Journal of Medicinal Plants Research Vol. 5(2), pp. 280-288, 18 January, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Role of *Nigella sativa* in ameliorating chloramphenicol induced tissue damage in rats

Hossam Ebaid[1,2]*, Mohamed A. Dkhil[1,3], Wafaa S. Zahran[2], Moustafa A. El Feki[2] and Mohamed S. Gabry[3]

[1]Department of Zoology, College of Science, King Saud University, Saudi Arabia.
[2]Department of Zoology, Faculty of Science, El-Minia University, Egypt.
[3]Department of Zoology and Entomology, Faculty of Science, Helwan University, Egypt.

Accepted 30 November, 2010

***Nigella sativa* ascribed to have many medicinal properties. The study aimed to investigate whether *N. sativa* oil could decrease the side effects induced by the antibiotic, chloramphenicol. Rats were assigned into the following: The control group (first group); the second and the third groups which were orally administrated chloramphenicol for 21 days at a dose of 86 mg/kg body weight. Then the third group was treated with *N. sativa* oil for 30 days at a dose of 13.5 mg/ 150 g; the forth group was synergistically administrated both chloramphenicol and *N. sativa* for 21 days. Administration of *N. sativa* oil was extended to 30 days more in this group. There was a decrease in erythrocyte, hemoglobin and hematocrit with a progressive increase of leukocyte count in drug treated group. A decrease in neutrophils and lymphocyte with an increase in nucleated immature red cells as well as myeloblasts and myelocytes. On the other hand, *N. sativa* showed a time dependent improvement in blood parameters. A gradual decrease in the counts of immature stages was realized with the administration of *N. sativa*. Pathological changes in spleen included splenomegaly, lymphocytic depletion, enlargement of the marginal zone, wide trabeculae, reticular cells, pyknotic nuclei and cells in different stages of megakaryopoiesis. A marked depletion in cortical lymphocytes, disturbed lobular pattern, increased reticuloepithelial cells and dilated blood vessels in the thymus of group 2. *N. sativa* showed only a slight improvement of the damaged spleen tissues, while a time dependent repair in thymic tissue and both the cellular and humoral immunity was observed. In conclusion, *N. sativa* had an obvious protective effect and decreased the side effects of chloramphenicol.**

Key words: *Nigella sativa*, chloramphenicol, pathological changes.

INTRODUCTION

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. *Nigella sativa* had been employed for thousands of years as a spice and food preservative (Salem, 2005). It is found that *N. sativa* is an important medicinal herb; its oil is used as a natural remedy for a wide range of diseases, including various allergies (Kalus et al., 2003). Seeds of *N. sativa* are used for the treatment and prevention of a number of diseases and conditions that include asthma and diarrhea (Ali and Blunden, 2003). Many studies have been carried out on the pharmacological effects of *N. sativa* that have uncovered their anti-inflammatory and immunological effects (Hajhashemi et al., 2004). *N. sativa* contains both fixed and essential oils, proteins, alkaloids and saponin. Thymoquinone, the major component of the essential oil, is the biologically active gradient of these seeds (Ali and Blunden, 2003). It probably has an important role in the pharmacological effects (Hajhashemi et al., 2004).

The pharmacological actions include protection against nephrotoxicity and hepatotoxicity induced by either

*Corresponding author. E-mail: hossamebaid@yahoo.com. Tel. 0966-14675754.

disease or chemicals. It protects hepatic tissue from deleterious effects of toxic metals such as lead, and attenuates hepatic lipid peroxidation following exposure to chemicals such as carbon tetrachloride (Kapoor, 2009). *N. sativa* oil decreases blood pressure and increases respiration (Ali and Blunden, 2003). Treatment of rats with the seed extract increased both the packed cell volume and haemoglobin, and decreased plasma concentrations of cholesterol, triglycerides and glucose (Ali and Blunden, 2003). The oil and seed enhance the oxidant scavenger system (Salem, 2005). Thus, *N. sativa* seed is a promising source for active ingredients that would be with potential therapeutic modalities in different clinical settings. The efficacy of the active ingredients, however, should be measured by the nature of the disease (Salem, 2005; Alenzi et al., 2010). It is of immense therapeutic benefit in diabetic individuals as it accentuates glucose-induced secretion of insulin besides having a negative impact on glucose absorption from the intestinal mucosa (Kapoor, 2009).

