

Protective and Treatment Effect of *Nigella sativa* Oil on Dexamethasone Immunosuppressed Rat Lingual Mucosa, Blood Cell Count and Glutathione Level in Blood

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Abstract

Background: Oral health is important for general health. *Nigella sativa* (NS) has important role in oral health and disease treatment.

Objective: To investigate the protective and treatment effect of *Nigella sativa* oil on dexamethasone immunosuppressed rat lingual mucosa.

Material and Methods: Thirty males, Wister albino rats were used in this study. Rats were divided into five groups, each comprised 6 rats. Group I was used as negative control. Group II, the rats were injected by dexamethasone 5 mg/kg I.P. for 2 weeks daily. Group III, the rats were injected by dexamethasone 5 mg/kg I.P. and *Nigella sativa* oil 5 ml/kg intraoral for 2 weeks daily. Group IV, the rats were given only *Nigella sativa* oil 5 ml/kg intraoral for 2 weeks. Group V, the rats were injected by dexamethasone 5 mg/kg I.P. for 4 weeks but treated by *Nigella sativa* oil 5 ml/kg intraoral after the first 2 weeks. Complete blood cell count and blood glutathione level were measured. The rats were euthanized The tongues were prepared for immunohistochemical localization of T-lymphocytes using CD3 antibody and α -smooth muscle actin. One-way ANOVA followed by LSD Post-hoc test were used

Results: Immunohistochemical results of CD3 stain revealed strong reaction in NS group (IV), moderate in group I, III, V and weak in dexamethasone group (II). Strong reaction of α -smooth muscle actin in the wall of blood vessels with neovascularization in the lamina propria of NS group (IV). Moderate reaction in other groups.in the lamina propria.

One-way ANOVA statistical test revealed total significant differences between the different groups. The highest mean of total leucocyte count was in the control group (9.9500 ± 2.35181) and the lowest mean was in dexamethasone group (5.2167 ± 1.3765). The highest value of lymphocyte count was in NS group (0.8267 ± 0.05538) and the lowest count was in dexamethasone group (0.5817 ± 0.08773). The highest count of RBCs was in NS group (8.7583 ± 0.41950) and the lowest count was in dexamethasone group (5.8683 ± 1.27080). The highest value of haemoglobin was in NS group (15.1333 ± 0.75807) and the lowest value was in dexamethasone group (11.6667 ± 0.23381). The highest count of neutrophils was in dexamethasone group (0.3333 ± 0.08214) and the lowest count was in *Nigella sativa* group (0.1200 ± 0.04336). The highest value of glutathione level in blood was in NS group (1.9517 ± 0.12449) and the lowest value was in dexamethasone group (0.9033 ± 0.12707). There was significant improvement in lymphocyte, RBCs, haemoglobin in group III and V and increase in glutathione level. NS significantly increases lymphocyte count, glutathione level in blood and T lymphocytes in lingual mucosa.

Conclusions: NS has protective, treatment and immunomodulatory effect.

Keywords: *Nigella sativa* (NS); Dexamethasone Dexta; CD3; Glutathione

Introduction

Prophet Muhammad (peace be upon him) said: "This black seed is a cure for every disease except death" [1]. Currently there is a world-wide growing interest on the use of medicinal herbs in the treatment of various diseases due to their promising results and fewer side

effects. According to the World Health Organization (WHO), 60 - 80% of the world's population particularly in developing countries, depends on herbal treatment for their primary health care and treatment. Moreover, the WHO has encouraged the use of medicinal plants as a resource to generate effective health care programs. One of the top ranked evidence-based herbal medicines, which has been described as the miracle herb of the century is *Nigella sativa* (NS) [2-4].

Nigella sativa, is a member of Ranunculaceae family growing on the Mediterranean coasts, is an annual herb forms a fruit capsule containing seeds [5].

