Journal of Animal and Veterinary Advances 6 (5): 601-605, 2007

Effects of *Nigella sativa* L. and *Urtica dioica* L. On the Numbers of Mast Cells in the Thymus, Spleen and Mesenteric Lymph Nodes of Rats with Carbon Tetrachloride Toxicity

Mecit Yoruk, Turan Karaca and Sema Uslu
Department of Histology and Embryology, Faculty of Veterinary Medicine,
University of Yuzuncu Yil, Van, Turkey

Abstract: This study investigated the effects of *Nigella Sativa* L. (NS) and *Urtica Dioica* L. (UD) on the number of Mast Cells (MCs) in Carbon tetrachloride (CCl_4)-treated rats. A total of 35 healthy male Sprague-Dawley rats were divided into 5 equal groups. All groups, expect control group, were injected subcutaneously with 0.8 mL CCl_4 per kg body weight twice a week for 45 days and then CCl_4 + UD-treated, CCl_4 + NS-treated and CCl_4 + UD + NS-treated rats received a daily intraperitoneal injection of 0.2 mL kg^{-1} NS or/and 2 mL kg^{-1} UD oils for 45 days starting on day 46. Thymus, spleen and mesenteric lymph nodes were sampled, stained with 1% aqueous toluidine blue and examined microscopically. The thymus, spleen and lymph nodes of CCl_4-exposed animals contained significantly increased numbers of MCs compared with control group, CCl_4 + UD-treated, CCl_4 + NS-treated and CCl_4 + UD + NS-treated rats ($p<0.05$). However, when exposed rats were also treated with UD, NS or UD + NS, a decrease in MCs was observed. These results demonstrate that UD and NS decrease the number of MCs induced by CCl_4 in the lymphoid tissues of rats.

Key words: Nigella sativa, *Urtica* dioica, thymus, spleen, mesenteric lymph node, mast cell, carbon tetrachloride

INTRODUCTION

Herbal products have a long history of use based on religious and cultural traditions in which plants are viewed as health remedies (Huxtable, 1992). People living in widely separated cultures with no obvious means of communication are known to use the same plants for similar health problems (Fransworth, 1984).

Nigella sativa (*N. sativa*), commonly known as black seed or black cumin, is an important medicinal herb. *Nigella sativa* seeds are commonly eaten alone or in combination with honey and are included in many food preparations. *Nigella sativa* L. contains >30 of a fixed oil and 0.40-0.45 w w^{-1} of a volatile oil. The volatile oil possesses insecticidal, bronchodilating, immunopotentiating (Haq *et al*., 1999; Kadi and Kandil, 1987) antibacterial (Hanafy and Hatem, 1991) hypotensive (Zaoui *et al*., 2000) choleretic, antitumoral (Salomi *et al*., 1992) antifungal (Khan *et al*., 2003) antihelminthic and antiasthmatic properties (Tahir *et al*., 1993). The effects of black seed have recently been evaluated in clinical and animal studies (Tennekoon *et al*., 1991).

Urtica Dioica L. (UD), stinging nettle, is a plant belonging to the plant family *Urtica*ceae. It is endemic in many parts of Turkey and the seeds have long been widely used in folk medicine, particularly in the therapy of advanced cancer patients (Davis, 1965). Some beneficial properties of this plant such as anti-inflammatory effects (Obertreis *et al*., 1996; Riehemann *et al*., 1999) and stimulation of proliferation of human lymphocytes (Wagner *et al*., 1989) have been reported.

Carbon tetrachloride (Ccl_4) is a selective hepatotoxic chemical agent that can cause liver cirrhosis, a disease in which 3 are a variety of systemic immunological abnormalities, such as polyclonal hypergammaglobulinaemia, auto-antibody production, decreased cellular immunity and decreased natural killer activity (Kita *et al*., 2001; Nakamura *et al*., 1983). Immunotoxicity can be detected in primary lymphoid organs (bone marrow and thymus) and/or in secondary lymphoid organs such as the spleen, lymph nodes and Peyer's patches (Bice and Muggenburg, 1988; Bice *et al*., 1980). Several authors (Bice and Muggenburg, 1988; Sopori *et al*., 1989) have demonstrated the autonomy of the immune compartments. Mast cells are a group of long-living cells of bone marrow origin that are commonly found in the skin and the gastrointestinal and respiratory systems. They are known to be present in normal and pathological livers (Grizzi *et al*., 2003). A number of studies have indicated that MCs are associated with hepatic fibrosis as they promote fibroblast growth, collagen synthesis and may inhibit extracellular matrix

Corresponding Author: Turan Karaca, Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Yüzüncü Yil, Kampüs, 65080, Van, Turkey

degradation by means of inhibitors of metalloproteases (Farrell *et al*., 1995; Neubauer *et al*., 2001; Armbrust *et al*., 1997). Mast cell density is a valid index of acute liver inflammation.

