Chapter-III

Farnesol attenuates 1, 2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic responses in the colon of Wistar rats
1. Background

Colon cancer is the major health hazard related with high mortality and it is the second most common cause of cancer-related death (O’Brien et al. 2007). Colon carcinogenesis is a multistep process and is thought to arise by the accretion of genetic alterations involving a variety of oncogenes and tumor suppressor genes that transform normal colonic epithelium into an invasive carcinoma (Janne and Mayer 2000). Colon cancer is frequently a pathological consequence of persistent oxidative stress and inflammation (Bartsch and Nair 2002; Terzic et al. 2010). Oxidative stress is a state which occurs when the balance between the productions of reactive oxygen species (ROS) overcomes the endogenous antioxidant defense system and inflammation is a complex biological response of tissues to pathogens and damaged cells (Ferrero-Miliani et al. 2007).

Several epidemiological studies suggest that diet is considered as one of the major factor associated with increased risk for colon cancer incidence and mortality (Correa and Haenszel 1978; Doll 1980; Burstein 1993). Many experimental animal models have supported the idea that high fat diet augments the incidence of colon carcinogenesis (Bansal et al. 1978; Reddy et al. 1976; Reddy et al. 1977) whereas low fat and high fiber (present in fruits and vegetables) diet, decreases the risk of colon cancer (Hioki et al. 1997). Many natural products present in the high fiber diets have been reported to possess chemopreventive properties against cancer (Wattenberg et al. 1979). Therefore, chemoprevention is a logical and current strategy to reduce the mortality from colon cancer because numerous chemopreventive agents are present in the diet (Corpet and Tache 2002).
Farnesol is a 15-carbon naturally occurring sesquiterpene and it may be endogenously generated in the cells by enzymatic dephosphorylation of farnesyl pyrophosphate, a metabolic precursor of squalene yielding sterols and other isoprenoid compounds (Crick et al. 1997; Meigs and Simoni 1997). Dietary sources of farnesol are the plant products (He et al. 1997) including fruits and berries such as apricots, peaches, plums, blueberries, cranberries, raspberries and strawberries, vegetables such as tomatoes (Tatman and Mo 2002), herbs such as lemon grass and chamomile (Horn et al. 2005) and it is also obtained from the essential oils of ambrette seeds, and citronella (Zhao et al. 2004). Studies from our laboratory have revealed that farnesol is a potent antioxidant and protects the kidneys and lungs against oxidative damage induced by Ferric-Nitrilo Acetate (Fe-NTA) and Cigarette Smoke Extract (CSE) respectively (Jahangir et al. 2006; Qamar and Sultana 2008). Previous studies also have been shown that farnesol exhibits significant anti-tumor and anti-carcinogenesis effects in vivo that might be due to the inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (Burke et al. 2002; Rao et al. 2002; Wiseman et al. 2007). Thus farnesol results in the inhibition of cholesterol formation due to HMG CoA reductase inhibition and may alter the cell/mitochondrial membrane integrity thereby leads to the apoptosis favourably in tumor cells as compared to normal cells (Rao et al. 2002; Khan et al. 2007; Buttke and Sandstrom 1994; Slater et al. 1995; Heitman et al. 1983). Tumor cells are highly proliferating cells requires high levels of cholesterol to maintain the cell/mitochondrial membrane integrity (Burke et al. 2002; Rao et al. 2002). Apoptosis, one of the forms of programmed cell death, characterized by chromatin condensation, nuclear fragmentation, membrane blebbing, cytoskeletal rearrangement and cell shrinkage. It is involved in many physiological and pathological processes and helps to regulate tissue homeostasis by eliminating potentially deleterious cells (Oravec et al. 1986). Oxidative stress is known to be involved in the
induction of apoptosis and antioxidants have been reported to diminish the extent of apoptotic tissue damages (Sengottuvelan et al. 2006; Fiala 1977).

