis, it can be concluded that DMH induced cellular damage resulting in colon cancer can be ameliorated upon treatment with piperine (50 mg kg^{-1}).

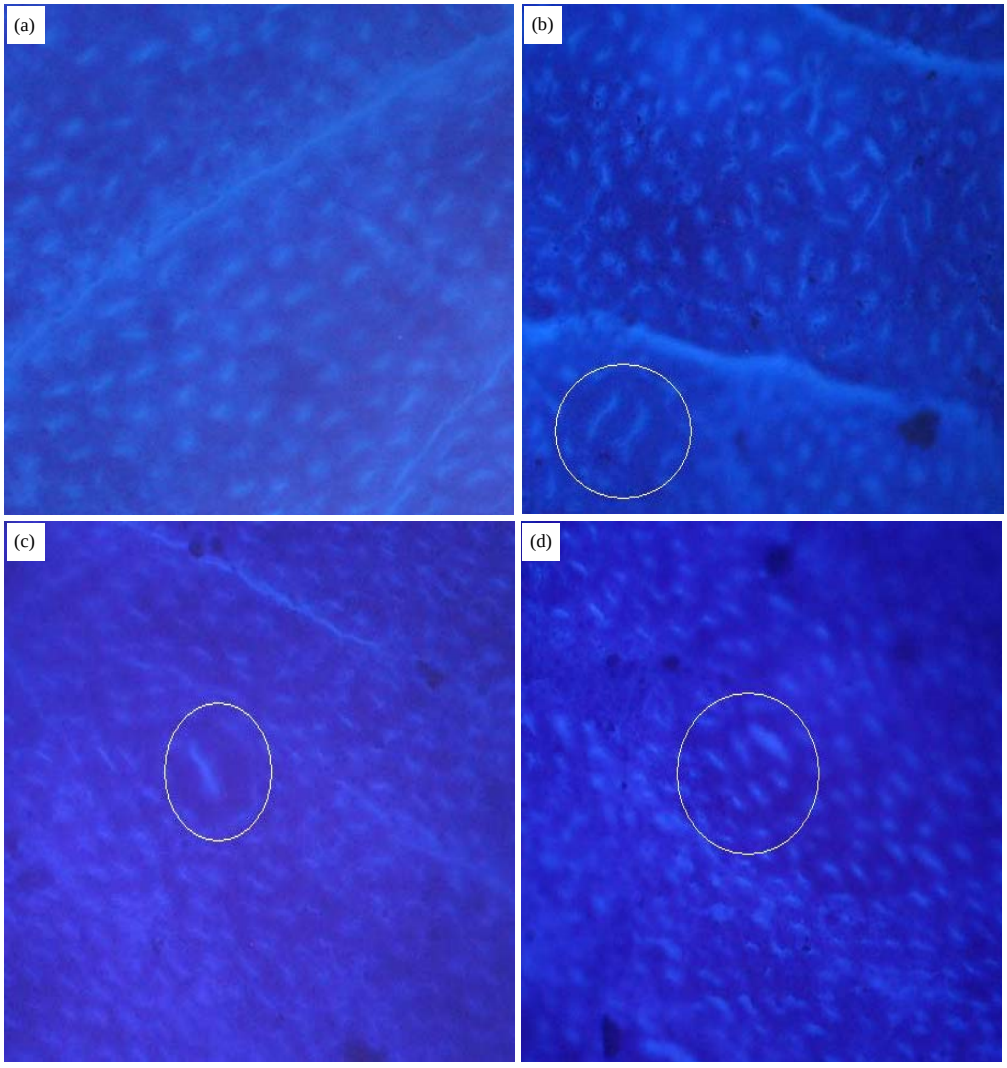

Fig. 1(a-d): Methylene blue stained colonic tissues (40X) showing normal colon and colonic tissue with aberrant crypt foci, (a) Normal colon, (b) Colonic ACF with 1 crypt, (c) Colonic ACF with 2 crypts and (d) Colonic ACF with $\geq$4 crypts

DISCUSSION

In the present study, it was demonstrated that treatment with piperine restored the DMH induced damage to the colon, evidenced by decreased ACF formation and restored oxidative parameters. DMH is a known environmental pollutant that consists of carcinogenic properties which produces its toxicity through the metabolic activation in the liver by producing active electrophilic carbonium ion, is known to elicit oxidative stress[4]. Piperine, a well known drug, has been reported to possess many therapeutic activities, where chemo- preventive against various tumours like lung cancer, skin cancer and buccal carcinogenesis.

