Original Article

Histopathological Efficacy Of Nigella Sativa Oil For Prevention Of 5-Fluorouracil-Induced Mucositis In Rats

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Abstract

This study aimed to assess the histopathological efficacy of Nigella sativa (N. Sativa) oil to prevent 5-fluorouracil (5-FU)-induced mucositis in rats. In this animal study, mucositis was induced in 96 albino Wistar rats by injection of 5-FU. The rats were then randomly divided into three groups: control, placebo, and pre-treatment with N. Sativa. The rats in the third group received 100 mg/kg N. sativa on day 1 and 65 mg/kg on day 3. The / buccal mucosa of all rats was scratched with an 18-gauge needle at 3 and 5 days. Groups 2 (placebo) and 3 (pre-treatment) received 400 µL kg/day placebo and N. Sativa oil intraperitoneally respectively daily, starting from 15 days prior to chemotherapy until the last day of the experiment. Biopsy specimens were obtained from the buccal mucosa of 8 rats in each group at 3, 6, 9 and 12 days for histopathological analysis. The severity of mucositis was evaluated by measuring three parameters namely epithelial changes, level of inflammation (based on the percentage of inflammatory cells present) and connective tissue changes. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests. The severity of mucositis in the pre-treatment group was significantly lower than that in the placebo and control groups at 3 (P=0.010) and 6 (P=0.012) days; however, this difference was not significant at other time points (P>0.05). Intraperitoneal injection of N. Sativa oil can decrease the severity of 5-FU-induced mucositis in rats.

Keywords: Nigella sativa; Chemotherapy; 5-Fluorouracil; Mucositis; Rats

Introduction

Chemotherapy-induced mucositis is a common debilitating complication of chemotherapy. In brief, its pathogenesis involves DNA destruction and the generation of free oxygen species by chemotherapy. The oxygen-free radicals activate the transcription enzymes and factors such as the nuclear factor kappa B (NF-κB) and cause the over-expression of some genes related to production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin (IL)-1B and IL-6. These cytokines degrade the basal membrane and submucosa and lead to

apoptosis. Following tissue injury and wound formation, the destructed tissue becomes susceptible to bacterial colonization and development of secondary infection [1]. No definite treatment or preventive measure has been confirmed for chemotherapy-induced mucositis [1]. However, according to the mechanism and pathogenesis of mucositis, it seems that the following strategies may be effective for its prevention or management [1]: (I) elimination of reactive oxygen species, (II) inhibition of inflammation and production/release of cytokines and NF-κB, and (III) inhibition of apoptosis at the cellular level. Among the anti-cancer medications, 5-fluorouracil (5-FU) plays a prominent role in development of mucositis. It is an antimetabolite commonly used in the chemotherapy protocolfor most prevalent cancer types in Iran [1-3]. Following treatment with 5-FU, over 40% of patients develop mucositis; out of which 10% to 15% develop severe grade-3 or 4 mucositis [4]. Nigella sativa (N. Sativa) is a valuable medicinal plant in phytotherapy. Several evidence-based investigations confirm its analgesic, antimicrobial, anti-inflammatory and anti-oxidative properties [5-8]. N. Sativa contains potent antioxidants such as tocopherol, carvacrol, T-anethole, thymoquinone and 4-terpineol [9]. The synergistic effect of N. Sativa constituents is responsible for its higher efficacy compared with the pure form of its active ingredients [9]. The presence of quinones such as thymoquinone and thymohydroquinone and a phenolic compound namely thymol in the composition of N. sativa oil, is responsible for it's the unique properties. The majority of the medicinal properties of this herb are attributed to thymoquinone [10]. N. Sativa decreases the serum level of pro-inflammatory cytokines such as IL-1B and TNF-alpha and several pro-inflammatory mediators, which can lead to a reduction in the level of malondialdehyde and increase in the level of anti-oxidative enzymes such as superoxide dismutase and consequent exertion of their anti-oxidative effects [6, 9-11]. Aside from its therapeutic effects, N. Sativa oil increases the level of anti-oxidative enzymes and prevents oxidative stress as such [10, 11]. Previous studies have confirmed the optimal anti-inflammatory, anti-oxidative and antimicrobial properties of N. Sativa. Also, evidence shows that N. Sativa oil can enhance gingival wound healing by accelerating the proliferation of gingival fibroblasts and regulating the fibroblast growth factor [5, 6, 10-13]. Animal studies on rats have confirmed the efficacy of pre-treatment with thymoquinone in reducing biochemical markers of oxidative stress on the surface of red blood cells [14]. Also, evidence shows that pre-treatment with N. Sativa oil for 21 days significantly increases the anti-oxidative capacity of blood and plasma and also increases the level of catalase, glutathione peroxidase and superoxide dismutase activity [14, 15]. The efficacy of N. Sativa oil for preventing chemotherapy-induced mucositis has not been previously evaluated. Thus, this study aimed to assess the efficacy of N. Sativa oil for the prevention of 5-FU-induced mucositis in rats.