1, 2-dimethylhydrazine (DMH) is a colon specific carcinogen and it has been widely used to induce colon cancer in rodents (Park et al. 1989). DMH undergoes metabolism mainly in the liver and to some extent also in the colon and the ultimate metabolite thus formed in the liver delivered to the colon via, blood or bile, as glucoronide conjugates (Halliwell and Gutteridge 1990; Wei and Frenkel 1993). In vivo transformation of DMH results in the formation of azomethane and N-oxidation of azomethane leads to the formation of azoxymethane. Further, hydroxylation of azoxymethane leads to the formation of methyazoxymethanol which is an unstable compound and readily yields highly reactive electrophilic methylidiazonium ion. The latter leads to the formation of methyl free radicals and DMH also generate hydroxyl radical or hydrogen peroxide in the presence of metal ion which are known to elicit oxidative stress due to imbalance between the production of ROS and endogenous antioxidants (Latham et al. 1999; Pozharisski et al. 1979). It has been reported earlier that these ROS are mainly responsible for the damaging effects of the DMH in colonic tissue (Latham et al. 1999). DMH also cause covalent modification of DNA by 8-hydroxy-2’-deoxyguanosine (8-OHdG) adduct formation which is a marker of oxidative DNA damage (Rogers and Nauss 1985) and it has been well accepted that oxidative DNA damage plays an important role in carcinogenesis (Weisburger et al. 1977). DMH-induced colon tumorigenesis reflects many of the same cell kinetic and similar molecular and histopathological alterations to the sporadic colon tumors of human (Kawanishi and Yamamoto 1991; Dudeja and Brasitus 1990).

The present study was intended to explore the anticipatory effects of farnesol against DMH induced oxidative stress, inflammation and apoptotic responses in the colon of wistar rats.
2. Treatment Regimen

To study the effect of prophylactic treatment with farnesol on 1, 2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic tissue damage in colon, 30 male Wistar rats were randomly allocated to 5 groups of 6 rats each. The rats of group I (control group) and II received corn oil orally at the dose of 5ml/kg body weight once daily for 7 days, which was used as a vehicle for farnesol. Group III received farnesol orally at the dose of 50 mg/kg body weight once daily for 7 consecutive days. Group IV and V (only dose 2 of farnesol) received farnesol at the dose of 100 mg/kg body weight once daily for 7 days. Group II, III and IV rats were given a single injection of DMH (dissolved in 1mM EDTA solution pH adjusted to 6.5 with 1mM NaOH) at the dose of 40 mg/kg body weight, subcutaneously in the groin on day 7 after 1 hr of the last treatment of farnesol. Group I and V rats also received subcutaneous injection of 1mM EDTA solution, (pH adjusted to 6.5 with 1mM NaOH), which was used as the vehicle for DMH. All the rats were anaesthetized with mild anaesthesia and sacrificed by cervical dislocation after 24 hr of the DMH injection.

3. Results

3.1. Effect of prophylactic treatment of farnesol on the activities of glutathione dependent enzymes in colonic tissue

The activities of GPx, GR and GST decreased significantly (p<0.001) in Group II as compared to Group I. Farnesol pretreatment at the dose of 50mg/kg b.wt. significantly increased the activities of GPx (p<0.01), GST (p<0.001), and GR (p<0.01) in Group III as compared to Group II. Higher dose of farnesol (100mg/kg b.wt.) also showed significant increase in the activities of GPx (p<0.001), GST (p<0.001), and GR (p<0.01) in Group IV as compared to
Group II. However, the activities of these enzymes in Group V did not change significantly as compared to Group I. (Table 1)

3.2. Effect of prophylactic treatment of farnesol on the activities of colonic antioxidant enzymes

DMH treatment caused significant decrease in the activities of SOD (p<0.01), Catalase (p<0.001) and QR (p<0.001) in Group II as compared to Group I. Farnesol pretreatment (50mg/kg b.wt.) significantly increased the activities of SOD (p<0.05), Catalase (p<0.05), and QR (p<0.01) in Group III as compared to Group II. Higher dose of farnesol (100mg/kg b.wt.) also showed significant increase in the activities of SOD (p<0.01), catalase (p<0.01), and QR (p<0.01) in Group IV as compared to Group II. However, the activities of these enzymes in Group V did not change significantly as compared to Group I. (Table 2)

3.3. Effect of prophylactic treatment of farnesol on the level of colonic GSH

The level of GSH was depleted significantly (p<0.001) in DMH treated group (Group II) as compared to control group (Group I). Farnesol pretreatment showed a significant increase in the level of GSH in Group III (p<0.001) and Group IV (p<0.001) when compared with group II. Group V exhibited no significant changes in the level of GSH as compared to control group. (Fig.1)