The aim of the present study was to investigate the effects of essential oils derived from NS and UD on the numerical distribution of Mast Cells (MCs) in thymus, spleen and mesenteric lymph nodes in CCl_4-treated rats.

MATERIALS AND METHODS

Plant materials and extraction procedure: The NS and UD seeds used in the experiment were purchased from a local herbal shop in Van, Turkey. Voucher specimens have been retained at the Department of Biochemistry, Yuzuncu Yil University, Van, Turkey for future reference. The seeds of NS were powdered in a mixer, placed in a distillation flask and the volatile oil with 0.2% yield was collected by steam distillation. The fixed oil of UD was extracted with the help of a rotary evaporator apparatus using diethyl ether as solvent.

Treatment of rats: A total of 35 healthy male Sprague-Dawley rats, weighing 150-200 g and averaging 5.5 months of age were randomly allotted into one of 5 experimental groups: Control, A (Ccl_4 + NaCl treated), B (Ccl_4 + UD treated), C (Ccl_4 + NS treated) and D (Ccl_4 + UD + NS treated), each containing 7 animals. All groups received Ccl_4 (Merck, M = 153.82 g moL^{-1}, 1 litre = 1.59 kg, Germany at a rate of 0.8 mL kg^{-1} body weight subcutaneously, twice a week for 45 days starting on day 1), expect control group. Thereafter, B, C and D groups received daily intraperitoneal injections of 0.2 mL kg^{-1} NS or/and 2 mL kg^{-1} UD oils for 45 days starting on day 46, while group A received only 2 mL kg^{-1} normal saline solution. All rats were housed in macrolon cages under standard laboratory conditions (light period 7 a.m. to 7 p.m., 21 ± 1°C, rat food and tapwater freely available). All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health. The experiment lasted 45 + 45 days.

Histological analysis: Rats were sacrificed at the end of the experiment. The spleen, thymus and mesenteric lymph nodes were dissected and fixed in Mota's fixative (Basic Lead Acetate (BLA) at room temperature for 48 h, set in paraffin and 6 μm thick cross-sections were processed routinely for light microscopy (Kanter *et al*., 2004) by staining with toluidine blue (1% aqueous solution). Mast cells were identified by the characteristic metachromatic staining of secretory granules by toluidine blue. Tissue sections were examined under light microscopy (×400) and the number of MCs counted in random high-power fields using a Nikon Optiphot II light microscope incorporating a square graticule in the eyepiece (eyepiece×10, objective ×40, a total side length of 0.225 mm). Mast cell density was assessed by counting the number of cells in 200 high power fields in lymphoid tissue preparations of each group. The MC density in each site was calculated and recorded as MC numbers mm^{-2}.

Statistical analysis: The data were expressed as the mean±Standard deviation (SE). The Bartlett test was used in order to determine whether the data were heterogeneous or homogeneous. The Bonferroni multiple comparison procedure was then used to identify differences between means. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

At the end of the experiment, no morphological differences were found in MCs in the lymphoid tissues of CCl_4-treated rats in the experimental groups. The MCs varied in shape from ovoid to elongated.

Thymus: In all group, MCs were extremely large in the subcapsular areas. However, MC density was higher in close proximity to the arterial vessels and medulla region of thymus than in the cortical region (Fig. 1 and 2). In thymus Ccl_4-Group Animals (Group A), the number of degranulated MCs was increased in the medullary sinuses and around blood vessels and there were MCs in high numbers (82.7±12.0; mean±SE per 1 mm^2) ($p<0.05$) compared with the other experimental groups and control group.

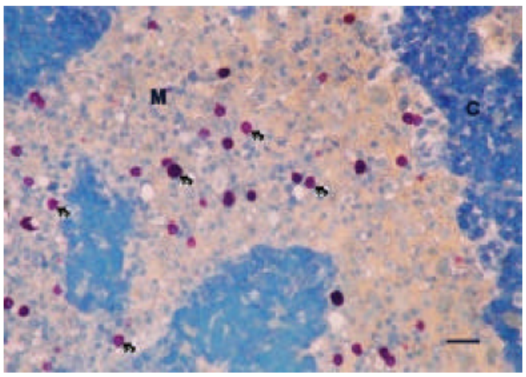

Fig. 1: Toluidine blue staining, thymus of rats CCl_4 group (Group A), mast cells (arrows), C: Cortex and M: Medulla, Bar = 60 μm