To detect the morphological changes in colonic mucosa, biomarkers such as, aberrant crypts (AC) are employed. The ACFs can be identified with their increased size, thicker epithelial lining and an increased peri-cryptal zone[30]. In the more advanced stages of carcinogenesis, the crypts have increased in size, number and correlates with the incidence of colorectal adenomas[8]. The ACF formation is also known to be a putative indicator of colon carcinogenesis and efficacy of anti-carcinogenic effects[32].

In agreement with the previous reports, it has been concluded that the groups treated with DMH had reported a significantly high number of ACs, ACF and crypt multiplicity[5,11,32]. The protective effect of piperine was evidenced by the decrease in total number of AC per animal with the decreased number of AC per focus. Upon the administration of DMH, there was an increase in the level of malondialdehyde (MDA). Concurrent treatment with piperine significantly attenuated its level in the colonic tissues.

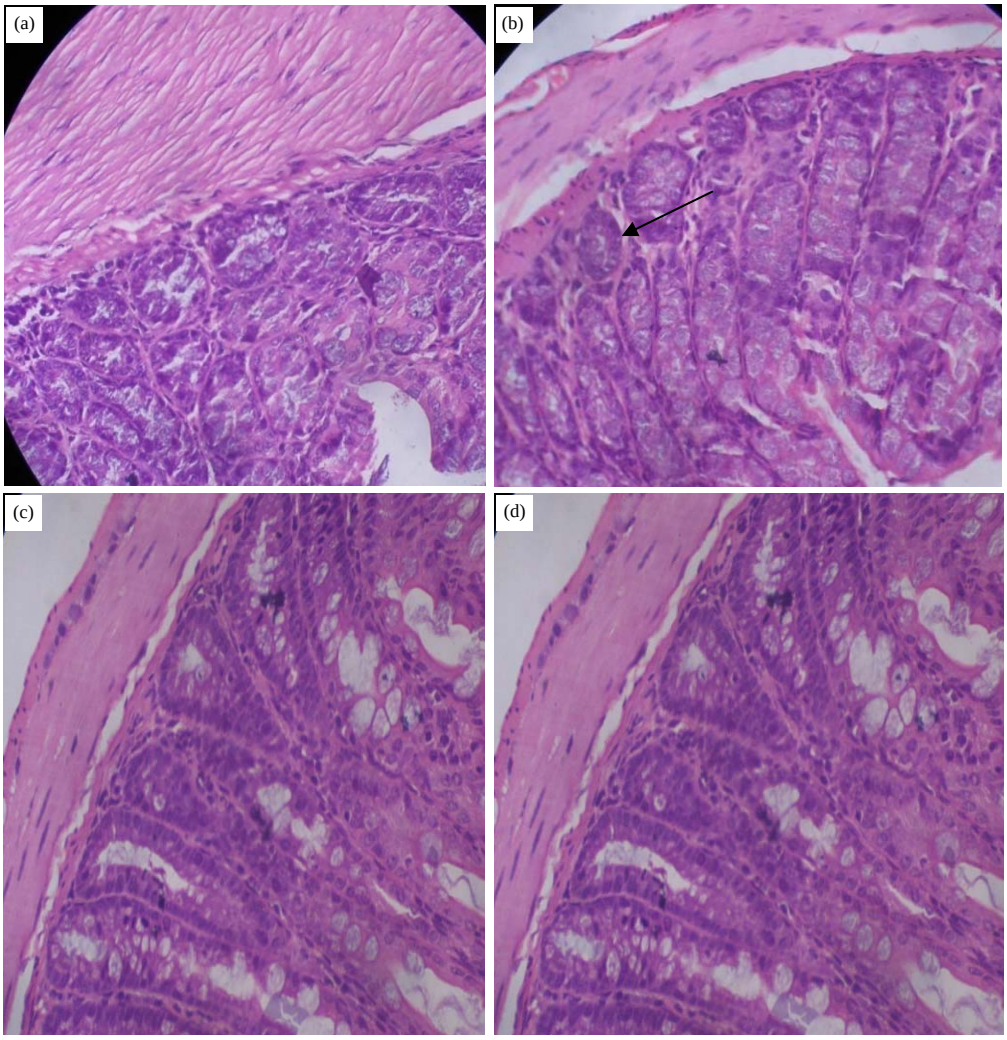

Fig. 2(a-d): H and E stained colonic tissues (40X). Showing normal colon and colonic tissue treated with DMH and DMH and piperine, (a) Colonic tissue of control group, (b) DMH treated mice, exhibiting dysplasia, indicated by the arrow marks, (c) Colonic tissue, treated with piperine (25 mg kg^{-1}) exhibiting moderate dysplasia and (d) Colonic tissue, treated with piperine (50 mg kg^{-1}) exhibiting mild dysplasia