3.4. Effect of prophylactic treatment of farnesol on the level of MDA and H$_2$O$_2$ content in colonic tissue

The level of MDA and H$_2$O$_2$ content were significantly (p<0.001) enhanced in Group II as compared to Group I. Farnesol pretreatment (50mg/kg b.wt.) significantly (p<0.01) decreased
the level of MDA and H_2O_2 in Group III as compared to Group II. Higher dose of farnesol (100mg/kg b.wt.) also significantly (p<0.001) decreased the level of MDA and H_2O_2 content in Group IV as compared to Group II. No significant difference was found in the level of MDA and H_2O_2 between Group I and Group V. (Fig 2 & 3)

3.5. Effect of prophylactic treatment of farnesol on Caspase-3 activity in colonic tissue
Caspase-3 activity was significantly (p<0.01) increased in DMH-treated group (Group II) as compared to control group (Group I). Caspase-3 activity decreased significantly (p<0.05) in Group IV only but non-significantly in Group III as compared to Group II. Pretreatment with only farnesol did not show any significant difference in caspase-3 activity between Group I and Group V. (Fig 4)

3.6. Effect of prophylactic treatment of farnesol on Cytochrome P450 (Cyt P450) activity in colonic tissue
Cyt P450 activity was significantly (p<0.01) increased in DMH-treated group (Group II) as compared to control group (Group I). Cyt P450 activity decreased significantly (p<0.01) in Group III and IV as compared to group II. Farnesol pretreatment did not show any significant difference between Group I and Group V. (Fig 5)

3.7. Histopathological findings
Histological sections of vehicle treated control group (Group I) exhibited normal histoarchitecture of the colon with mild inflammatory cells infiltration. In DMH-treated group (Group II), irregular glandular structure, regional destruction of the mucosa with intense inflammatory cells infiltration in mucosal and submucosal layers as well as submucosal edema were examined. In group III (dose1 of farnesol + DMH) and group IV (dose2 of farnesol +
DMH), histological sections showed that farnesol supplementation prevent the mucosal damage and remarkable reduction in the inflammatory cells infiltration and submucosal edema. Group V (only dose2 of farnesol) tissue sections displayed normal histology as similar to that of Group I (vehicle treated control group). (Fig 6)

4. Discussion

A current upsurge in classifying natural products as cancer chemopreventive agents is gaining much attention of many investigators because natural products like fruits, vegetables, medicinal plants, and herbs have many pharmacological properties and have potential to fight against numerous human diseases associated with oxidative stress. Several natural compounds of dietary sources have been reported to inhibit various diseases including cancer (Steinmetz and Potter 1991; Wattenberg 1996) and farnesol is one of them, belongs to the category of isoprenoid alcohol. Farnesol has been reported to possess chemopreventive properties against initial phases of hepatocarcinogenesis (Ong et al. 2006). We have found in the present study that farnesol supplementation modulates DMH-induced oxidative stress by decreasing the tissue lipid peroxidation and enhancing the overall antioxidant status.

DMH is a procarcinogen and after metabolic activation it results in the formation of methyl free radical which is known to induce oxidative stress. DMH also generates hydroxyl radical or hydrogen peroxide in the presence of metal ion that may play a part in the initiation of lipid peroxidation (Pence 1991; Kawanishi and Yamamoto 1991).

Lipid peroxidation is a consequence of oxidative stress and remarkable elevation in the level of malondialdehyde (MDA), a lipid peroxidation product, was observed after treatment with DMH.
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(Dudeja and Brasitus 1990). It has already been reported that MDA acts as a mutagen and tumor promoter (Seven et al. 1999; Mukai and Goldstein 1976; Yau 1979). In the present study, DMH-treated rats showed a remarkable increase in the level of MDA and farnesol significantly attenuated its level in colonic tissue. Thus, farnesol exhibited the protective efficacy against DMH-induced lipid peroxidation in colonic tissue. Studies from our laboratories have also shown that farnesol diminished the level of MDA in the kidney and lung after subsequent treatment with Fe-NTA and CSE respectively (Jahangir et al. 2006; Qamar and Sultana 2008). Apart from lipid peroxidation, the level of reduced glutathione (GSH) depleted following DMH treatment.