Nigella sativa oil contains thymoquinone (TQ) which is the main active component and other constituents. The oil content of NS seeds ranges from 0.1% to 1.5% [6,7].

The oil and seed components particularly thymoquinone TQ, show valuable immunomodulatory properties. Also the active ingredients illustrated anti-tumor and antimicrobial properties against different cancers and microbes. TQ exhibits gastroprotective mechanism. TQ is potent against free radical damage. TQ has been shown to inhibit carcinogenesis by decreasing lipid peroxidation protecting the cells and tissues and increasing the cellular antioxidant capacity [8-10].

Nigella sativa is a powerhouse of antioxidants. Different ingredients of NS have been found to act synergistically thereby increasing their antioxidant capacity. Antioxidant status in plasma along with the cellular structure and function of membrane is restored by effectively reducing the concentrations of plasma lipid peroxidation markers producing supportive and protective effect against cancer [9,11,12].

Further investigations are required to study the mechanism of actions of *Nigella sativa* seeds and its constituents by which they exert their therapeutic effects regarding the oral tissues which are important for the general health. Also, attempt to unveil some of the potential mechanisms exhibited by *Nigella sativa* in preventing problems related to immunosuppression is a main concern. Therefore, the aim of this study was to investigate the protective and treatment effect of *Nigella sativa* oil on dexamethasone immunosuppressed rat lingual mucosa, blood cell count and blood glutathione level.

Material and Methods

The experimental processes were performed under the protocols of the Ethics Committee of the Faculty of Dentistry, Mansoura University, Mansoura, Egypt

Study design: Thirty males, pathogen-free, Wister albino rats, weighing 190 - 220g and aged 3 months, were used in this study. They were housed in fit cages at suitable animal house in Mansoura, Egypt. They received water and a standard diet and were kept in a 12-h light/12-h dark cycle. Rats were randomly divided into five groups, each comprised 6 rats.

- I) Group I was used as negative controls; these rats were fed and kept under the same housing conditions as test rats.
- II) Group II, the rats were injected by dexamethasone (AMRIYA for pharmaceutical industries Alexandria - Egypt) 5 mg/kg I.P. for 2 weeks daily.
- III) Group III, the rats were injected by dexamethasone 5 mg/kg I.P. and *Nigella sativa* oil 5 ml/kg intraoral for 2 weeks daily.
- IV) Group IV, the rats were given only *Nigella sativa* oil 5 ml/kg intraoral for 2 weeks daily.
- V) Group V, the rats were injected by dexamethasone 5 mg/kg I.P. for 4 weeks but treated by *Nigella sativa* oil 5 ml/kg intraoral daily after the first 2 weeks.

The samples of blood were taken for complete blood cell count and glutathione level in blood [13] before euthanization. The rats were euthanized by cervical dislocation after 2 weeks except the 5th group after 4 weeks. The tongues were dissected and were cut posterior to the sulcus terminalis. Each tongue was divided into 2 halves to obtain longitudinal sections of the tongues. The specimens were processed into paraffin blocks at the Pathology Department, Faculty of Medicine, Mansoura University. They were prepared for histological examination using hematoxylin and eosin (H&E) stain (Sigma Aldrich) for routine examination.

Immunohistochemical stains for immunohistochemical localization of T-lymphocytes using CD3 antibody in tongue specimens of the different groups (CELL MARQUE, MillipoRe SIGMA) and Immunolabeling of α -SMA was performed using the Dako kit. According to the manufacturer's instructions and recommendations. Briefly, after deparaffinization of the sections in xylene, they were rehydrated in ethanol and water. Then were immersed in 0.1% H₂O₂ for 30 minutes to avoid the activity of the endogenous peroxidase enzyme. Then they were washed with phosphate-buffered solution (PBS) and were immersed in ethylene diaminetetraacetic acid (EDTA) buffer or sodium

citrate for 10 minutes at 95°C. Then they were cooled to room temperature and were incubated for 20 minutes in 10% normal goat serum to avoid the background activity. After the addition of primary antibodies for (Cd3 or α -SMA), they were incubated with mouse or rabbit secondary antibody for 30 minutes at room temperature, and washed in PBS. Finally they were stained with diaminobenzidine tetrahydrochloride solution (DAB), and counter stained by haematoxylin stain [14,15]. 6 areas were selected from different sections and different specimens were examined by 2 observers. The qualitative of the intensity of the staining were weak (+), moderate (++) and strong (+++) [16].