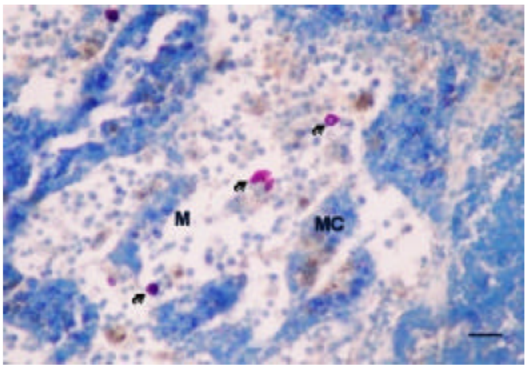

Fig. 2 Toluidine blue staining, thymus of rats CCl_4+UD group (Group B), mast cells (arrows), C: Cortex and M: Medulla, Bar = 60 μm

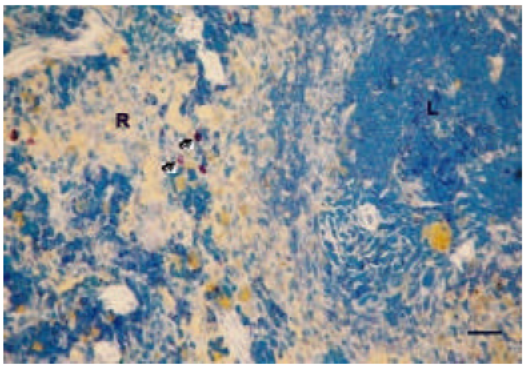

Fig. 3: Toluidine blue staining, spleen of rats CCl4+NS (Group C), mast cells (arrows), L: splenic corpuscle, R: red pulp, Bar = 60 μm

Spleen and mesenteric lymph nodes: In the spleen in particular, MCs were localised in sinuses of red pulp, where they were found predominantly around blood vessels, whereas MCs were not observed the white pulp (Fig. 3). In the control, groups A, B, C and D spleens, Mcs were distributed into experimental groups as follows: 21.6±6.1, 33.6±10.3, 16.2±3.5, 22.8±7.5 and 20.1±5.4 (mean±SE per 1 mm^2), respectively. Similarly, for mesenteric lymph nodes MCs were not present within lymph follicles in any of the groups. The MCs of lymph nodes were usually localised in the subcapsular areas, capsule and trabeculae and medulla region. Some of the MCs in the tissues of the CCl_4-treated experimental group A were degranulated (Fig. 4 and 5). The distribution of MCs in control and experimental groups was: 46±7.8, 157.4±16.7,62.5±12.6,80.0±15.4 and50.8±6.8 (mean±SEper 1 mm^2), respectively.

The numbers of MCs in the thymus, spleen and mesenteric lymph nodes of the four groups are shown in

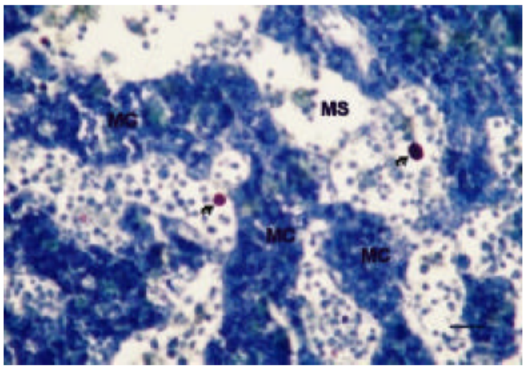

Fig. 4: Toluidine blue staining, mesenteric lymph node of rats CCl4+NS (Group C), mast cells (arrows), MC: Medullary cord, MS: Medullary sinus, Bar = 60 μm

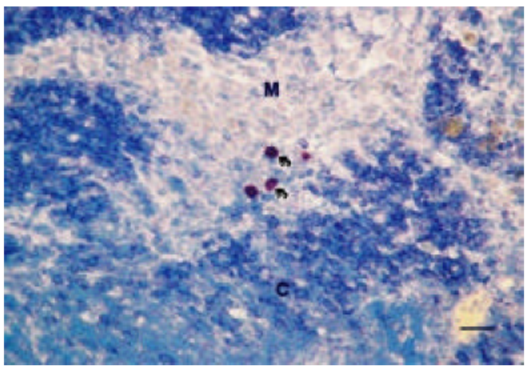

Fig. 5: Toluidine blue staining, mesenteric lymph node of rats CCl_4+UD+NS group (Group D), mast cells (arrows), M: Medullary sinus, MC: Medullary cord, Bar = 60 μm

