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Carvacrol exhibits anti-oxidant and anti-inflammatory effects against 1, 2-dimethyl hydrazine plus dextran sodium sulfate induced inflammation associated carcinogenicity in the colon of Fischer 344 rats

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ABSTRACT

Chronic inflammation is one of the remarkable etiologic factors for various human ailments including cancer. The well known hypothesis is that persistent inflammation in colon can increase the risk of colorectal cancer (CRC). In this study, a pharmacological evaluation of carvacrol, a phenolic monoterpene constituent of essential oils produced from aromatic plant Oreganum vulgarea sp. on colitis associated colon cancer (CACC) induced by 1,2 Dimethylhydrazine (DMH) and dextran sodium sulfate (DSS) in male Fischer 344 rat model was studied. F344 rats were given three subcutaneous injections of DMH (40 mg/kg body wt) in the first week and were given free access to drinking water containing 1% DSS for the next one week followed by 7–14 days of water as three cycles. Carvacrol was administrated before and after tumor induction at a concentration of 50 mg/kg body weight (o.p). Carvacrol treated groups promotes the endogenous antioxidant system and suppress the inflammation in DMH/DSS induced animals. An increased antioxidant status and restoration of histological lesions in the inflamed colonic mucosa was observed in carvacrol treated rats. This effect was confirmed biochemically by reducing free-radical accumulation and suppressing expression of pro-inflammatory mediators. In this study, Carvacrol significantly increased the anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) glutathione (GSH) levels and reduced lipid peroxides (LPO), myeloperoxidase (MPO) and nitric oxide (NO) as compared to DMH/DSS induced rats. These dramatic changes facilitate the suppression of pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS), and interleukin-1 beta (IL-1β) in CACC induced rats. Taken together, these findings suggest that Carvacrol may play a beneficial role in DMH/DSS induced experimental rat model and serve as an excellent dietary antioxidant as well as anti-inflammatory agent. It may represent novel therapeutic interventions against colon cancer triggered by chronic inflammation.

1. Introduction

Gastrointestinal cancer is the most lethal cancer worldwide and a leading cause of cancer-related mortality. Chronic inflammation is one of the important underlying etiologies of colorectal cancer (CRC) [1,2]. The gastrointestinal tract is more susceptible to toxic chemical exposure may execute the chronic inflammation and later it becomes tumor in gastrointestinal tract [3]. Chronic inflammation is a culprit in the process of inflammation-dysplasia sequence which is mirrored with human inflammatory bowel disease (IBD) [4]. Reactive oxygen species (ROS) have high impact on physiological and pathological status by inducing oxidative stress mediated inflammatory response in several tissues [5]. Increased status of ROS is also an important factor to develop colitis associated colon cancer (CACC) [6] and it becomes into oxyradical overload disorder [7]. Oxidative stress in colonic mucosa was counter balanced through endogenous anti-oxidant defense portal systems including superoxide dismutase, catalase and glutathione peroxidase myeloperoxidase to decompose ROS which generate in response to inflammatory stimuli [8]. Altered biochemical processes due to

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overload of free radicals leads to elevated expression and abnormal activation of diverse range of pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS) and interleukin- 1 beta (IL-1β) in gastrointestinal tract [9].

To evaluate the free radical scavenging capacity and anti-inflammatory properties, male F344 rats were induced with 1, 2-dimethylhydrazine (DMH) and dextran sodium sulfate (DSS). This animal model greatly resembles the histopathological alteration and molecular characteristic features of IBD in human [10]. DMH is an environmental pollutant [11] and its metabolite azoxymethane (AOM) are potent genotoxic agents which can trigger oxidative stress via macromolecule methylation of colonic epithelial cells and facilitate inflammation associated cancer in gastrointestinal tract [12]. DSS is a non-genotoxic, synthetic polysaccharide composed of dextran with sulfated glucose units, well reported as to develop colitis in rodents [13].

Carvacrol (CAR, 2-methyl-5-isopropylphenol) is a phenolic monoterpenes abundantly present in the essential oils produced by aromatic plants and spices of lamiaceae family [14]. Carvacrol has been reported with many pharmacological potential such as anti-oxidant, anti-inflammatory [16] and anti-cancer properties [17–20]. Till now, there are no reports available on the possible anti-antioxidant as well as anti-inflammatory effect of carvacrol. Therefore, the present work aims to evaluate the gastroprotective action of carvacrol in DMH/DSS induced colitis associated colon cancer using F344 rat model.