Materials and Methods

This animal study evaluated 96 albino Wistar rats between 8 to 10 weeks that weighed 250 to 300 g. The rats were virgin, albino Wistar with no sign/symptom of disease or infection and similar age and weight that were selected by a veterinarian. The rats that had signs/symptoms of disease before the study or development of a disease condition unrelated to chemotherapy during the experiment were excluded. The excluded rats were replaced. The rats were purchased from the animal center of Shahid Beheshti University of Medical Sciences, School of Dentistry. After purchase, the rats were kept in polycarbonate cages under similar standard conditions of 24 h light/24 h dark cycles, 22±2°C temperature and ad libitum access to food and water for acclimation for one week. The rats were standardized in terms of weight, age, storage conditions, access to food and water, and method of induction of mucositis. After one week, the rats were randomly divided into three groups control, placebo and pre-treatment with N. Sativa oil (n=32). Each group was randomly divided into 4 subgroups (n=8) for histopathological assessment at 3, 6, 9 and 12 days after the induction of mucositis (completion of chemotherapy). The 5-FU was obtained from Ebewe pharma (Australia) in 250 mg vials. The N. Sativa oil (Barich Essence) was purchased in 30 mL vials. To ensure the purity of the purchased N. Sativa oil, it was analyzed at the Medicinal Plant Research Center of Shahid Beheshti University by gas chromatography-mass spectrometry at 60°C for 5 min followed by 250°C for 2 min, and its constituents were isolated (Table 1). Its composition was then compared with the constituents reported in similar studies [6, 7, 16], and its purity was confirmed. After measuring its density, its concentration was estimated to be 0.9%. The placebo was also obtained from the same company (Barich Essence).

Table 1. Constituents of N. Sativa oil purchased from Barich Essence

Compound	Area %
n-Nonanal	4.96
Thymol	50.81
Carvacrol	10.11
2-Undecenal	3.36
Heptadecane	1.69
1-Nonadecane	3.94
Methyl Palmitate	1.63
Heneicosane	3.63
Nonadecane	11.91

First, a pilot study was carried out to ensure the correct methodology and the selected doses. The pilot study was conducted on five rats. Next, tissue specimens were obtained from the buccal mucosa of two healthy rats to serve as a reference for comparison. In the main study, the rats were randomly divided into three groups (n=32) of control, placebo and pre-treatment with N. Sativa oil. The pre-treatment group received an intraperitoneal injection of 400 µL/kg of N. Sativa oil once a day for 15 days to prevention of chemotherapy-induced mucositis [5]. The placebo group received the same amount of placebo oil intraperitoneally on a daily basis for 15 days. The control group did not undergo any intervention during the first 15 days. After completion of the prevention period, all rats in all groups underwent chemotherapy with 5-FU and received 100 mg/kg of 5-FU on day 1 and 65 mg/kg 5-FU on day 3 intraperitoneally with an insulin syringe with <30° angle. It was injected in the inferior quadrant of the abdomen slightly away from the midline and almost parallel to the rat's leg. In the pre-treatment and placebo groups, the injection of N. Sativa oil and placebo continued until the experiment's final day. On days 3 and 5 following the initiation of chemotherapy, anesthesia was induced by intramuscular injection of 3 mg/kg xylocaine hydrochloride (Merck, Germany) and 90 mg/kg ketamine hydrochloride (Merck, Germany). A 0.5 cm scratch with the same depth was created in the left buccal mucosa of the rats in all groups using an 18-gauge needle (Ava Pezeshk, Iran) twice with a linear movement [17]. The same person created all scratches. Next, the 32 rats in each group were randomly divided into 4 subgroups (n=8) for sacrifice at 3, 6, 9 and 12 days after completing chemotherapy with 5-FU. At each time point, the rats were first anesthetized and then sacrificed. The biopsy specimens were harvested from the left buccal mucosa of the rats using a #6 biopsy punch (Ava Pezeshk, Iran). The tissue specimens were fixed using 10% formalin, routinely embedded in paraffin and sectioned into 5-µm slices. They were then stained with hematoxylin and eosin and observed under a light microscope (Eclipse; Nikon, Japan). The severity of mucositis was evaluated by measuring three parameters: epithelial changes, inflammation level (based on the percentage of inflammatory cells present), and connective tissue changes [18, 19]. A calibrated oral pathologist observed the specimens and scored the histopathological changes as follows:

Histopathological epithelial changes [19]:
Score 0: Normal mucosa
Score 1: Focal or disseminated changes in the basal layer along with atypical nuclei and ≥ two squamous cells with dyskeratotic changes
Score 2: Thinning of the epithelial lining (2 to 4 cell layers) and ≥ 3 squamous cells with dyskeratotic changes in the epithelium
Score 3A: Epithelial loss without any breakage in keratinization or presence of atrophic eosinophilic epithelium
Score 3B: Subepithelial formation of vesicles or bullae
Score 4: Complete loss of epithelium and the underlying keratinized layer, ulceration