Statistical analysis

Statistical analysis was performed blindly using SPSS version 17.0 (SPSS, Chicago, IL, USA). One-way ANOVA was used to compare more than two groups and descriptive statistics for the different groups and the different parameters. Followed by LSD Post-hoc statistical results for the different groups and different parameters. A value of $P < 0.05$ was considered to indicate statistical significance.

Immunohistochemical results

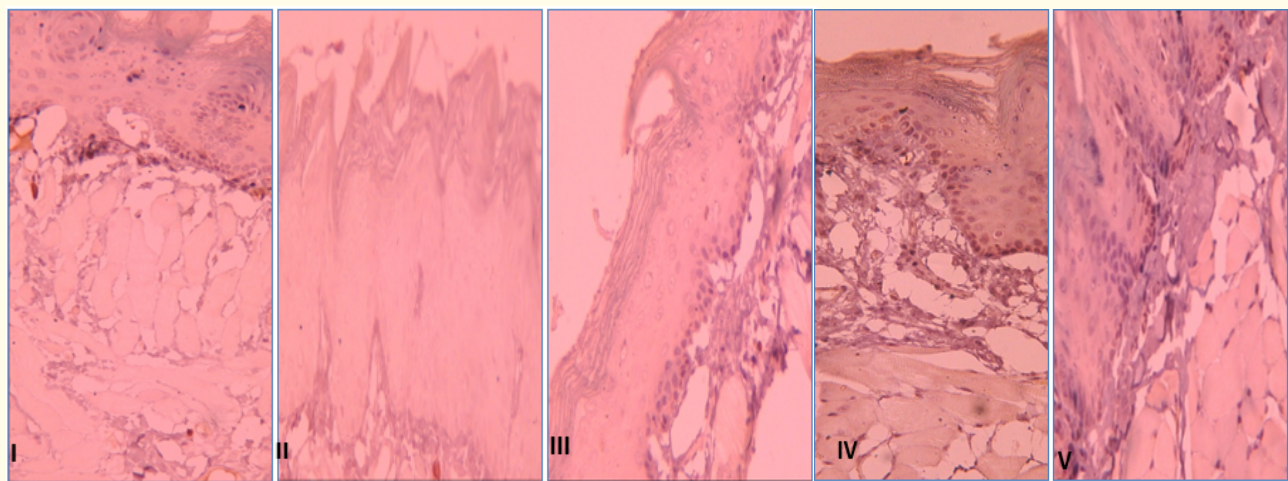


Figure 1: Photomicrograph of group (I) showing moderate CD3 immunoreactive cells in the lamina propria and epithelium (brown colour). Group(II): Weak immunoreactive cells in the lamina propria and negative in the epithelium. Group (III) moderate immunoreactive cells in the lamina propria and epithelium. Group (IV): Strong immunoreactive cells in epithelium and lamina propria. Group (V): Moderate immunoreactive cells.