2. Materials & methods

2.1. Animals

The experimental protocols involving animals were approved by the Institutional Animal Ethics Committee (IAEC No:07/01/2012) and the experiments on animals were performed in accordance with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines. Experiments were performed on male Fischer 344 Rats (5 weeks old) procured from the National Center for Laboratory Animal Science (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India.

2.2. Chemicals

1, 2-Dimethylhydrazine Dihydrochloride and Carvacrol were purchased from Sigma Chemical Company, St Louis, MO, USA. Dextran sulfate sodium salt was purchased from MP Biomedical, USA. Goat-anti-rabbit secondary antibodies were purchased from Bangalore Genei. All other chemicals used were of analytical grade.

2.3. Induction of colitis associated colon cancer

For induction of colitis associated colon cancer, male F344 rats ($n = 6$) received three subcutaneous injections of DMH (40 mg/kg b.wt) in the first week of the experimental period. DSS was dissolved in normal drinking water at a concentration of 1% (w/v). The rats received 1% DSS orally followed by DMH as three cycles which consist of 7 days of DSS and 14 days of normal drinking water. The control rats were given subcutaneous dose of saline alone.

2.4. Experimental design

Animals were divided into five groups, each group consisting of 6 animals; Group I received normal drinking water and served as control. Group II received CAR, 50 mg/kg b.w (this dose of carvacrol was fixed based on the effective dosage fixation studies) for the entire period of the experiment as drug control. Group III–V received DMH in the first week and DSS was administrated in three cycles. Group IV received CAR (50 mg/kg b.w) for one week prior to the induction as pre-treatment. Similarly, Group V received CAR for one week after the induction period as post-treatment. The experiment was terminated after the end of the experimental period of 12 weeks, and all the animals were killed by cervical dislocation after an overnight fast. Blood was collected in heparinized tubes and plasma was separated by centrifugation at 3500 rpm for 10 min. Colonic tissues were homogenized in 0.1 M Tris–HCl buffer (pH 7.4) and was used for further analysis.

2.5. Evaluation of biochemical parameters

The total protein in the colonic tissues was measured by method of Lowry et al. [21]. The activities of colon cancer marker enzymes such as 5′-ND [22] c-GT [23], CD [24] were assayed both in serum and tissue. CEA, AFP [25], ALP [26] and LDH [27] were assayed in the serum. MPO [28] was estimated in tissue samples and NO [29] was estimated in both tissue and serum. The activities of antioxidant enzymes - superoxide dismutase (SOD) [30], catalase (CAT) [31], glutathione peroxidase (GPx) [32] glutathione reductase (GR) [33] were estimated in the colon tissue homogenate and serum. The levels of lipid peroxidation (LPO) products were estimated in serum and colon tissue homogenate [34]. The non-enzymic antioxidants reduced glutathione (GSH) [35], vitamin C [36] and vitamin E [37] was assayed in colon tissue.

2.6. Histopathological evaluation

Histological evaluation was performed in colon tissues and a portion of the specimen was fixed in 10% formalin and embedded in paraffin wax, sectioned at 4 μm thickness and were stained with haematoxylin & Eosin. Light microscopy was used to evaluate pathological changes of colon in control and experimental groups of animals.

2.7. Mast cell staining

Histochemical analysis of mast cells were carried out as reported earlier [38]. Briefly, 4 μm thickness tissue sections were dewaxed in xylene and rehydrated through decreasing concentrations of ethanol to distilled water. The sections were stained with toluidine blue for 2 min and washed with distilled water followed by staining with light green SF for 30 s and washed using distilled water and dehydrated in increasing concentrations through alcohol series, xylene and mounted using DPX.