Table 1: Numbers of mast cells* in the lymphoid organs (thymus, spleen, mesenteric lymph nodes) of rats exposed to CCl_4 (Groups A-D) plus extracts of essential oils from Nigella Sativa (NS) and/or *Urtica* Dioica (UD) (Groups B-D)

	Organ		
Group	Thymus	Spleen	M. Lymph Nodes
Control	48.4±8.5^c	21.6±6.1^b	46±7.8^c
A: CCl_4	82.7±12.0^a	33.6±10.3^a	157.4±16.7^a
B: CCl_4 + UD	71.8±9.4^b	16.2±3.5^c	62.5±12.6^c
C: CCl_4 + NS	66.3±8.6^b	22.8±7.5^b	80.0±154^b
D: CCl_4 + UD + NS	51.2±14.5^c	20.1±5.4^b	50.8±6.8^c

*All samples stained with toluidine blue (1% aqueous solution). Values are mean±Standard deviation (SE), each group n = 7, Means followed by different letters within columns are significantly different ($p<0.05$)

Table 1. The numbers of MC in thymus, spleen and mesenteric lymph nodes of the animals exposed to CCl_4 (Group A) were significantly ($p<0.05$) higher than for CCl_4 + UD (Group B), Ccl_4 + NS (Group C), CCl_4 +UD+NS

(Group D) and control group. Although, in thymus, 3 were no differences in the number of MCs at Group B and C compared with other groups. Similarly, there were no differences in the number of MCs at Group C and D compared with other groups at the spleen. MC numbers were significantly increased in the Group A at mesenteric lymph nodes ($p<0.05$). Our study is the first to show a linear change in the number of MCs in lymphoid tissues as a result of CCl_4 exposure. In a previous study (Karaca and Simsek, 2007) we demonstrated that the numbers of MCs in the ovary of lead-exposed rats were decreased by treatment with *Spirulina*.

Lymph node sinuses of unstimulated rats contain macrophages, lymphocytes, plasma cells and MCs (Karaca and Simsek, 2007). Mast cells appear mainly in the medullary sinusoids of mesenteric lymph nodes to varying extents and occasionally in the other sinuses Majeed, 1994). In our study, in contrast to Majeed (1994) we found that MCs were largely localised in the subcapsular area, capsule and trabeculae. We also found that NS or UD treatments (alone or combination) for 45 days decreased the elevated numbers of MCs in CCl_4-treated rats; MCs were significantly decreased in organ compartments of thymus, spleen and lymph nodes of group B (CCl_4 + UD-treated), group C (CCl_4 + NS-treated) and group D (CCl_4 + UD + NS-treated) compared with Group A (CCl_4 + NaCl-treated) ($p<0.01$). To our knowledge, this is the first study showing the effect of UD and NS oils on the numbers of MC in the lymphoid tissues of CCl_4-treated rats.

CONCLUSION

It is concluded that since oils derived from *Nigella sativa* and *Urtica dioica* decrease the numbers of mast cells in rats showing symptoms of carbon tetrachloride toxicity, these herbal plants should be further studied for use in the treatment of allergic diseases.

REFERENCES

Armbrust, T., D. Batusic, R. Burkhardt and G. Ramadori, 1997. Mast Cells distribution in human liver disease and experimental rat liver fibrosis. Indications for Mast Cell participation in development of liver fibrosis. J. Hepatol., 26: 1042-1054.

Bice, D.E. and B.A. Muggenburg, 1988. Localized immune memory in the lung. Am. Rev. Respir. Dis., 138: 567-561.

Bice, D.E., D.L. Harris and B.A. Muggemburg, 1980. Regional immunologic responses following local deposition of antigen in the lung. Exp. Lung Res., 1: 33-41.

Davis, P.H., 1965, 1982. Flora of Turkey and the East Aegean Islands. Edinburgh: Edinburgh University Press, 1: 98-103, 7: 633-635.

El-Kadi, A. and O. Kandil, 1987. The black seed (Nigella sativa) and immunity: its effect on human T cell subset. Fed. Proc., 46: 1222.

El-Tahir, K.E.H., M.M.S. Ashour and Al-Harbi, 1993. The respiratory effects of the volatile oil of the black seed (Nigella sativa) in guinea-pigs: Elucidation of the mechanism (s) of action. Gen. Pharmacol., 24: 1115-1122.

Farrell, D.J., J.E. Hines, A.F. Walls, P.J. Kelly, M.K. Bennett and Burt, 1995. Intrahepatic Mast Cells in chronic liver diseases. Hepatology, 22: 1175-1181.