Histopathological degree of inflammation:
Score 0: No inflammation
Score 1: Mild inflammation (10% to 30%)
Score 2: Moderate inflammation (20% to 50%)
Score 3: Severe inflammation (over 50%)

Histopathological connective tissue changes:
Score 0: Normal connective tissue
Score 1: Granulation tissue formation
Score 2: Fibrosis
Score 3: Necrosis
Finally, each rat received three scores indicative of the severity of mucositis. Since the value of all three parameters was the same and all three were reliable, the three scores were summed to calculate the total mucositis grade for each rat. Accordingly, the total mucositis grade was categorized as mild (total score of 1 to 5), moderate (total score of 5 to 10) or severe (total score of 10 to 14) for each rat.

Statistical Analysis
Data were analyzed using SPSS version 24 (SPSS Inc., IL, and USA). The non-parametric Kruskal-Wallis test was used to compare the groups. Pairwise comparisons were carried out using the Mann-Whitney test at $P<0.05$ level of significance.

Results

In this study, 96 rats were divided into three groups: placebo, control and pre-treatment, and histopathological changes in all three groups were examined on days 3, 6, 9 and 12. The Kruskal-Wallis test revealed significant differences between the groups regarding the epithelial changes (P=0.000) and degree of inflammation (P=0.001); however, no significant difference was noted among the three groups regarding the connective tissue changes (P=0.215). Pairwise comparisons of the groups by the Mann-Whitney test revealed that the score of epithelial changes in the pre-treatment group was significantly lower than that in control (P=0.001) and placebo (P=0.001) groups, while the difference between the control and placebo groups was not significant regarding epithelial changes (P=0.83). The degree of inflammation was significantly lower in the pre-treatment group compared with the placebo (P=0.003) and control (P=0.000) groups; however, the difference between the control and placebo groups was not significant (P=0.21) (Table 2).

Table 2. Frequency of epithelial change, degree of inflammation and connective tissue change scores in the three groups

Epithelial changes				**Degree of inflammation**				**Connective tissue changes**			
Score	control	placebo	pretreatment	Score	control	Placebo	pretreatment	Score	control	Placebo	Pretreatment
0	0 0%	0 0%	5 6.15%	0	11 34.4%	19 59.4%	27 84.4%	0	1 3.1%	3 9.4%	10 31.3%
1	0 0%	0 0%	0 0%	1	9 28.1%	5 15.6%	2 6.3%	1	9 28.1%	6 18.8%	4 12.5%
2	1 3.1%	2 6.3%	6 18.8%	2	7 21.9%	4 12.5%	0 0%	2	1 3.1%	1 3.1%	0 0%
3A	1 3.1%	0 0%	1 3.1%	3	5 15.6%	4 12.5%	3 9.4%	3	21 56.6%	22 68.8%	18 56.3%
3B	5 7.15%	0 0%	7 21.9%								

4	25 1.78%	30 93.7%	13 40.6%

A: Epithelial changes at different time points:

Epithelial changes at 3 (P=0.000) and 6 (P=0.038) days after chemotherapy were significantly different among the three groups, while no significant difference was noted at 9 (P=0.134) and 12 (P=0.175) days. Pairwise comparisons revealed significant differences between the pre-treatment and the other two groups at 3 and 6 days (P<0.05), while no significant difference was noted at 9 and 12 days (P>0.05). The difference between the placebo and control groups was not significant at any time point (P>0.05).

B: Inflammatory changes at different time points:

The degree of inflammation was significantly different among the three groups at 3 days after chemotherapy (P=0.010). It was lower in the pre-treatment group while the difference was not significant at 6 (P=0.070), 9 (P=0.317) or 12 (P=0.494) days. Pairwise comparisons of the groups on day 3 revealed that the inflammatory changes in the pre-treatment group were significantly lower than those in the control and placebo groups (P<0.05). However, no significant difference was noted in this respect at other time points (P>0.05). The changes were not significantly different between the control and placebo groups at any time point (P>0.05).

C: Connective tissue changes:

The degree of connective tissue change at 3 days after chemotherapy was significantly different among the three groups and was significantly lower in the pre-treatment group (P=0.003); however, this difference was not significant at 6 (P=0.231), 9 (P=0.592) or 12 (P=0.368) days. Pairwise comparisons revealed a significantly lower degree of connective tissue change in the pretreatment group than the control and placebo groups at 3 days (P<0.05). However, the placebo and control groups were not significantly different at 3 days. No significant difference was noted between the three groups at 6, 9 or 12 days (P>0.05). The Kruskal-Wallis test was used to analyze the total mucositis grade. Table 3 presents the frequency of different total mucositis grades. Table 4 shows the severity of mucositis in the three groups at different time points. The results revealed a significant difference among the three groups regarding the severity of mucositis at 3 (P=0.010) and 6 (P=0.012) days. No significant difference was noted between the groups at 9 (P=0.300) or 12 (P=0.146) days. Pairwise comparisons of the groups revealed significant differences between the pre-treatment group and the placebo and control groups at 3 and 6 days (P<0.05); however, no significant difference was noted at 9 and 12 days (P>0.05). A comparison of the control and placebo groups revealed no significant difference at any time point (P>0.05), (Table 3, 4).