2.8. Immunohistochemical analysis of iNOS and IL-1 beta

Paraffin embedded colonic tissue sections of 4 μm thickness were rehydrated first in xylene and then in graded ethanol solution. The slides were then blocked with 3% BSA in TBS (Tris buffered saline) for 2 h. The sections were then immunostained with respective primary antibodies of Rabbit polyclonal iNOS (Santa Cruz Biotech, USA, 1:100 dilution), and rabbit polyclonal IL-1β (Santa Cruz Biotech, USA, 1: 100 dilution); incubated overnight at 4 °C. After washing the slides thrice with TBS, the sections were then incubated with goat anti-rabbit secondary antibody (Bangalore Genei, India), diluted 1:200 in TBS and incubated for 2 h at room temperature. Sections were then washed with TBS and incubated for 5–10 min in a solution of 0.02% diaminobenzidine (DAB) containing 0.01% hydrogen peroxide. Counter staining was performed using haematoxylin, and the slides were visualized under a light microscope (Olympus CH 20iBIMF).

Table 1
Carvacrol effect on pathophysiological and cancer marker enzymes.

Parameters	Group I	Group II	Group III	Group IV	Group V
5′ Nucleotidase (5′ND)	6.43 ± 0.84	7.28 ± 0.68[ns]	9.06 ± 1.08[a]	5.42 ± 0.68[b]	6.92 ± 0.25[c]
Glutamyltransferase (cGT)	8.21 ± 0.46	8.43 ± 0.33[ns]	14.28 ± 0.63[a]	7.92 ± 0.39[b]	8.61 ± 0.74[c]
Carcinoembryonic antigen (CEA)	7.16 ± 0.23	7.14 ± 0.26[ns]	12.51 ± 1.24[a]	6.35 ± 0.43[b]	7.59 ± 0.52[c]
Alpha-fetoprotein (AFP)	0.16 ± 0.04	0.12 ± 0.04[ns]	0.74 ± 0.08[a]	0.28 ± 0.059[b]	0.36 ± 0.080[c]
Cathepsin-D (CD)	17.34 ± 0.08	15.63 ± 0.72[ns]	22.42 ± 2.30[a]	17.82 ± 1.60[b]	21.58 ± 2.15[c]
Alkaline phosphatase (ALP)	76.58 ± 4.80	81.65 ± 5.30[ns]	117.08 ± 9.25[a]	86.27 ± 6.13[b]	88.74 ± 7.76[c]
Lactate dehydrogenase (LDH)	78.32 ± 4.76	83.72 ± 6.18[ns]	125.73 ± 8.20[a]	89.04 ± 7.18[b]	90.20 ± 6.23[c]

Units: 5′-ND, nmol of Pi liberated/min/mg protein; c-GT, nmol of p-nitroaniline formed/min/mg protein; CEA, ng/ml of protein, AFP,IU/ml of protein; CD, nmol of tyrosine liberated/min/mg protein/hr; ALP, l mol of phenol liberated/min/mg protein; LDH, l mol of pyruvate liberated/min/mg protein.
Data represents as mean ± SD (n = 6); Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant.
[ns]CAR alone vs control.
[a] DMH/DSS vs control.
[b] CAR + DMH/DSS (Pre-treatment) vs DMH/DSS.
[c] DMH/DSS + CAR. (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$.

2.9. Statistical analysis

All the data were evaluated with SPSS/13 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. $p < 0.05$ was considered to indicate statistical significance. All these results were expressed as mean ± SD for six animals in each group.

3. Results

3.1. Effect of carvacrol on cancer marker enzyme levels

Colon cancer diagnostic markers (5′-ND, c-GT, CEA, AFP and CD) and pathophysiological enzymes (ALP and LDH) levels were remarkably elevated in DMH/DSS–induced rats when compared with control (Group I) rats as shown in Table 1, whereas in carvacrol pre-treated and post-treated groups significantly ($p < 0.05$) reduced these marker levels as compared to DMH/DSS induced rats (Group III).

3.2. Colonic antioxidant enzyme activities of carvacrol

The activity of antioxidant enzymes in colonic tissues and hemolysate of control and experimental group of animals is shown in Table 2. A significant ($p < 0.05$) decrease in the activities of SOD, CAT, GPx and GR was evident in DMH/DSS-induced rats (Group III) when compared with control (Group I). Carvacrol treated groups (Group IV & V) exhibited more prominent activities of antioxidant