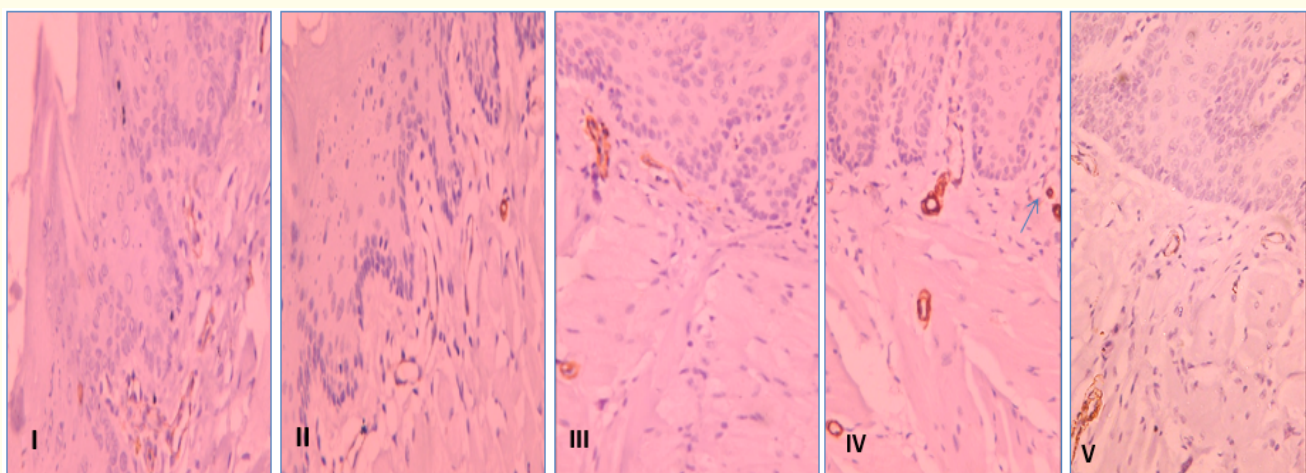


Figure 2: Photomicrograph of group I, II, III, V: Moderate reaction in the blood vessel walls. Group IV: Strong reaction in the blood vessel walls and neovascularization in the lamina propria (arrow).

The immunohistochemical positive results were detected as brown deposits using Cd3 (cytoplasmic and in the cell membrane) α -SMA intermediate filaments. The immunohistochemical sections of the tongue specimens revealed the followings: Immunohistochemical localization of T-lymphocytes using CD3 antibody showed moderate reaction in control group in the epithelium and lamina propria. Weak reaction in dexamethasone group only in the lamina propria. Moderate reaction in groups III and V in which dexamethasone is protected and treated by NS respectively. Strong reaction in NS group in epithelium and lamina propria. Strong reaction of α -smooth muscle actin in the wall of blood vessels with neovascularization in the lamina propria of NS group (IV). Moderate reaction in other groups.in the lamina propria.

Statistical results

One-way ANOVA statistical test revealed total significant differences between the different groups. by descriptive statistics for the different groups and the different parameters (Table 1 and figure 3). The highest mean of total leucocyte count was in the control group (9.9500 ± 2.35181) and the lowest mean was in dexamethasone group (5.2167 ± 1.3765). The highest value of lymphocyte count was in *Nigella sativa* group (0.8267 ± 0.05538) and the lowest count was in dexamethasone group (0.5817 ± 0.08773). The highest count of RBCs was in *Nigella sativa* group (8.7583 ± 0.41950) and the lowest count was in dexamethasone group (5.8683 ± 1.27080).The highest value of haemoglobin was in *Nigella sativa* group (15.1333 ± 0.75807) and the lowest value was in dexamethasone group (11.6667 ± 0.23381). The highest count of neutrophils was in dexamethasone group (0.3333 ± 0.08214) and the lowest count was in *Nigella sativa* group (0.1200 ± 0.04336). The highest value of glutathione level in blood was in *Nigella sativa* group (1.9517 ± 0.12449) and the lowest value was in dexamethasone group (0.9033 ± 0.12707). There was significant differences between groups when P value < 0.05.