Table 3. Frequency of different total mucositis grades.

		Day 3		Day 6		Day 9		Day 12	
Group	Degree	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Control	Mild	0	0%	0	0%	0	0%	0	0%
	Moderate	1	12.5%	1	12.5%	1	12.5%	0	0%
	Severe	7	87.5%	7	87.5%	7	87.5%	8	100%
Placebo	Mild	0	0%	0	0%	0	0%	0	0%
	Moderate	1	12.5%	2	25%	1	12.5%	0	0%
	Severe	7	87.5%	6	75%	7	87.5%	8	100%
Pre-treatment	Mild	5	62.5%	2	25%	0	0%	0	0%
	Moderate	2	25%	3	37.5%	0	0%	1	12.5%
	Severe	1	12.5%	3	37.5%	8	100%	7	87.5%

Table 4. Mean severity of mucositis in the three groups at different time points

Group	Day 3	Day 6	Day 9	Day 12
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Control	10.3 (2.50)	11.25 (1.66)	10.87 (1.246)	12.25 (1.28)
Placebo	10.37 (2.50)	10.62 (2.199)	10.75 (1.16)	11.87 (1.35)
Pretreatment	4.62 (3.11)	7.87 (2.47)	10.62 (.517)	11.00 (1.51)

Trend of changes:

In the control and placebo groups, the degree of epithelial changes from beginning to day 3 was significantly higher than in the N. Sativa oil pre-treatment group. However, this difference decreased on day 9 and became insignificant on 9 and 12 days (Table 1). The mean severity of inflammation on day 3 in N. Sativa oil pre-treatment group was significantly lower than that in the control and placebo groups. In all three groups, inflammation showed a descending trend from day 3 to day 9, and in all three groups, degree of inflammation was minimal on day 9. The degree of inflammation increased again from day 9 to 12 (Table 2). The severity of connective tissue changes on day 3 was significantly lower in the pre-treatment group compared with the other two groups. The severity of inflammation in the connective tissue had an ascending trend in the control and placebo groups; however, in the pretreatment group, the changes had a descending trend after day 9. On day 12, the severity of connective tissue changes in N. Sativa oil pre-treatment group was lower than that in the control and placebo groups; however, this difference was not significant (Table 3).

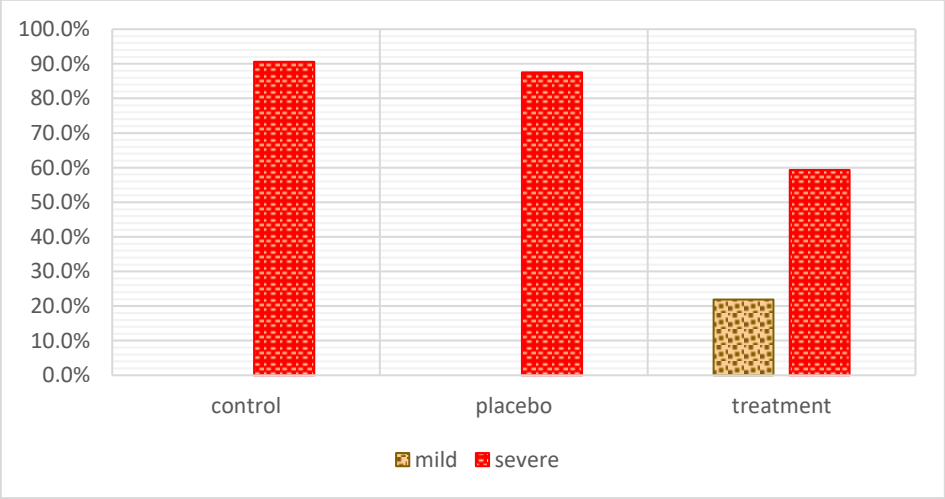

Chart 1: Frequency of different degrees of mucositis (Total mucositis grade) in percentage in different groups.