GSH is a low molecular weight tripeptide a cellular antioxidant. It protects the peroxidation of lipid membrane by conjugating with the electrophile such as 4-Hydroxy-3-nonenal (HNE), formed during lipid peroxidation and thus gets depleted in this conjugation reaction (Forman et al. 2009). This conjugation of GSH via sulphahydryl (-SH) group to electrophile is catalysed by an antioxidant enzyme i.e., glutathione-s-transferase (GST), and thus the GST activity decreased in this process (Sharma and Sultana 2004; Douglas 1987; Chadha et al. 2007). The levels of free radicals generated by the DMH kept low by the endogenous non-enzymatic (GSH) and enzymatic (SOD, GPx, GR, Catalase etc.) antioxidants and these antioxidants could play a role in protection of the colonic tissue by scavenging the free radicals. Thus the concentrations of antioxidants and the activities of the antioxidant enzymes could be down-regulated by the DMH treatment (Arutiunian et al. 1997; Rajeshkumar and Kuttan 2003; Dreher and Junod 1996). The protective efficacy of farnesol against DMH induced oxidative stress can be attributed to its ability to induce antioxidant/detoxifying enzymes in rats viz., GR, QR, and GST etc. as reported previously (Horn et al. 2005; Joo and Jetten 2010; Chaudhary et al. 2009).
The findings of the present study demonstrated that the level of GSH and the activities of GST and glutathione-associated enzymes (GPx, GR) were decreased significantly in the colon of DMH treated rats. Farnesol supplementation augmented the level of GSH and the activities of GST and glutathione redox cycle enzymes in colonic tissue. These results are in agreement with the previous studies from our lab (Jahangir et al. 2006; Qamar and Sultana 2008).

The activities of other antioxidant enzymes like SOD, catalase and quinone reductase were also reduced by DMH treatment. Farnesol pretreatment significantly enhanced the activities of all these antioxidant enzymes in colonic tissue. The results of the present study are in accordance of the previous findings which has been shown that the activities of these antioxidant enzymes decreased in colonic tissue after DMH treatment (Chadha et al. 2007). H$_2$O$_2$ content formed in the colonic tissue is associated with oxidative DNA damage and it may lead to or play a role in cancer development (Stone et al. 1994). In the present finding DMH increased the basal level of H$_2$O$_2$ in the PMS of colonic tissue and the basal level of H$_2$O$_2$ was significantly reduced in the PMS of colonic tissue of farnesol treated rats due to preservation of antioxidants enzymes.

DMH is an alkylating agent which damages cellular DNA by forming adduct. Some cells are repaired by DNA repair enzymes but a few are not and these cells with damaged DNA undergo apoptotic removal (Rogers and Pegg 1977; Lane 1992). Apoptosis is a tightly regulated state of programmed cell death (Goncu and Parlak 2008) and caspases play a key role for initiation and execution of cell death. Caspases are the cysteine-dependent enzymes and are activated by oxidative stress (Ghavami et al. 2009). Caspase-3 is the main executioner caspase because it can be activated through both intrinsic and extrinsic pathway. Activated caspase-3 leads to DNA fragmentation and cleavage of specific cellular proteins like PARP, actin and lamins during
apoptosis (Wen et al. 2002). The present study has demonstrated that the caspase-3 activity significantly up regulate in DMH treated group and pretreatment with farnesol significantly down regulate the caspase-3 activity.

Histological evaluation showed that farnesol suppressed the inflammatory responses in the colon by decreasing the intense infiltration of the inflammatory cells in the mucosal and submucosal layers. It also reduced the severity of submucosal edema and regional destruction of mucosal layer induced by DMH in the colon of rats. Previous studies have shown that farnesol diminished the extent of inflammation in mice (Cohen 1981; Marcuzzi et al. 2008) but the exact mechanism is still unclear. Previously, it has been demonstrated that farnesol reduced the levels of both, Th1 cytokine gamma interferon (IFN-γ) and Th1-inducing cytokine interleukin-12 (IL-12) (Chaudhary et al. 2009) but increased the level of the Th2 cytokine IL-5. IL-5 repressed the expression of both IL-12 and IFN-γ (Navarathna Dhammika et al. 2007). It may be a plausible molecular mechanism of farnesol against DMH induced inflammation in the colon of rat.