Superoxide dismutase (SOD) and catalase (CAT) are crucial enzymatic involved in the mechanism of anti-oxidant and act against toxic oxygen free radicals such as superoxides (O_2^-) and hydroxyl (·OH) ions in biological systems[2]. The SOD and CAT are involved in the direct elimination of these reactive oxygen species (ROS), hence, have been known as the primary antioxidant enzymes[11]. The data has shown that the activity of SOD, CAT and GSH were decreased in groups treated with DMH. The depleted levels of these enzymes on DMH administration suggested that these may also be involved in detoxification and possibly repair mechanism in colonic mucosa.

Treatment with Piperine resulted in enhanced activity of these enzymes, due to its ability to scavenge for free radicals and toxic carcinogenic metabolites[16].

It was proven that low levels of SOD, CAT and GSH activity in pre-cancerous tissues promoted growth of cancer and its infiltration into surrounding tissues, which is necessary for invasion and metastasis[32].

Histo-pathological studies revealed severe dysplasia and focal congestion in muscularis mucosa and sub-mucosal layers in animals treated with DMH. Histological evaluation of colonic tissues of mice treated with piperine exhibited mild dysplasia with very mild focal congestion. Colonic tissues harvested were evaluated by Transmission electron microscopy to study the cellular and sub-cellular organelles of the cell. The TEM analysis revealed that upon treatment with DMH, there was an alteration in the cellular integrity such as rapidly proliferating cells and condensed chromatin.

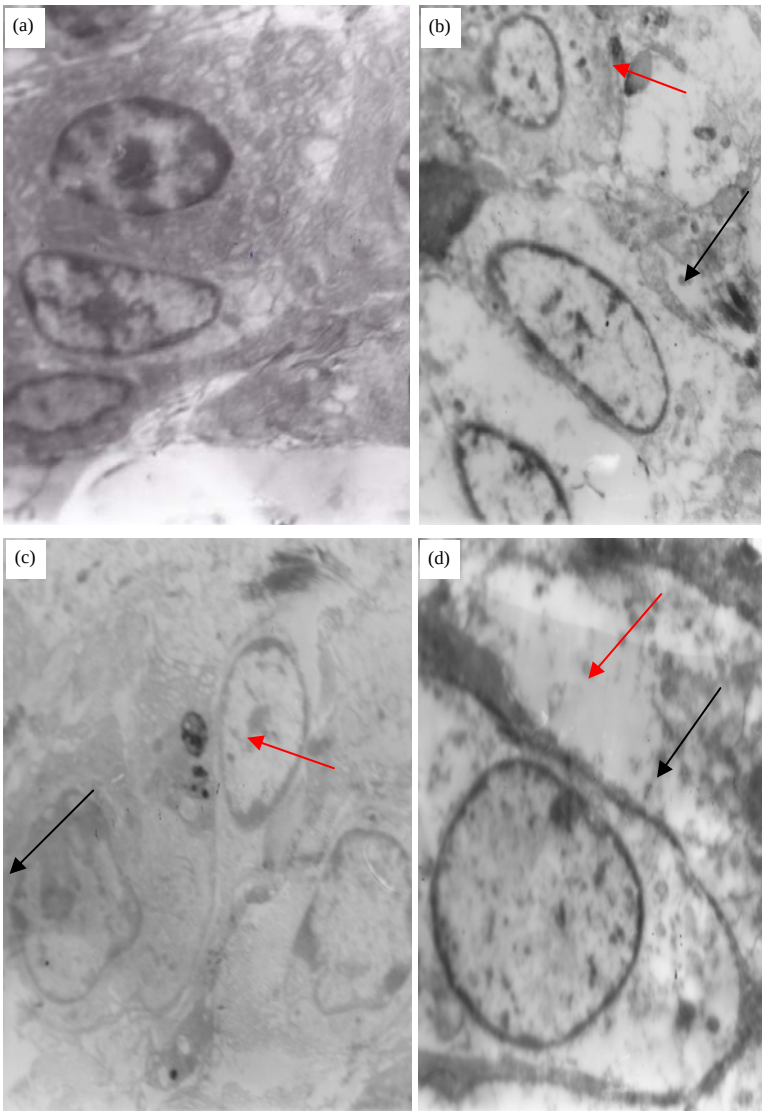

Fig. 3(a-d): TEM analysis colonic tissues, showing normal colon and colonic tissue treated with DMH and DMH and piperine, (a) Colonic tissue of control group, (b) DMH treated mice, exhibiting marginated chromatin (black arrow) and shrunken mitochondria (red arrow), (c) Colonic tissue, treated with piperine (25 mg kg^{-1}) depicting condensed chromatin and indistinct nucleus (black and red arrows) and (d) Colonic tissue, treated with piperine (50 mg kg^{-1}) exhibiting mitochondrial matrix (black arrow) and endoplasmic reticulum (red arrow)