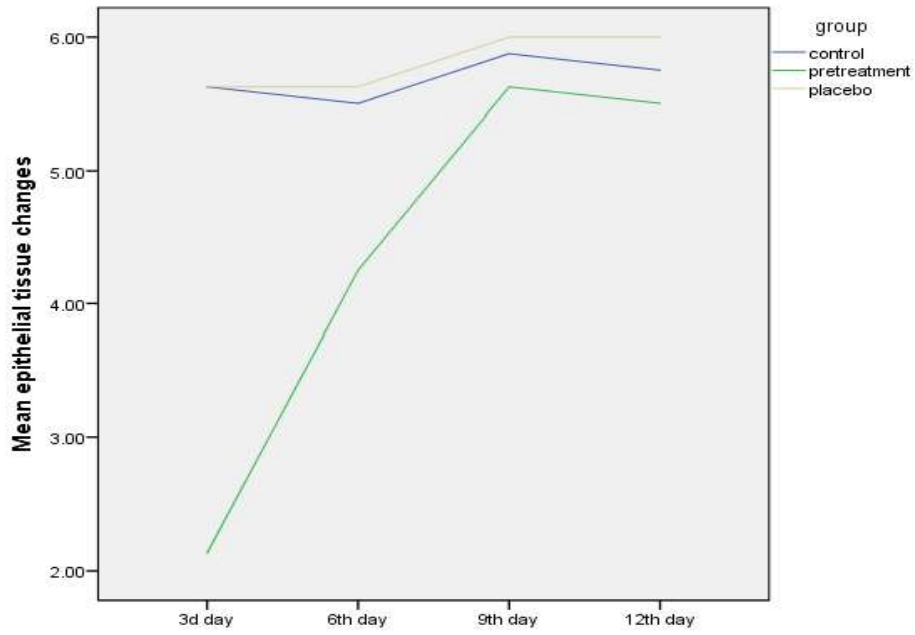

Chart 2: Comparison of the mean epithelial tissue changes between different groups.

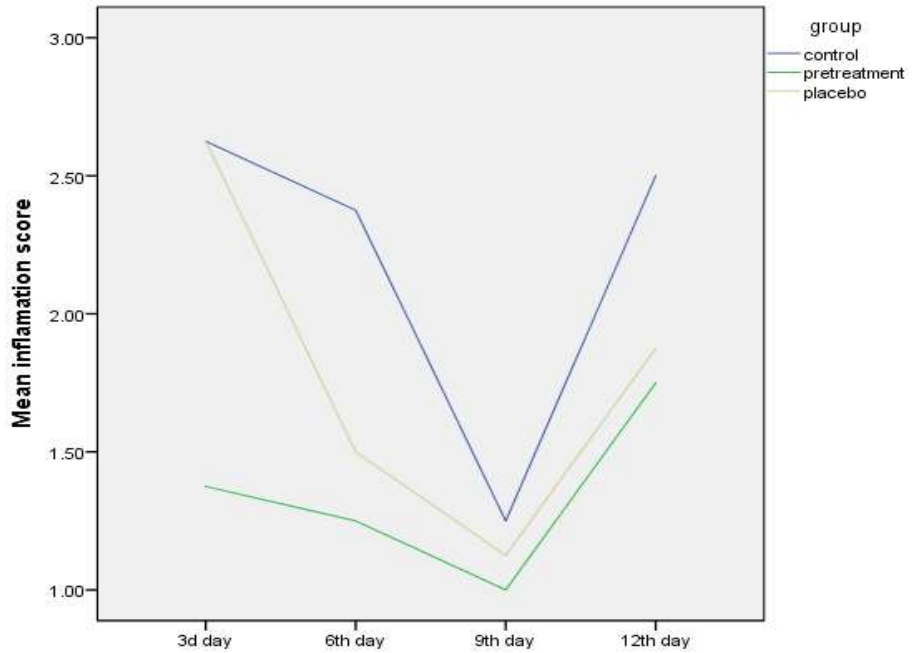

Chart 3: Comparison of the mean inflammation score between different groups.

Histopathological analysis:

The tissue specimens were histopathologically evaluated at 3, 6, 9 and 12 days after chemotherapy. Figures 1 and 2 shows the histopathological changes of the tissue specimens at 3 to 12 days after chemotherapy. As shown, in N. Sativa oil pre-treatment group, an increase in epithelial thickness was noted. Epithelial separation was noted in some parts. The rate of inflammation was relatively low, despite ulcers and granulation tissue. In the control and placebo

groups, inflammation was noted along with complete epithelial loss and formation of subepithelial vesicles. On day 6, the specimens in N. Sativa oil pre-treatment group showed some ulcers while the epithelium was almost normal in other parts. However, in the control and placebo specimens, microbial colonies, higher levels of inflammation and subepithelial vesicles were noted (Figure 1). On day 9, the severity of inflammation decreased in all groups. On days 9 and 12, the histopathological differences between the groups were insignificant (Figure 2). The charts (No 1-3) show frequency of different degrees of mucositis (Total mucositis grade) in percentage, epithelial changes and inflammation scores in different groups.