The precise mechanism of chemopreventive action of farnesol against colon carcinogenesis remains to be elucidated but the plausible mechanism of the protection of farnesol may be through the induction of the antioxidant enzymes. From the findings of the current study it can be concluded that farnesol supplementation effectively suppressed the initial phases of colon carcinogenesis probably by reducing the oxidative damage, inflammatory and apoptotic responses induced by DMH in rats. The results also shows that farnesol have low toxicity as well as its availability from dietary sources made this compound to be more important for further studies. Farnesol can be a potent supplement that prevents colon cancer in human subjects.
Table: 1 Effects of farnesol and DMH on the activities of glutathione peroxidase (GPX), glutathione-S-transferase (GST) and glutathione reductase (GR)

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>GPx</th>
<th>GST</th>
<th>GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>252.598 ± 11.8</td>
<td>236.45 ± 11.12</td>
<td>11.621± 1.377</td>
</tr>
<tr>
<td>II (DMH only)</td>
<td>168.798 ± 13.099***</td>
<td>172.15 ± 11.82***</td>
<td>6.336 ± 0.667***</td>
</tr>
<tr>
<td>III (DMH + D1 )</td>
<td>196.02 ± 10.659##</td>
<td>205.42 ± 11.75###</td>
<td>8.46 ± 1.007##</td>
</tr>
<tr>
<td>IV (DMH + D2)</td>
<td>214.208 ± 9.076###</td>
<td>219.81 ± 11.45###</td>
<td>8.891 ± 0.632##</td>
</tr>
<tr>
<td>V (D2 only)</td>
<td>253.833 ± 11.202</td>
<td>237.12 ± 11.27</td>
<td>11.908 ± 1.036</td>
</tr>
</tbody>
</table>

Table.1 Values of glutathione metabolizing enzymes (GR and GPx) and GST are expressed as mean ± S.D. (n = 6). GPx as nmol NADPH oxidized/min/mg protein, GST as nmol CDNB conjugate formed/min/mg protein and GR as nmol NADPH oxidized/min/mg protein. Significant difference was indicated by ***p<0.001 when compared with control animals (Group I), and ##p < 0.01 and ###p<0.001 when compared with Group II.
Table: 2 Effects of farnesol and DMH on the activities of Catalase (CAT), Superoxide dismutase (SOD) and Quinone reductase (QR)

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Catalase</th>
<th>SOD</th>
<th>QR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>3.016 ± 0.252</td>
<td>14.544 ± 1.308</td>
<td>52.953 ± 7.328</td>
</tr>
<tr>
<td>II (DMH only)</td>
<td>1.968 ± 0.276***</td>
<td>11.381 ± 1.498**</td>
<td>28.937 ± 6.415***</td>
</tr>
<tr>
<td>III (DMH + D1)</td>
<td>2.487 ± 0.387#</td>
<td>13.693 ± 0.792#</td>
<td>44.213 ± 6.787##</td>
</tr>
<tr>
<td>IV (DMH + D2)</td>
<td>2.649 ± 0.349##</td>
<td>14.215 ± 0.766##</td>
<td>47.678 ± 7.662##</td>
</tr>
<tr>
<td>V (D2 only)</td>
<td>3.126 ± 0.221</td>
<td>14.602 ± 1.875</td>
<td>53.155 ± 7.457</td>
</tr>
</tbody>
</table>

Table.2 Values of antioxidant enzymes are expressed as mean ± S.D. (n = 6). Catalase as µmol $H_2O_2$ consumed/min/mg protein, SOD as Units/mg protein and QR as nmol DCPIP reduced/min/mg protein. Significant differences were indicated by **$p < 0.01$ and ***$p < 0.001$ when compared with control animals (Group I), and #$p < 0.05$ and ##$p < 0.01$ when compared with Group II.
Fig 1. Effects of farnesol and DMH on the colonic GSH level

Fig.1 Effect of prophylactic treatment of farnesol on colonic GSH content. Values are expressed as mean ± S.D. (n=6) measured as nmol DTNB conjugate formed/g tissue. GSH content was significantly decreased in Group II (**p<0.01) as compared to Group I. Farnesol pretreatment significantly increased the level of colonic GSH in Group III (#p<0.05) and Group IV (###p<0.001) as compared to Group II.

GP1- Vehicle Treated Control Group
GP2- DMH Treated Group
GP3- DMH + Dose 1 of Farnesol (50 mg/kg b.wt.)
GP4- DMH + Dose 2 of Farnesol (100 mg/kg b.wt.)
GP5- Only Dose 2 of Farnesol (100 mg/kg b.wt.)
**Fig 2.** Effects of farnesol and DMH on the colonic MDA level

![Graph showing effects of farnesol and DMH on colonic MDA level]

**Fig.2** Effect of prophylactic treatment of farnesol against DMH-induced lipid peroxidation product (MDA level) in colon of wistar rats. Values are expressed as mean ± S.D. (n=6) measured as nmol MDA formed/min/g tissue. MDA content was significantly (***p<0.001) increased in DMH treated group (Group II) as compared to Group I. Pretreatment with farnesol significantly (##p<0.01, ###p<0.001) attenuated the MDA content in Group III and IV as compared to Group II.