Fransworth, N., 1984. How can the well be dry when it is full of water? Econ. Botany, 38: 4-13.

Grizzi, F., B. Franceschini, N. Gagliano, C. Moscheni, G. Annoni, C. Vergani, P.L. Hermonat, M. Chiriva-Internati and Dioguardi, 2003. Mast cell density, hepatic stellate cell activation and TGF-b1 transcript in aging Sprague-Dawley rats during early acute liver injury. Toxicol. Pathol., 31: 173-178.

Grizzi, F., B. Franceschini, M. Chiriva-Internati, Y. Liu, P.L. Hermonat and Dioguardi, 2003. Mast Cells and human hepatocellular carcinoma. World J. Gastroenterol., 9: 1469-1473.

Hanafy, M.S.M. and M.E. Hatem, 1991. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). J. Ethnopharmacol., 34: 275-278.

Haq, A., P.I. Lobo, M. Al-Tufail, N.R. Rama and S.T. Al-Sedairy, 1999. Immunomodulatory effect Nigella sativa proteins fractionated ion-exchange chromatography. Int. J. Immunopharmacol., 21: 283-295.

Huxtable, R.J., 1992. The myth of beneficent nature: The risk of herbal preparations. Ann. Int. Med., 117: 165-166.

Kanter, M., M. Yoruk, B. Ozbay, T. Karaca, S. Acar and Coskun, 2004. Distribution of mast cells in lung tissues of rats exposed to biomass smoke. Scand. J. Lab. Anim. Sci., 31: 67-72.

Karaca, T. and N. ªim°ek, 2007. Effects of Spirulina on the number of ovary mast cells in lead-induced toxicity in rats. Phytother. Res., 21: 44-46.

Khan, M.A., M.K. Ashfaq, H.S. Zuberi, M.S. Mahmood and Gilani, 2003. The in vivo antifungal activity of the aqueous extract from Nigella sativa seed. Phytother. Res., 17: 183-186.

Kita, H., I.R. Mackay, J. van De Water and M.E. Gershwin, 2001. The lymphoid liver: Considerations on pathways to autoimmune injury. Gastroenterology, 120: 1485-1501.

Majeed, S.K., 1994. Mast cell distribution in rats. Arzneimittel-Forschung, 44: 370-374.

Nakamura, T., T. Morizane, T. Watanabe, K. Tsuchimoto, Y. Inagaki, N. Kumagai and M. Tsuchiya, 1983. Decreased natural killer activity in patients with liver cirrhosis. Int. J. Cancer, 32: 573-575.

Neubauer, K., B. Saile and Ramadori, 2001. Liver fibrosis and altered matrix synthesis. Can. J. Gastroenterol., 15: 187-193.

Nopajaroonsri, C., S.C. Luk and G.T. Simon, 1971. Ultrastructure of the normal lymph node. Am. J. Pathol., 65: 1-24.

Obertreis, B., T. Ruttkowski, T. Teucher, B. Behnke and H. Schmitz, 1996. Ex-vivo in-vitro inhibition of lipopolysaccharide stimulated tumor necrosis factor-alpha and interleukin-1 beta secretion in human whole blood by extractum *Urticae* dioicae foliorum. Arzneimittel-Forschung, 46: 389-394.

Riehemann, K., B. Behnke and K. Schulze-Ostho, 1999. Plant extracts from stinging nettle (*Urtica* dioica), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB. FEBS Lett., 442: 89-94.

Salomi, N., S.C. Nair, K.K. Jayawarahanan and C.D. Varghese, 1992. Antitumor principles from Nigella sativa seeds. Johns Hopkins Al. Mag., 63: 33-36.

Sopori, M.L., S. Cherian, R. Chilukuri and G.M. Shopp, 1989. Cigarette smoke causes inhibition of the immune response to intratracheally administered antigens. Toxicol. Applied Pharmacol., 97: 489-499.

Tennekoon, K.H., S. Jeevathayaparan, A.P. Kurukula-Sooriya and E.H. Karunanayake, 1991. Possible hepatotoxicity of Nigella sativa seeds and Dregea volubilis leaves. J. Ethnopharmacol., 31: 283-289.

Wagner, H., F. Willer and B. Kreher, 1989. Biologically active compounds from the aqueous extract of *Urtica* dioica. Planta Med., 55: 452-445.

Zaoui, A., Y. Cherrah, M.A. Lacaille-Dubois, A. Settaf, H. Amarouch and Hassar, 2000. Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat. Therapie, 55: 379-382.