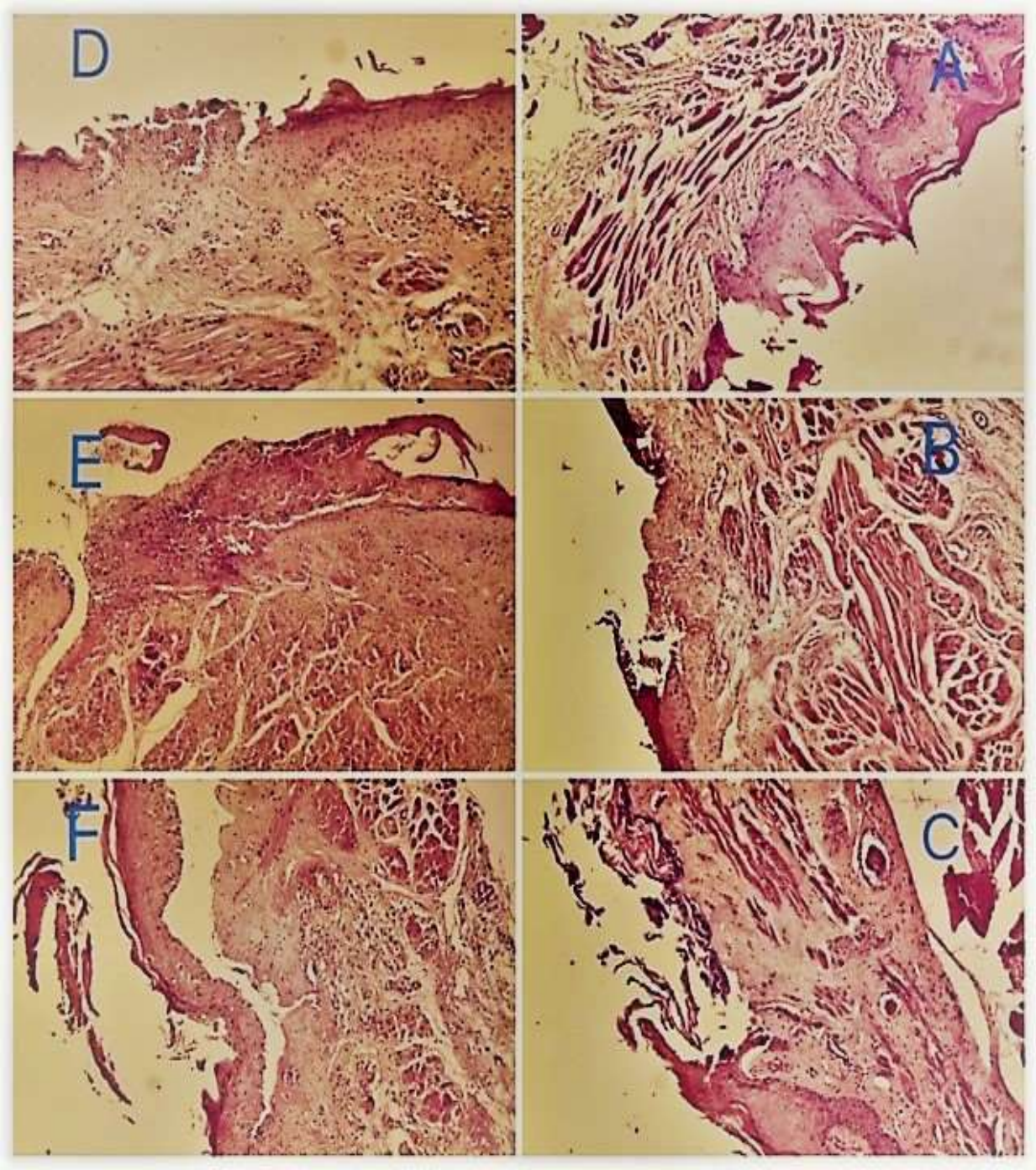

Figure 1: Buccal mucosa specimens in the three groups on day 3. (A) The pretreatment group shows increased epithelium thickness and epithelial separation in some parts. (B) Control group shows epithelial loss along with subepithelial vesicles. (C) Placebo group shows epithelial loss along with the formation of subepithelial vesicles. On day 6, (D) Pre-treatment group shows normal mucosa (except for some small ulcers), (E) control group shows epithelial loss along with accumulation of bacterial colonies on the epithelial surface, (F) placebo group shows epithelial loss along with inflammation and formation of subepithelial bulla.