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		Mean \pm SD					
Groups		Total leucocyte count	Lymphocytes	RBCs	Haemoglobin	Neutrophils	Glutathione
Control		9.9500 \pm 2.35181	0.7850 \pm 0.06473	7.5200 \pm 0.40812	13.1167 \pm 0.77309	0.1500 \pm 0.05514	1.7250 \pm 0.14516
Dexamethasone		5.2167 \pm 1.3765	0.5817 \pm 0.08773	5.8683 \pm 1.27080	11.6667 \pm 0.23381	0.3333 \pm 0.08214	0.9033 \pm 0.12707
Dexa+Nigella		7.3333 \pm 2.13791	0.7150 \pm 0.08313	6.4767 \pm 0.57486	12.4667 \pm 0.28048	0.2133 \pm 0.07789	1.4383 \pm 0.13452
Nigella		8.7333 \pm 1.95107	0.8267 \pm 0.05538	8.7583 \pm 0.41950	15.1333 \pm 0.75807	0.1200 \pm 0.04336	1.9517 \pm 0.12449
Dexa+ (Dexa+Nigella)		7.7000 \pm 1.03150	0.7300 \pm 0.05865	6.9033 \pm 0.79887	12.8667 \pm 0.51251	0.2133 \pm 0.05820	1.3433 \pm 0.04502
ANOVA	F ratio	5.513	10.247	12.484	31.716	9.536	65.402
	P value	0.003	0.000	0.000	0.000	0.000	0.000

Table 1: One-way ANOVA and descriptive statistics for the different groups and the different parameters.

*The mean difference is significant at the 0.05 level.

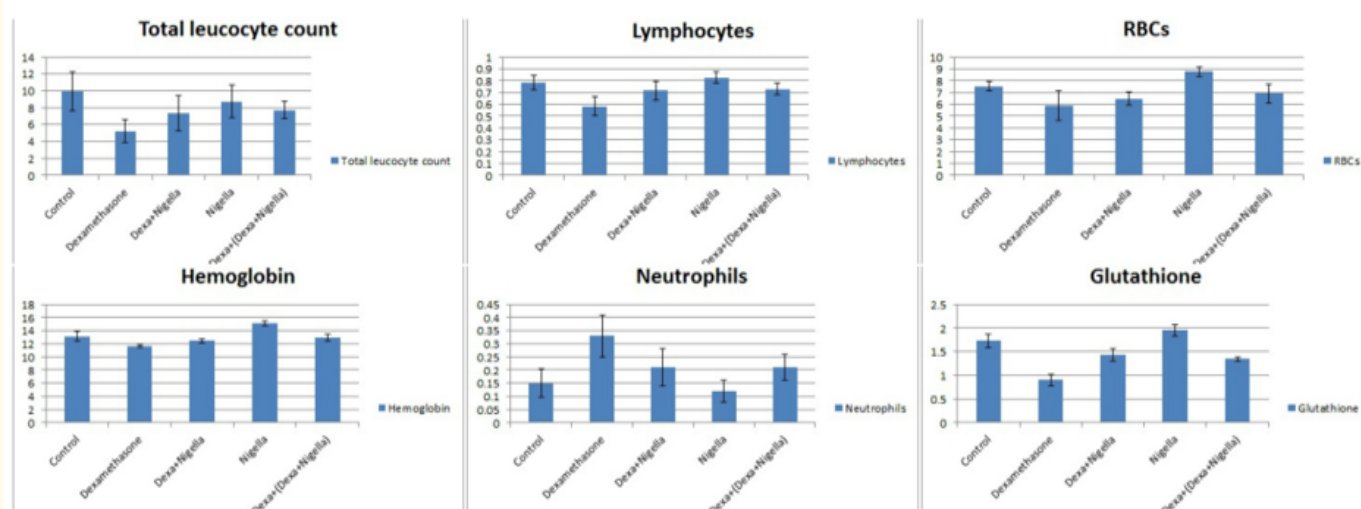


Figure 3: One-way ANOVA and descriptive statistics for the different groups and the different parameters.