GP1- Vehicle Treated Control Group

GP2- DMH Treated Group

GP3- DMH + Dose 1 of Farnesol (50 mg/kg b.wt.)

GP4- DMH + Dose 2 of Farnesol (100 mg/kg b.wt.)

GP5- Only Dose 2 of Farnesol (100 mg/kg b.wt.)
Fig 3. Effects of farnesol and DMH on colonic $H_2O_2$ content

Fig. 3 Effect of prophylactic treatment of farnesol on $H_2O_2$ formation in colonic tissue of wistar rats. Values are expressed as mean ± S.D. ($n = 5$) measured as nmol $H_2O_2$ formed/h/g tissue. $H_2O_2$ content was significantly elevated in Group II (***p<0.001) as compared to Group I. Pretreatment with farnesol significantly decreased $H_2O_2$ content in Group III (##p<0.01) and Group IV (###p<0.001) as compared to Group II.

GP1- Vehicle Treated Control Group

GP2- DMH Treated Group

GP3- DMH + Dose 1 of Farnesol (50 mg/kg b.wt.)

GP4- DMH + Dose 2 of Farnesol (100 mg/kg b.wt.)

GP5- Only Dose 2 of Farnesol (100 mg/kg b.wt.)
**Fig 4.** Effects of farnesol and DMH on the activity of Caspase-3 in the colon

![Graph showing effects of farnesol and DMH on Caspase-3 activity in the colon.](image)

**Fig.4** Effect of prophylactic treatment of farnesol on caspase-3 activity in the colon of DMH-induced rats. Values are expressed as mean ± S.D. (n=6) and measured as absorbance/mg protein. Caspase-3 activity was significantly increased in the Group II (**)p<0.01) as compared to Group I. Group III exhibited no significant change in caspase-3 activity as compared to group II. Pretreatment with higher dose of farnesol (Group IV) significantly (#p<0.05) decreased the caspase-3 activity as compared to group II. There is no significant difference in the caspase-3 activity between Group V and Group IV.

GP1- Vehicle Treated Control Group

GP2- DMH Treated Group

GP3- DMH + Dose 1 of Farnesol (50 mg/kg b.wt.)

GP4- DMH + Dose 2 of Farnesol (100 mg/kg b.wt.)

GP5- Only Dose 2 of Farnesol (100 mg/kg b.wt.)
Fig 5. Effects of farnesol and DMH on the activity of Cytochrome P450 in the colon

Fig.5 Effect of prophylactic treatment of farnesol on Cytochrome P450 activity in the colon of DMH-induced rats. Values are expressed as mean ± S.D. (n=6) and measured as absorbance/mg protein. Caspase-3 activity was significantly increased in the Group II (**p<0.01) as compared to Group I. Group III exhibited no significant change in caspase-3 activity as compared to group II. Pretreatment with higher dose of farnesol (Group IV) significantly (#p<0.05) decreased the caspase-3 activity as compared to group II.

GP1- Vehicle Treated Control Group
GP2- DMH Treated Group
GP3- DMH + Dose 1 of Farnesol (50 mg/kg b.wt.)
GP4- DMH + Dose 2 of Farnesol (100 mg/kg b.wt.)
GP5- Only Dose 2 of Farnesol (100 mg/kg b.wt.)

**Fig 6.** Effects of farnesol and DMH on the colonic histoarchitecture

**Fig.5 Histological Studies:** Photomicrographs of histological sections of colonic mucosa. (A) Vehicle treated control group, (B) DMH-treated group, (C) DMH + Dose 1 of Farnesol (50
mg/kg b.wt.), (D) DMH + Dose 2 of Farnesol (100 mg/kg b.wt.), (E) Only Dose 2 of Farnesol (100 mg/kg b.wt.).

(A) Normal histology of the rat colon with mild inflammatory cells infiltration (normal appearance of the mucosal crypts of Lieberkuhn, submucosa, and muscular layers)

(B) Regional destruction of the mucosal layer with intense inflammatory cells infiltration in mucosal and submucosal layers as well as submucosal edema formation.

(C)-(E) Farnesol supplementation prevent the mucosal damage and ameliorates inflammatory cells infiltration as well severity of the submucosal edema.

Insets at the right panel show a magnified view (40x magnifications) of the insets showed at the left panel (10x magnifications).