Even though clinical studies have reported the relation between the colon carcinogenesis and oxidative stress in a few case studies there lacks a definite etiology of the disease. For further investigations, various pathways of treating colorectal cancer via oxidative stress with the help of biomarkers should be looked into.

CONCLUSION

In this study, Piperine was treated against DMH induced colorectal cancer in mice and upon treatment, it had shown significant protective effects. The altered oxidative stress markers and the altered histo-architecture of the isolated colons were significantly repaired due to piperine. The findings of the present study had demonstrated that, treatment with piperine exhibits significant protective effect by virtue of its antioxidant property. Evidence obtained from this study provides an encouraging warrant to further the research regarding the protective effects of piperine and also the definite etiology and possible use of biomarkers for the detection of colorectal cancer.

SIGNIFICANT STATEMENT

In this study, it was discovered that piperine demonstrates a chemo-protective effect against DMH induced colon cancer. Using markers such ACFs (aberrant crypt foci) and various biochemical parameters, the therapeutic activity of piperine was assessed against the damage caused by DMH. In this study, piperine's efficacy was concluded due to its anti-oxidant property and the role of antioxidants as a method of treatment may provide a new direction in understanding the etiology of many disorders including colon cancer.

REFERENCES

1. Langman, M.J.S., 1971. Epidemiology of cancer of the oesophagus and stomach. Br. J. Surg., 58: 792-793.
2. Amersi, F., M. Agustin and C.Y. Ko, 2005. Colorectal cancer: Epidemiology, risk factors and health services. Clin. Colon Rectal Surg., 18: 133-140.
3. Cappell, M.S., 2005. The pathophysiology, clinical presentation and diagnosis of colon cancer and adenomatous polyps. Med. Clin., 89: 1-42.
4. Wei, E.K., E. Giovannucci, K. Wu, B. Rosner, C.S. Fuchs, W.C. Willett and G.A. Colditz, 2004. Comparison of risk factors for colon and rectal cancer. Int. J. Cancer, 108: 433-442.
5. Aranganathan, S. and N. Nalini, 2009. Efficacy of the potential chemopreventive agent, hesperetin (citrus flavanone), on 1,2-dimethylhydrazine induced colon carcinogenesis. Food Chem. Toxicol., 47: 2594-2600.
6. Bird, R.P., 1987. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. Cancer Lett., 37: 147-151.
7. Lee, B.M., S.K. Lee and H.S. Kim, 1998. Inhibition of oxidative DNA damage, 8-OHdG and carbonyl contents in smokers treated with antioxidants (Vitamin E, Vitamin C, β-carotene and red ginseng). Cancer Lett., 132: 219-227.
8. Wang, J.G., D.F. Wang, B.J. Lv and J.M. Si, 2004. A novel mouse model for colitis-associated colon carcinogenesis induced by 1,2-dimethylhydrazine and dextran sulfate sodium. World J. Gastroenterol., 10: 2958-2962.
9. Rosenberg, D.W., C. Giardina and T. Tanaka, 2009. Mouse models for the study of colon carcinogenesis. Carcinogenesis, 30: 183-196.
10. Byers, T., B. Levin, D. Rothenberger, G.D. Dodd, R.A. Smith and American Cancer Society Detection And Treatment Advisory Group on Colorectal Cancer, 1997. American cancer society guidelines for screening and surveillance for early detection of colorectal polyps and cancer: Update 1997. CA: Cancer J. Clin., 47: 154-160.
11. Sengottuvelan, M., R. Senthilkumar and N. Nalini, 2006. Modulatory influence of dietary resveratrol during different phases of 1,2-dimethylhydrazine induced mucosal lipid-peroxidation, antioxidant status and aberrant crypt foci development in rat colon carcinogenesis. Biochim. Biophys. Acta, 1760: 1175-1183.