LSD Post-hoc statistical results for the different groups and different parameters (Table 2) revealed in dexamethasone group There was significant decrease in total leucocyte count, lymphocyte count, RBCs, haemoglobin and glutathione level in blood. in comparison to the other 4 groups P value < 0.05 except in total leucocyte count in comparison with the 3rd group (nigella and dexa.) P value 0.057. There was significant increase in neutrophil count in dexamethasone group in comparison to all groups P value < 0.05. There was significant difference in total leucocyte count in dexa. and nigella group in comparison to the control group. P value = 0.021. There was non-significant difference in lymphocyte, RBCs, neutrophil count and haemoglobin between control and (dexa. and nigella) group i.e. there was improvement in dexamethasone results by taking *Nigella sativa* oil. There was significant difference in glutathione level p = 0.000. There was non-significant difference between control group I and gp. V (dexa. + dexa. and nigella) in lymphocyte, RBCs, neutrophil count and haemoglobin. (i.e. nigella has treatment effect to the decrease of lymphocytes during treatment with dexamethasone). There was signifi-

Groups	P value					
	Total leucocyte count	Lymphocytes	RBCs	Haemoglobin	Neutrophils	Glutathione
Control* Dexa.	0.000	0.000	0.001	0.000	0.000	0.000
Control* Dexa.+Nigella	0.021	0.101	0.026	0.055	0.104	0.000
Control* Nigella	0.262	0.320	0.010	0.000	0.432	0.003
Control* Dexa.+(Dexa.+Nigella)	0.044	0.193	0.175	0.447	0.104	0.000
Dexa.* Dexa.+Nigella	0.057	0.003	0.181	0.020	0.004	0.000
Dexa* Nigella	0.003	0.000	0.000	0.000	0.000	0.000
Dexa.* Dexa.+(Dexa+Nigella)	0.027	0.001	0.027	0.001	0.004	0.000
Dexa.+Nigella* Nigella	0.199	0.012	0.000	0.000	0.020	0.000
Dexa.+Nigella* Dexa.+(Dexa+Nigella)	0.732	0.718	0.343	0.228	1.000	0.185
Nigella* Dexa+(Dexa+Nigella)	0.339	0.027	0.000	0.000	0.020	0.000

Table 2: LSD Post-hoc statistical results for the different groups and different parameters.

*: The mean difference is significant at the 0.05 level.

Discussion

The study of any herbal drug becomes more significant when it improves some disease condition and/or counteract the side effects of drugs. NS is a powerhouse of antioxidants [11], NS is a miracle herb, the researches are increasing to evaluate its beneficial effects and the mechanism its action to be cure from all diseases except death as Mohammad (peace be upon him) said [1].

Complete blood pictures were measured as homeostasis of hematological parameters is essential for assuring the general health status of any living organism [17].

CD3 is initially expressed in the cytoplasm of pro-thymocytes, the stem cells from which T-cells arise in the thymus. CD3 antigen begins to migrate to the cell membrane. The antigen is found bound to the membranes of all mature T-cells, and in virtually no other cell type, his high specificity, combined with the presence of CD3 at all stages of T-cell development, makes it a useful immunohistochemical marker for T-cells in tissue sections [18] T cell, or T lymphocyte plays a central role in cell-mediated immunity [19].