Hamdy and Taha (2009) examined whether *N. sativa* oil and its active constituent thymoquinone attenuate oxidative stress in the heart and brain in an experimental model of diabetes mellitus using streptozotocin. The increase in heart and brain nitric oxide and malondialdehyde concentrations was attenuated by posttreatment of rats with *N. sativa* oil and thymoquinone. The lowered levels in glutathione and catalase were improved by either *N. sativa* oil or thymoquinone administration. *N. sativa* decreases DNA damage and thereby prevents initiation of carcinogenesis in colonic tissue secondary to exposure to toxic agents such as azoxymethane. We have previously studied the protective effect of *N. sativa* against chloramphenicol on the bone marrow (Ebaid, 1996; Zahran et al., 1996). Therefore, this study is an investigation of the histological changes of spleen and thymus, the peripheral blood and immunological parameters. Over use of the antibiotics can increase the risk of cancer. Chloramphenicol causes mitochondrial stress and decreased ATP biosynthesis, and accelerates cancer progression (Li et al., 2010). In addition, it is known that this antibiotic causes bone marrow suppression and aplastic anemia. Thus, chloramphenicol was chosen in this study to challenge the health of the animal models either with *N. sativa* or without *N. sativa.*

MATERIALS AND METHODS

Animal groups

Rats were divided into 4 groups. The first group was the control group inoculated with 2 ml of saline for 21 days. The second group was orally administrated chloramphenicol (Cidocetine) daily for 21 days at a dose of 86 mg/kg body weight. The third group was orally administrated chloramphenicol daily for 21 days and then treated with *N. sativa* oil for 30 days at a dose of 13.5 mg/ 150 g as previously described by Salomi et al. (1991). The fourth group was orally administrated both chloramphenicol and *N. sativa* daily for 21 days. In this group, administration of chloramphenicol was stopped after 21 days but administration of *N. sativa* oil was continued for 30 days more. We have previously examined the effect of N. sativa on albino rats (Zahran et al., 1996). The experiments were approved by State authorities and followed the Egyptian rules for animal protection.

Histological examination

Pieces of spleen and thymus were quickly removed, then fixed in Carnoy's fixative fluid. Following fixation, specimens were dehydrated, embedded, and then sectioned to 5×10^{-6} thickness. For histological examinations, sections were stained with Haematoxylin and Eosin. Many slides have been carefully examined for each animal (each group contained 5 animals and for each animal at least 3 slides from different areas of the organ were examined).

Plaque and rosette forming cell assays

To detect B-lymphocytes, spleen was removed and cell suspension was prepared and 0.2 ml of this suspension, 0.1 ml serum and 0.05 ml coated erythrocytes were added and incubated for 1.5 h at 37°C. Then 0.1 ml of human complement was added and the mixture was incubated at 37°C for 1 h. Number of the hemolytic plaque was examined microscopically. T-lymphocytes were detected by their ability to form rosettes with sheep erythrocytes. Then, 0.2 ml of spleen suspension was added to 0.2 ml of 10% antigen coated sheep erythrocytes and incubated for 2 h. Cells were gently re-suspended and number of rosettes was counted by hemocytometer (Onoé et al., 1980).

Passive hemagglutination test

The hemagglutination assay was carried out as described by McGarey and Allerd (1994). Hemagglutination tests were performed in 96-well U-bottomed microtiter plates using 20 µl of serially diluted antibodies (serum from control and treated animals) in phosphate buffer. Sheep erythrocytes were first coated with the chloramphenicol (antigen injected) and then 20 µl of 2% of these sheep red cell suspension were applied to each well in the plate. The suspension was incubated for 2 h at 37°C. Titers were considered to the last minimal dilution of the treated and control sera which could achieve clear hemagglutination with the applied sheep erythrocytes.