Table 2
Effect of Carvacrol on antioxidant enzymes in colon and hemolysate of control and experimental groups of rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
Colon					
SOD	9.63 ± 0.68	9.51 ± 0.067[ns]	5.81 ± 0.46[a]	7.83 ± 0.54[b]	6.41 ± 0.38[c]
CAT	21.54 ± 1.42	21.73 ± 1.64[ns]	13.53 ± 1.14[a]	17.30 ± 1.75[b]	15.67 ± 1.63[c]
GPx	2.13 ± 0.08	2.68 ± 0.07[ns]	1.05 ± 0.08[a]	2.14 ± 0.05[b]	1.91 ± 0.09[c]
GR	1.78 ± 0.06	1.67 ± 0.05[ns]	0.60 ± 0.04[a]	1.43 ± 0.08[b]	0.75 ± 0.03[c]
GST	108.72 ± 9.63	94.31 ± 8.68[ns]	70.57 ± 8.39[a]	98.35 ± 8.94[b]	86.12 ± 9.21[c]
Hemolysate					
SOD	3.64 ± 0.08	3.20 ± 0.14[ns]	0.83 ± 0.04[a]	2.24 ± 0.08[b]	2.04 ± 0.06[c]
CAT	1.82 ± 0.05	1.12 ± 0.04[ns]	0.75 ± 0.06[a]	1.14 ± 0.04[b]	1.02 ± 0.05[c]
GPx	19.76 ± 1.56	21.08 ± 0.09[ns]	11.67 ± 0.73[a]	18.62 ± 1.08[b]	14.36 ± 1.25[c]
GR	46.43 ± 3.75	47.04 ± 1.53[ns]	32.52 ± 3.26[a]	43.23 ± 3.92[b]	39.20 ± 3.74[c]
GST	4.46 ± 0.18	4.72 ± 0.20[ns]	1.08 ± 0.67[a]	4.29 ± 0.08[b]	3.98 ± 0.06[c]

Colon tissue: SOD: 50% inhibition of adrenaline auto oxidation/min; CAT: lM H_2O_2 consumed/mg protein/min; GPx: lg GSH utilized/mg protein/min; GR: nM NADPH oxidized/mg protein/min., GST: μmol CDNB-GSH conjugate formed/min/Hb.
Hemolysate: SOD: units/mg Hb; CAT: lM H_2O_2 consumed/mg Hb; GPx: lg GSH utilized/min/mg Hb; GR: lM NADPH oxidized/min/mg Hb. Values are given as mean ± SD for groups of six rats each.
Data represents as mean ± SD (n = 6); Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant.
[ns]CAR alone vs control.
[a] DMH/DSS vs control.
[b] CAR + DMH/DSS (Pre-treatment) vs DMH/DSS.
[c] DMH/DSS + CAR. (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$.

Table 3
Effect of Carvacrol on non-enzymic antioxidants in the colon of control and experimental groups of rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
GSH	1.72 ± 0.03	1.68 ± 0.07[ns]	0.69 ± 0.06[a]	1.47 ± 0.12[b]	1.32 ± 0.12[c]
Vitamin C	1.25 ± 0.04	1.28 ± 0.12[ns]	1.08 ± 0.13[a]	1.76 ± 0.13[b]	1.43 ± 0.14[c]
Vitamin E	0.64 ± 0.03	0.76 ± 0.07[ns]	0.51 ± 0.02[a]	0.73 ± 0.14[b]	0.68 ± 0.10[c]

Enzyme activities are expressed as GSH: μg/mg protein; Vitamin C: μg/mg protein; Vitamin E: μg/mg protein.
Data represents as mean ± SD (n = 6); Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant.
[ns]CAR alone vs control.
[a] DMH/DSS vs control.
[b] CAR + DMH/DSS (Pre-treatment) vs DMH/DSS.
[c] DMH/DSS + CAR. (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$.

enzymes as compared with DMH/DSS group (Group III). The non-enzymatic antioxidants such as GSH, vitamin E and vitamin C were estimated in colonic tissues of control and experimental groups of rats is showed in Table 3. A significant ($p < 0.05$) decrease in the levels of GSH, vitamin E and vitamin C was evident in DMH/DSS induced rats (Group III) when compared with control (Group I). Carvacrol administration to the treatment group of rats (Group IV &V) significantly ($p < 0.05$) increased the levels of these antioxidants as compared with DMH/DSS-induced group.