12. Baskar, A.A., K.S. Al Numair, M.G. Paulraj, M.A. Alsaif, M.A. Muamar and S. Ignacimuthu, 2012. β-sitosterol prevents lipid peroxidation and improves antioxidant status and histoarchitecture in rats with 1,2-dimethylhydrazine-induced colon cancer. J. Med. Food, 15: 335-343.
13. Nirmala, P. and M. Ramanathan, 2011. Effect of kaempferol on lipid peroxidation and antioxidant status in 1, 2-dimethyl hydrazine induced colorectal carcinoma in rats. Eur. J. Pharmacol., 654: 75-79.
14. Khan, R. and S. Sultana, 2011. Farnesol attenuates 1,2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic responses in the colon of Wistar rats. Chem.-Biol. Interact., 192: 193-200.
15. Manju, V. and N. Nalini, 2005. Chemopreventive efficacy of ginger, a naturally occurring anticarcinogen during the initiation, post-initiation stages of 1,2 dimethylhydrazine-induced colon cancer. Clin. Chim. Acta, 358: 60-70.
16. Mittal, R. and R.L. Gupta, 2000. *In vitro* antioxidant activity of piperine. Methods Find. Exp. Clin. Pharmacol., 22: 271-274.
17. Khajuria, A., N. Thusu, U. Zutshi and K.L. Bedi, 1998. Piperine modulation of carcinogen induced oxidative stress in intestinal mucosa. Mol. Cell. Biochem., 189: 113-118.
18. Sunila, E.S. and G. Kuttan, 2004. Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. J. Ethnopharmacol., 90: 339-346.
19. Hwang, Y.P., H.J. Yun, H.G. Kim, E.H. Han, J.H. Choi, Y.C. Chung and H.G. Jeong, 2011. Suppression of phorbol-12-myristate-13-acetate-induced tumor cell invasion by piperine via the inhibition of PKCα/ERK1/2-dependent matrix metalloproteinase-9 expression. Toxicol. Lett., 203: 9-19.
20. Bezerra, D.P., C. Pessoa, M.O. de Moraes, E.R. Silveira, M.A.S. Lima, F.J.M. Elmiro and L.V. Costa-Lotufo, 2005. Antiproliferative effects of two amides, piperine and piplartine, from *Piper* species. Zeitschrift Fur Naturforschung C, 60: 539-543.
21. Ahmad, N., H. Fazal, B.H. Abbasi, S. Farooq, M. Ali and M.A. Khan, 2012. Biological role of *Piper nigrum* L. (Black pepper): A review. Asian Pac. J. Trop. Biomed., 2: S1945-S1953.
22. Selvendiran, K. and D. Sakthisekaran, 2004. Chemopreventive effect of piperine on modulating lipid peroxidation and membrane bound enzymes in benzo(a)pyrene induced lung carcinogenesis. Biomed. Pharmacother., 58: 264-267.

23. Pradeep, C.R. and G. Kuttan, 2004. Piperine is a potent inhibitor of nuclear factor-κB (NF-κB), c-Fos, CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells. Int. Immunopharmacol., 4: 1795-1803.
24. Venkatasamy, R., L. Faas, A.R. Young, A. Raman and R.C. Hider, 2004. Effects of piperine analogues on stimulation of melanocyte proliferation and melanocyte differentiation. Bioorg. Med. Chem., 12: 1905-1920.
25. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
26. Fridovich, I., 2013. Superoxide Dismutase. In: Encyclopedia of Biological Chemistry, 2nd Edn., Lennarz, W.J. and M.D. Lane (Eds.)., Elsevier, USA., pp: 352-354.
27. Aebi, H., 1974. Catalase. In: Methods of Enzymatic Analysis (Second Edition), Volume 2, Bergmeyer, H.U. (Ed.). Academic Press, New York, USA., ISBN: 978-0-12-091302-2, pp: 673-684.
28. Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.
29. Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok and S.R. Tannenbaum, 1982. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. Anal. Biochem., 126: 131-138.
30. Tsikas, D., 2007. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 851: 51-70.
31. Gaudio, K.M., G. Thulin, T. Ardito, M. Kashgarian and N.J. Siegel, 1989. Metabolic alterations in proximal tubule suspensions obtained from ischemic kidneys. Am. J. Physiol.-Renal Physiol., 257: F383-F389.
32. Han, C. and Y. Gong, 2000. Experimental Studies on Cancer Chemoprevention by Tea Pigments. In: Dietary Anticarcinogens and Antimutagens, Johnson, I.T. and G.R. Fenwick (Eds.)., Woodhead Publishing Limited, UK., pp: 203-212.