Statistical analysis

MINITAB (Version 13.1, 2002) was used for statistical analysis. All data were first tested for normality (Anderson-Darling test) and homogeneity of variances. Data were normally distributed, and variances were homogeneous, thus one-way ANOVA was used to determine overall effects of treatments, followed by individual comparison using Tukey's pairwise comparison. Results are expressed as means ± S.D. Values of $P<0.05$ were considered statistically significant.

RESULTS

All investigated parameters of this study proved an ameliorative effect of *N. sativa* in a time dependent

Table 1. Changes in different blood parameters due to administration of chloramphenicol and *N. sativa*.

Group	Erythrocytes × 10^6 /mm	Hemoglobin (g/100 ml)	Hematocrit (%)	Leucocytes × 10^3/mm
1	7.83 ± 0.08	14.5 ± 0.3	46.1 ± 2.5*	6.8 ± 0.7
2	5.2 ± 0.27	8.0 ± 0.1	40.0 ± 0.95	10.3 ± 0.68
3	6.83 ± 0.11	12.4 ± 0.1	42.0 ± 0.1	7.5 ± 0.35
4	6.8 ± 0.13	14.2 ± 0.16	42.6 ± 0.15	7.1 ± 0.28

Values are means ± SD. * signifiicant value.

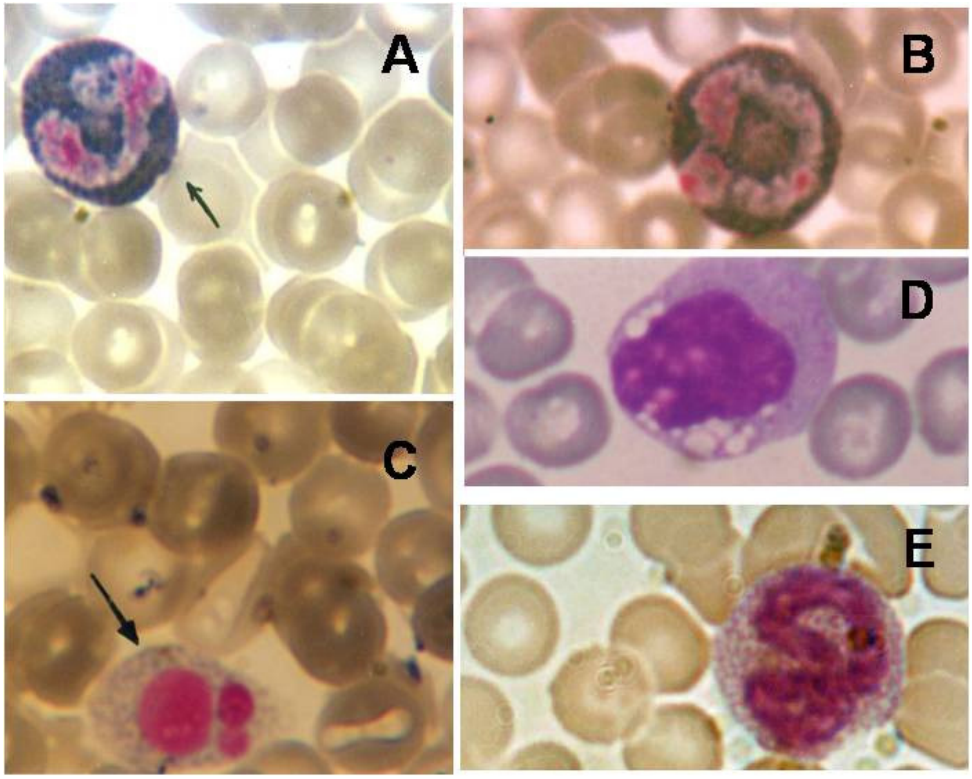

Figure 1. Stained blood films showing intensive basophilic cytoplasm with vacuolated bilobed nucleus (A) and vacuolated ring-shaped nucleus (B) from the drug-treated rats. A multinucleated erythroblast was showed in the blood film from drug + *N. sativa*-treated rats (C). Vacuolation in the nucleus and cytoplasm of an erythroblast (D) in the blood film of drug + *N. sativa*-treated rats. Blood film showing an easinophilic band cell (E). (Geimsa stain, ×1000).

manner. This conclusion was clearly reflected in the survival time of the rats from different groups. The survival time was significantly increased in the forth group compared to the drug treated group.