3.3. Carvacrol alters biochemical parameters

3.3.1. Colonic MPO activity

Fig. 1A depicts a significant ($p < 0.05$) rise in the levels of MPO in the DMH/DSS group which was found to be 163.15 ± 2.92 μg/mg of protein. There was a significant decrease in the levels of MPO in Carvacrol pre & post-treated groups (78.84 ± 25.48 μg/mg) and (83.26 ± 15.37 μg/mg) respectively, when compared to DMH/DSS induced group.

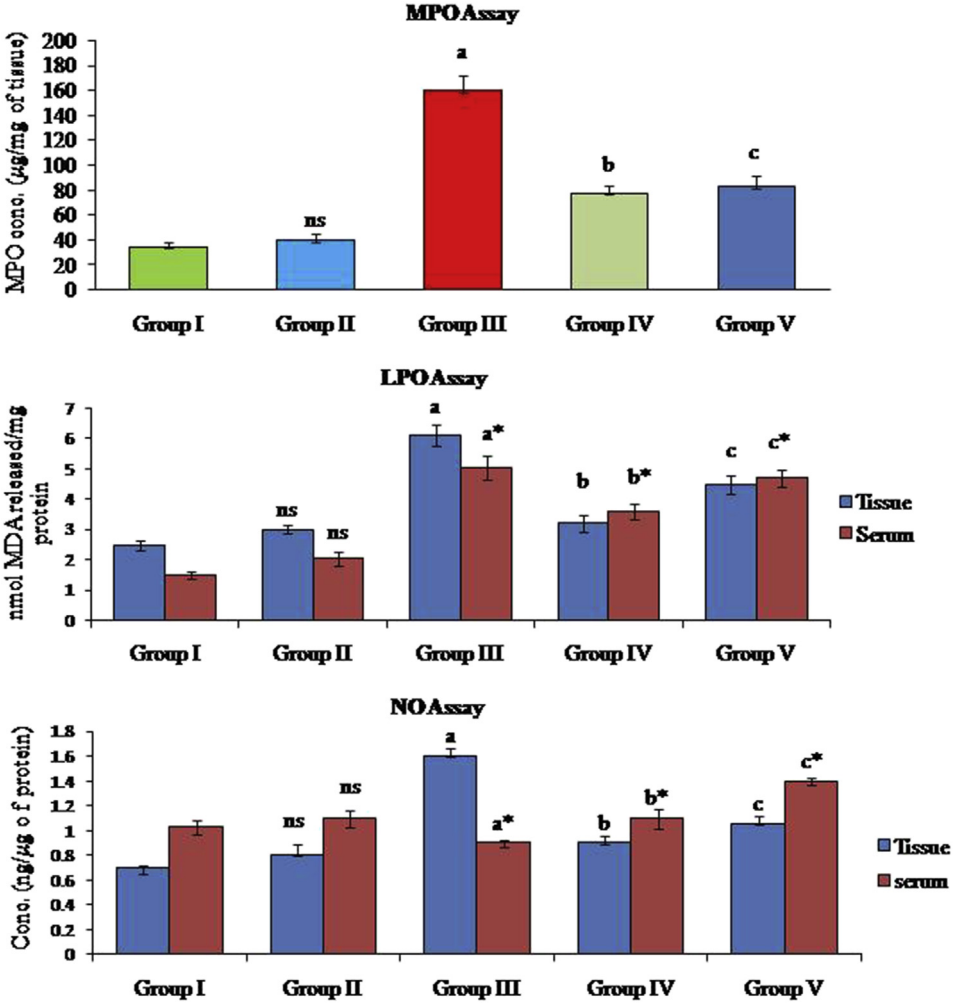

Fig. 1. A. Effect of carvacrol on the levels of MPO in tissue homogenates. Data represents as mean ± SD (n = 6); Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant ^{ns}CAR alone vs control; ^{a}DMH/DSS vs control; ^{b}CAR + DMH/DSS (Pre-treatment) vs DMH/DSS; ^{c}DMH/DSS + CAR. (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$. B. Effect of Carvacrol on the concentration of MDA in the colonic tissue homogenates and serum. Data represents as mean ± SD (n = 6); Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant, ^{ns}CAR alone vs control; ^{a,a*}DMH/DSS vs control; ^{b,b*}CAR + DMH/DSS (Pre-treatment) vs DMH/DSS; ^{c,c*}DMH/DSS + CAR (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$. C. Effect of Carvacrol on the concentration of NO in the colonic tissue homogenate and serum. Data represents as mean ± SD (n = 6); Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant, ^{ns}CAR alone vs control; ^{a,a*}DMH/DSS vs control; ^{b,b*} CAR + DMH/DSS (Pre-treatment) vs DMH/DSS; ^{c,c*}DMH/DSS + CAR (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$.