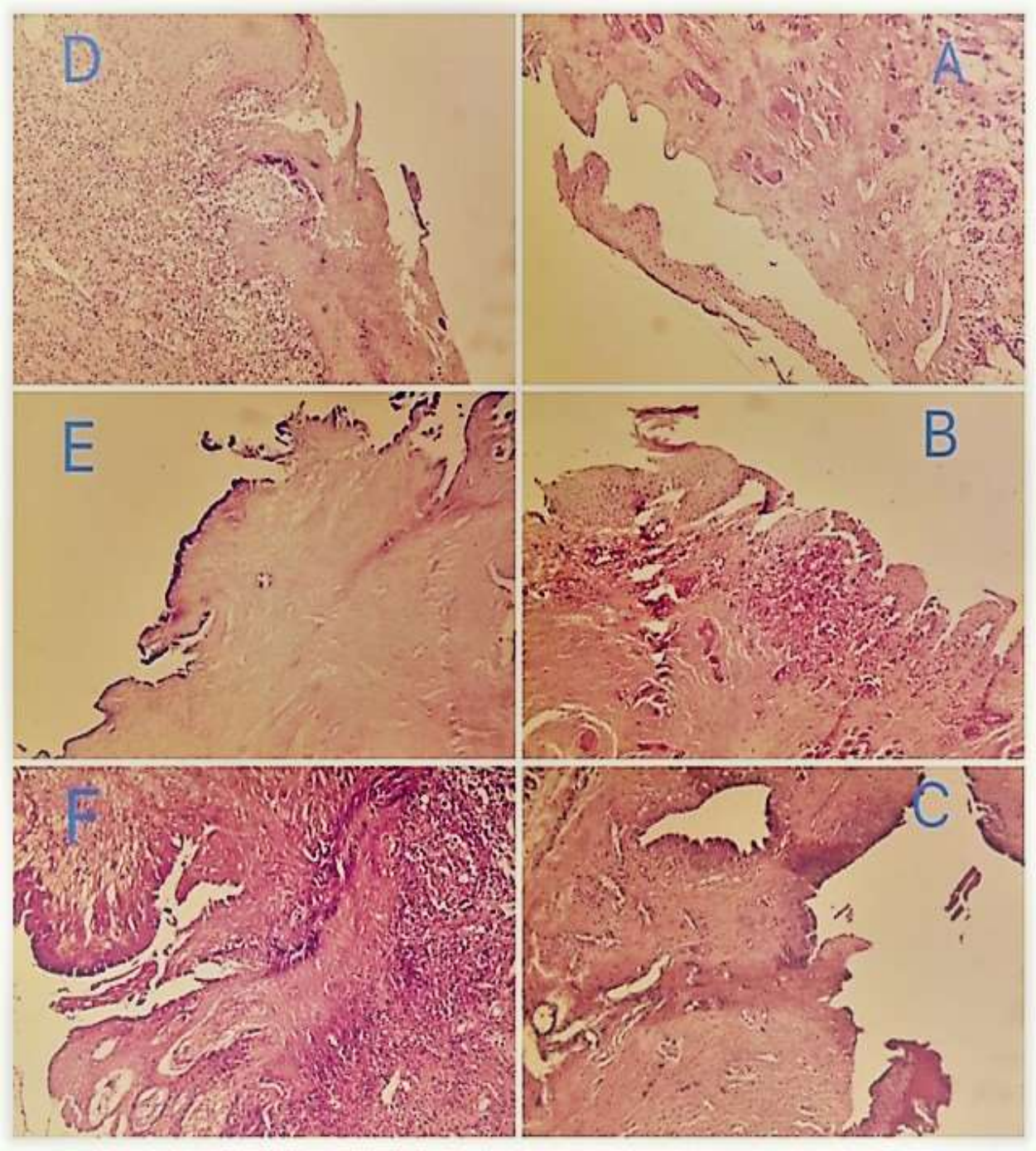

Figure 2: Buccal mucosa specimens in the three groups on day 9. (A) Pre-treatment group shows evidence of ulcer, subepithelial vesicle and necrosis with low level of inflammation. (B) The control group shows necrosis, subepithelial

vesicle, angiogenesis, and low level of inflammation. (C) Placebo group shows epithelial loss, subepithelial vesicle and necrosis, along with low degree of inflammation. On day 12, (D) pre-treatment group shows thin epithelium and chronic inflammation, (E) control group shows epithelial loss, no inflammation and presence of fibrosis and (F) placebo group shows scraped-off epithelium along with subepithelial vesicles and low degree of inflammation.

Discussion

This study assessed the efficacy of N. Sativa oil for the prevention of 5-FU-induced mucositis in rats. The results showed that the severity of mucositis in the pre-treatment group was significantly lower than that in the placebo and control groups at 3 (P=0.010) and 6 (P=0.012) days; however, this difference was not significant at other time points. Mucositis is the most important non-hematological complication of anti-cancer treatments [20], and no definite preventive or therapeutic measure has been suggested to prevent or manage this complication [1]. Destruction of endothelial cells in mucositis causes the early release of pro-inflammatory cytokines and generation of reactive oxygen species on the mucosal surface and subsequent activation of transcription factors such as NF-κB, over-expression of some specific genes, induction of apoptosis, and eventual epithelial ulceration [20]. Evidence shows that pre-treatment with thymoquinone significantly decreases pro-inflammatory mediators such as IL-1B, interferon-gamma, TNF-alpha, IL-6 and prostaglandin E2 and inhibits the cyclooxygenase and lipoxygenase pathways in arachidonic acid metabolism as well as other inflammatory mediators such as transcription factors and NF-κB. Evidence shows that the anti-inflammatory and anti-oxidative effects of N. Sativa oil are higher than those of pure thymoquinone [10].