Hematological changes

Chloramphenicol has significant effects on the bone marrow and blood cells. Results revealed that there was a decrease in erythrocyte count, hemoglobin concentration and hematocrit values in samples taken from drug treated rats. The leukocyte count was progressively increased in these animals. A time dependent improvement was noted with the administration of *N. sativa* in the forth group in particular (Table 1).

Stained blood films from the drug treated group revealed a decrease in neutrophils and lymphocytes with an obvious increase in nucleated polychromatophlic and vacuolated immature red cells (Figure 1). Also immature myeloblasts and myelocytes were observed (Figure 1, Table 2). A gradual decrease and increase in immature stages and neutrophils, respectively, were found as long as the administration of *N. sativa* in the second and third groups.

Table 2. Changes in leukocyte differential counts in the different groups showing the mature and the immature stages.

Groups	Mature stages					Immature stages			
	N	E	B	M	L	Band cells	Myelo-blast	Myelo-cyte	Pro-erythrocyte
1	25 ± 0.9	1.5 ± 0.1	0.1 ± 0.01	5.7 ± 0.2	67.7 ± 1.4	0	0	0	0
2	18 ± 0.9	0.9 ± 0.3	1.0 ± 0.2	10 ± 0.2	17.4 ± 0.52	2.0 ± 0.2	38.0 ± 6.2**	6.0 ± 1.5	6.7 ± 1.2
3	27.3 ± 2.0	0.9 ± 0.3	1.3 ± 0.15	6.0 ± 0.5	38.0 ± 1.5	7.0 ± 1.2	10.0 ± 3.7*	3.0 ± 0.8	6.5 ± 0.3
4	25.9 ± 0.36	0.8 ± 0.4	0	5.5 ± 0.26	50.0 ± 1.7	3.0 ± 0.4	5.7 ± 0.92	8.1 ± 0.2	1.8 ± 0.12

Values are means ± SD. * signifiicant value.

Histological changes of spleen

The drug treated group

Histological examination of spleen sections showed that the capsule was increased in thickness compared to that of the control spleen. It became moderately hyaline in appearance containing many cell types such as lymphocytes, macrophages and fibroblasts. Trabeculae were highly distributed with depletion in the spleen white pulp (Figure 2). The marginal zone became thick and rich in macrophages, showing active phagocytosis represented by many dark yellow to brown pigments. A large number of neutrophils were present in the splenic red pulp.

Numerous immature cells invaded the splenic pulps with various stages of the megakaryocytes which occasionally infiltrated the spleen (Figure 2).

The drug followed by N. sativa treated group

Morphologically, there was a marked splenomegaly. No marked change was observed in the capsule and trabecular density and distribution. The white pulp became much diffused with a great decrease in the lymphocytic population with many lymphocytes appearing with pyknotic nuclei. The red pulp was intensely congested with hemolyzed blood cells and dark brown granules. Also, a large number of fibroblasts were detected and the degree of fibrosis was relatively high (Figure 2).

The drug + *N. sativa* followed by *N. sativa*-treated group

Splenomegaly with no demarcation between white and red pulps still occurred. White pulp contained many pyknotic nuclei. Splenic infiltration with immature megakaryocytes was observed. Hemolysis was also present. Although, these results confirmed a large damage of spleen tissue by chloramphenicol, sections of this group showed a considerable improvement. Many normally appeared mitotic figures and a normally appeared capsule were observed. Therefore, the total histological architecture of the spleen was affected with only a slight improvement. Similar results were obtained when many sections were examined from different animals of this group (Figure 2).

Histological changes of thymus

The drug treated group

Thymus of this group showed many histological changes compared to the control one (Figures 3A and B). The boundaries between cortex and medulla were slightly demarcated. The width of medulla appeared to be predominant over the cortex. The cortical lymphocytes showed a marked depletion. Increased numbers of dilated small vessels were observed in both medulla and cortex. Also there was an interlobular hemorrhage (Figure 3C and D).