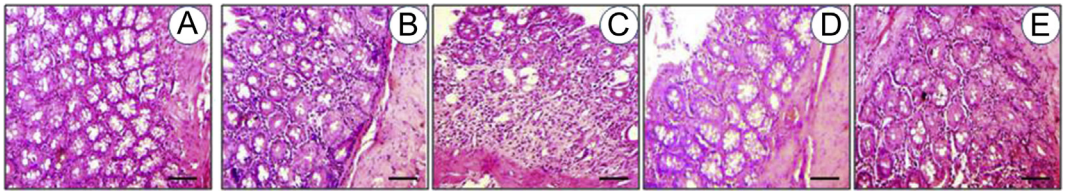

Fig. 2. Histopathological alterations in colon of control and experimental group of rats (40×). Scale bar 60 μm (A) Control, (B) CAR alone (C) DMH/DSS-induced (D) CAR + DMH/DSS (E) DMH/DSS + CAR.

3.3.2. Colonic MDA contents

Fig. 1B exhibits a significant ($p < 0.05$) rise in the levels of MDA in the DMH/DSS group, which was found to be 6.13 ± 0.078 nM/mg of protein. When compared to DMH/DSS group, the levels of MDA in tissues and serum were significantly ($p < 0.05$) reduced in carvacrol treated groups and found to be 3.6 ± 0.054, 3.0 ± 0.06 nM/mg and 4.1 ± 0.072, 3.2 ± 0.06 nM/mg of protein respectively in both treated groups.

3.3.3. Colonic NO level estimation

It is evident from Fig. 1C that when compared to the control group there was a significant ($p < 0.05$) rise in the levels of tissue and serum nitrite in the DMH/DSS group which was found to be 1.6 ± 0.082 and 0.9 ± 0.065 ng/μg of protein respectively. Compared to DMH/DSS induced group, Carvacrol pre & post treated groups showed a significant ($p < 0.05$) decrease in the levels of tissue and serum nitrite which were found to be