In general, N. Sativa oil is expected to decrease the production of pro-inflammatory mediators, inhibit the production of oxygen free radicals and inhibit NF-κB, and affect the first and second phases of mucositis. Moreover, it is expected to affect the fourth phase of mucositis due to its antimicrobial effects and consequently affect the fifth phase and increase the proliferation of fibroblasts. On the other hand, evidence shows that prophylactic use of thymoquinone, which is the active ingredient of N. Sativa oil, can decrease the hepatotoxicity of chemotherapy due to its anti-nitrosative anti-oxidative, anti-inflammatory and anti-apoptotic effects [21]. N. Sativa oil can inhibit the generation of free radicals that initiate mucositis due to its antioxidant activity [6, 9, 11, 22].

Although no previous study has evaluated the efficacy of N. Sativa oil for the prevention of chemotherapy-induced mucositis, its preventive effects on gastrointestinal ulcers (due to alcohol consumption), reduction of oxidative stress in patients with rheumatoid arthritis and Alzheimer's disease and hepatotoxicity of chemotherapy have been reported [21, 23-25]. The current results showed that pre-treatment with N. Sativa oil ould decrease the severity of mucositis by decreasing the degree of epithelial changes, the severity of inflammation, and the degree of connective tissue changes. This finding was in agreement with the results of Lotfy and Zayed [26] who showed the optimal efficacy of N. Sativa extract in decreasing the severity of chemotherapy-induced oral mucositis in albino rats. However, our study had some advantages compared with the study by Lotfy and Zayed [26]. Considering the significance of cytokines in the process of mucositis and to apply equal stress on rats in both groups, we also designed a placebo group with the same route of administration of placebo as the main medication. Also, we assessed the buccal mucosa at 3, 6, 9 and 12 days after chemotherapy, while Lotfy and Zayed [26] only assessed the mucosa after 14 days. Also, they only assessed the therapeutic effects of N. Sativa while we evaluated its preventive effect by its administration 15 days before chemotherapy. Last but not least, we assessed the epithelial changes, degree of inflammation and connective tissue changes altogether to assess the total severity of mucositis. To the best of the author's knowledge, no previous study has performed such a comprehensive assessment of mucositis. Our results revealed that pre-treatment with N. Sativa oil, irrespective of time, had no significant effect on the degree of chemotherapy-induced connective tissue changes. This finding was in line with that of Kavandi et al., [27] who showed that pre-treatment with N. Sativa oil had no significant effect on Behcet's disease (a connective tissue disease). However, another study showed that N. Sativa accelerated the granulation of tissue formation in diabetic rats [28]. This result was in line with our findings, since our results indicated an acceleration of granulation tissue formation in the pre-treatment group; also, connective

tissue changes in the pre-treatment group had significant differences with the other two groups on day 3. However, overall, the effect of N. Sativa on connective tissue was not significant during the 12-day study period. This difference may be due to the effects of chemotherapy on inflammatory changes and blood cells. The administered dosage of N. Sativa may also be responsible for the absence of a significant effect on the connective tissue. A higher dosage of N. Sativa may significantly affect the connective tissue. Duration of medication intake is another important factor that might have affected the results. Since epithelial healing occurs faster than connective tissue healing [29], a longer period may be required for the optimal effect of medication on the connective tissue. Our results indicated that the degree of connective tissue changes in the pre-treatment group decreased abruptly after day 9 while it had an ascending trend in the placebo and control groups. On day 12, the degree of connective tissue changes in the pre-treatment group was lower than in the other two groups, but not significantly. Studies with longer follow-ups may reveal significant changes in this parameter. Therefore, further studies with longer follow-ups are required to better elucidate this topicelucidate this topic. According to the current results, the effect of N. Sativa oil on the degree of epithelial change was significant in the first six days, and then the difference between the groups gradually decreased. Also, the results showed that the degree of inflammation in all groups significantly decreased on day 9, which can be due to the effect of chemotherapy on immunity and blood cells. Evidence shows that N. Sativa can regulate the function of the immune system by correcting the T4/T8 ratio and modifying the activity of the natural killer cells. Thus, by changing the dosage of N. Sativa, we may be able to further benefit from its immunomodulatory properties; however, further studies are warranted in this respect [30]. Insignificant change in the second week can be due to the poor immune system, bacterial accumulation and secondary infection, which need to be investigated in future studies. Although further cellular and molecular investigations are required on the effects of N. Sativa oil on chemotherapy-induced mucositis, it may be proposed as a potentially effective herbal medicine for the prevention of chemotherapy-induced mucositis. Considering the delayed effect of N. Sativa oil on the connective tissue changes, further long-term studies using a higher dosage of N. Sativa oil are recommended to assess its effect on the connective tissue better. Also, considering the significant reduction of inflammation on day 9 in all groups, further studies should focus on the effects of N. Sativa on inflammatory cells such as neutrophils, macrophages and mast cells.

Conclusion

Within the limitations of this study, the results indicated that intraperitoneal administration of N. Sativa oil could effectively prevent 5-FU-induced oral mucositis in rats in the short term.

Ethics Approval

The study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences, School of Dentistry (IR.SBMU.CRE.1397.074) and was conducted in accordance with the guidelines for the care and use of laboratory animals.

Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Disclosure

The authors report no conflicts of interest in this work.

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