The CD3 reaction is strong in NS group and there was significant increase in the number of lymphocytes in blood in comparison to all groups. This was in agreement previous studies that NS oil show valuable immunomodulatory properties by increasing the natural killer cells and T cell-mediated immune responses [8]. NS in clinical management of RA give modulation of T lymphocytes. The treatment also resulted in reduced CD8 (+), and increased CD4 (+) CD25 (+) T cell percentage [20]. Thymoquinone TQ has a beneficial effect in conditioning T cells *in vitro* for T-cell therapy against cancer and infectious disease [21]. NS oil 5 ml/kg BW decreased the number of platelet and neutrophils but increased the number of lymphocytes, increased phagocytic activity and IL-12 [22]. Interleukin-12 (IL-12) is one of the main cytokines in immune response regulation. It is produced by activated lymphocytes and accessory cells. *In vivo* study proved that IL-12 increase the activity of CD4 and CD8 lead the formation of CD4 T cell [23,24]. Macrophages have TLR4 receptor which serves to identify the presence of natural antigen from herbal or pathogen. The main roles of IL-12 in regulating the immune response Increase the proliferation and activity of macrophage and NK cells and activate B lymphocytes to produce antibodies. The biological effects of IL-12 are regulating the proliferation and differentiation of lymphoid, regulate the function of macrophages and dendritic cells, regulate the tolerance, memory and lymphocyte homeostasis [25]. Black cumin (*Nigella sativa*) contain evaporated oil, fatty acids, rich in sterols especially β -sitosterol, thymoquinone, dithymoquinone and saponins which have protective effect [26].

The weak CD3 reaction in dexamethasone group and decreased lymphocytes in blood can be explained as the dexamethasone is immunosuppressant. Increased neutrophils in spite of dexamethasone is anti-inflammatory because it delays apoptosis of neutrophils reduces the DNA cleavage and prolonged their viability and release immature neutrophils from the bone marrow [27].

Strong reaction of α -smooth muscle actin in the wall of blood vessels and neovascularization in the lamina propria in NS group can be explained by its action on VEGF which was significantly high and comparable in the NS-fed and the Ex-trained groups, indicating that both NS administration and exercise training are potential factors for the induction of capillary growth [28]. Dexamethasone has anti-angiogenic activity [29]. The decreased reaction to α -SMA in dexamethasone group could be attributed to dexamethasone effect which decrease the mRNA and protein expression in both the amount and assembly of α -SMA in cells [30]. The components of NS and its powerful antioxidant action and increasing glutathione, increasing IL12 are important mechanisms to the therapeutic effect of NS. In addition to its antibacterial action [31].

Glutathione

During body metabolism the production of free radicals is an integral part, but imbalance results is oxidative stress. The excessive lipid peroxidation results in destruction of cellular membranes that could leads to tissue damage. Antioxidants protect the body from the free radicals and lipid peroxidation. Glutathione level in blood was measured as an attempt to know the mechanism of action of NS. There was significant increase in NS group in comparison to all groups. This result was in agreement with many previous studies. Significant elevation in the gastric glutathione (GSH) with NS treatment, resulted in an increase in the antioxidant GSH levels thereby accelerating the gastric healing process [32]. This can be attributed to NS essential oil tends to normalize the level of lipid peroxidase (LPX), glutathione (GSH) superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes which are important cell antioxidant defense enzymes [33].

NS treatment arrested the decline in GSH concentration as a result of irradiation. The irradiation resulted in lipid peroxidation and pre-treatment of mice with NS significantly reduced radiation-induced lipid peroxidation. The NS administration reduced the delay in wound healing time, which may be due to enhanced synthesis of collagen and increased anti-oxidants and alleviation in the lipid peroxidation [34].

NS and essential oils are effective in improving the antioxidant indices (glutathione) against potassium bromate-induced oxidative stress [35]. TQ has been shown to inhibit carcinogenesis by decreasing lipid peroxidation and increasing the cellular antioxidant capacity [9]. Significant increase in lymphocyte count and increased reaction (moderate) to CD3 in groups III and V. Also increased the level of glutathione indicated and proved the protective and treatment effect of NS oil in dexamethasone-immunosuppressed rat lingual mucosa. These results make NS a good alternative for oral immune diseases, as well as its antioxidant and anti-neoplastic activities [36].

Conclusion

NS has protective, treatment and immunomodulatory effects. NS increases T cells in lingual mucosa and lymphocytes in blood.

Recommendation

Further preclinical and clinical studies at the cellular and molecular levels are recommended to investigate the mechanisms by which NS exerts its miraculous therapeutic effects. NS could lead to more effective and safer drugs.

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