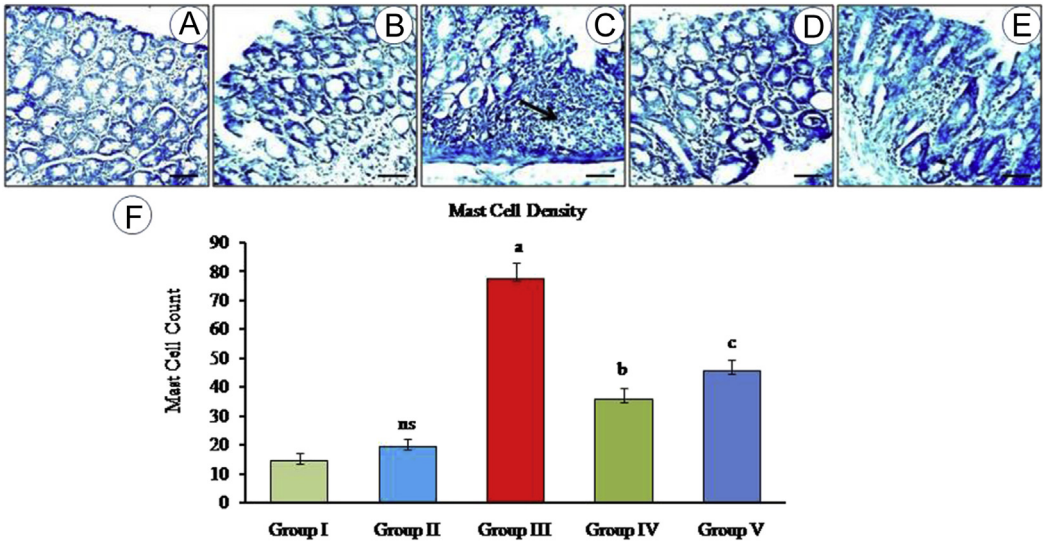

Fig. 3. Histochemical analysis of mast cells in the colon of control and experimental groups of rats (40×). Control and experimental groups of rat tissue sections were stained with toludine blue. Arrow shows mast cells (A). Control. (B). CAR alone. (C). DMH/DSS induced group. (D). CAR + DMH/DSS (pre-treated) (E). DMH/DSS + CAR (post-treated). (F). Quantitative analysis of total mast cell count. Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant, [ns]CAR alone vs control; [a]DMH/DSS vs control; [b]CAR + DMH/DSS (Pre-treatment) vs DMH/DSS; [c]DMH/DSS + CAR. (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

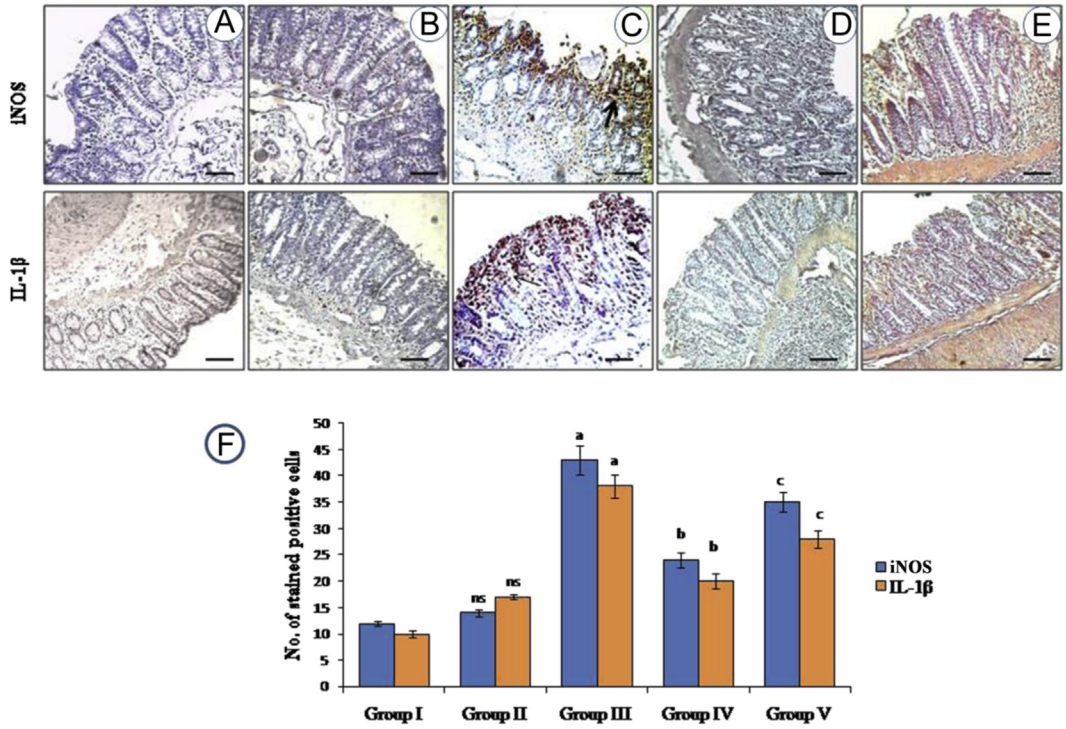

Fig. 4. Immunohistochemical expression of iNOS and IL-1β in rat colon (20×). (A) Control; (B) CAR alone; (C) DMH/DSS-induced: High level of iNOS positive cells (D) CAR + DMH/DSS (Pre-treated) (E) DMH/DSS + CAR (Post treated). (F) Quantitative analysis of iNOS and IL-1β stained positive cells. Values not sharing a common superscript letter (ns, a, b, c) differ significantly. ns, non-significant, [ns]CAR alone vs control; [a]DMH/DSS vs control; [b]CAR + DMH/DSS (Pre-treatment) vs DMH/DSS; [c]DMH/DSS + CAR. (Post-treatment) vs DMH/DSS. Values are statistically significant at $p < 0.05$.

0.97 ± 0.036, 1.1 ± 0.047 ng/μg and 1.06 ± 0.052, 1.4 ± 0.078 ng/μg of protein respectively.

3.4. Effect of carvacrol on histological alteration

The colon of control and carvacrol alone treated rats showed normal mucosa and sub mucosal layers and normal colonic architecture without apparent abnormality in Fig. 2A and B. DMH/DSS–induced rat sections in Fig. 2C showed clear deep ruptured/erosion and inflammation extended through the mucosa, muscularis mucosa and submucosa. In DMH/DSS induced rats (Group III), larger areas of thickened mucosal layer with severe infiltration of inflammatory cells and remarkable destruction of the mucosal epithelial architecture along with epithelial integrity was evident Fig. 2C. Carvacrol administration leads to the attenuation of these abnormalities. Significant ($p < 0.05$) reduction of mucosal epithelial cell damage and inflammation of sub-mucosal layers were evident in carvacrol treated rats Fig. 2D and E.

3.5. Effect of carvacrol on mast cell density

Carvacrol significantly ($p < 0.05$) decreases the number of mast cell in DMH/DSS-induced rats Fig. 3D and E. Mucosal mast cells present in the epithelium of gastrointestinal tract and its number is increased in the inflamed colon of DMH/DSS induced animals Fig. 3C.

Fig. 3F shows the histochemical staining of mast cells stained with toluidine blue in the colon of control and experimental groups of rats. DMH/DSS- induced group of rats showed significantly ($p < 0.05$) increased mast cell number when compared to control rats (Fig. 3A). Where as in both carvacrol treated groups, a significant level of decrease ($p < 0.05$) in mast cell density when compared to DMH/DSS-induced rats was observed.

3.6. Carvacrol ameliorates the inflammatory markers IL-1β and iNOS

Fig. 4 shows the immunohistochemical staining of IL-1 β and iNOS in colonic tissues in DMH/DSS induced CACC. In control and carvacrol alone groups of rats, negligible expression of IL-1β and iNOS expression was observed. However, in DMH/DSS-induced rats, an increased expression of IL-1β and iNOS positive cells were observed. In carvacrol treated rats, there was a decreased number of positive cells for IL-1β and iNOS expression when compared with DMH/DSS induced rats (Fig. 4C).

4. Discussion

The aim of this study is to evaluate the gastroprotective properties of naturally occurring essential oil, Carvacrol using laboratory animal model. CACC was developed in rats by DMH initiation followed by non-genotoxic substance, DSS, which is an established model [39]. During chemical insult, mast cells produce the toxic super oxide anions in the inflammatory environment. Mast cells recruits neutrophils to the inflammatory site to provoke the inflammatory reaction, generating excess ROS and enormous amount of proteolytic enzymes and cytokines which leads to tissue damage in CRC [40]. Dietary supplementation with essential oil not only inhibited the growth of ACF by decreasing the total number of ACF with multicrypts, but also affected the distribution of ACF in distal colon [41]. In mechanistic terms, the presence of phenolic hydroxyl group in carvacrol can make it a potent inhibitor of lipid peroxidation [42]. The administration of DSS induced an extensive inflammation and ulceration in the colon associated with an increased MPO activity [43]. Monoterpene derived essential oil compounds are reported to reduce MPO activity with a possible preventive action for gastrointestinal inflammation and ulceration [15]. The gradual increase in H_2O_2 level along with elevated MDA level observed in colons of DMH/DSS induced rats through the induction of lipid peroxidation as a result of depletion in the colonic antioxidant system. During inflammatory condition, activated macrophages, neutrophils and mast cells express receptors for the pro-inflammatory cytokines such as IL-1β and iNOS. IL-1β is the gate keeper of inflammation [44]. NO plays an important role in genesis of chronic inflammation and these molecules are contributed to the IBD as well as in experimentally induced CACC [45]. The apparent suppression of MPO and NO levels by carvacrol in DMH/DSS induced rats suggests its ability to prevent neutrophil infiltration and inflammation in the colon. Thus, carvacrol treatment effectively ameliorated the induction of oxidative stress, expression of pro-inflammatory cytokines, and ROS-related cellular and histological damage in the DMH/DSS-treated rats. Overall, the findings of the present investigation demonstrate the efficacy of carvacrol to prevent DMH/DSS-induced CACC. It is therefore reasonable to suggest that clinical application of carvacrol may be a candidate for dietary therapy for inflammation associated colon cancer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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