The drug followed by N. sativa treated group

Significant improvement was observed in thymus of this group compared to that of the first group. The basic histological architecture was nearly presented. Boundaries between cortex and medulla were easily demarcated. The cortex became much, dominating the medulla. Also the cortex appeared densely populated with lymphocytes. Mitotic figures were also observed in the cortex. However increased number of dilated blood vessels and interlobular hemorrhage were also noticed (Figures 4A and B).

The drug + *N. sativa* followed by *N. sativa*-treated group

The histological architecture of the thymus was

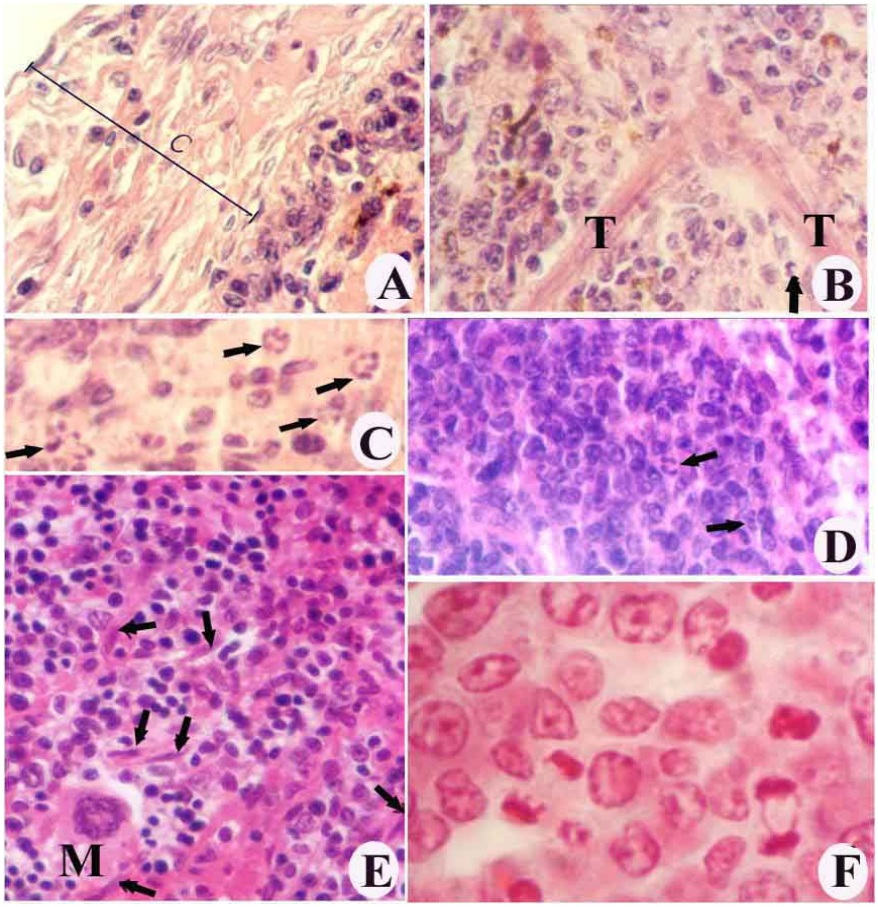

Figure 2. Microscopic sections of spleen showing a very thick capsule (A) (×500), distributed trabeculae in the red pulp with many dark yellow to brown pigments (B) (×500), a large number of neutrophils present in the splenic red pulp (C) (×1000) and the marginal zone became thick and rich in macrophages (arrows) (D) (×400) in the drug-treated group. The white pulp was lowered and many nuclei were pyknotic; splenic infiltration with immature megakaryocytes was observed; a large number of fibroblasts were detected (arrows) and so the degree of fibrosis was relatively high in spleen sections from drug followed by *N. sativa*-treated rats (E) (×400). Many mitotic figures were detected in sections from drug + *N. sativa* followed by *N. sativa*-treated rats (F) (×1000) showing a normal cellular activities with a little but marked improvement in spleen histology. Sections stained with hematoxylin and eosin.

resumed in this group to the control. Cortex and medulla were sharply demarcated and the lymphocytic population in the cortex seemed to be normal. However reticuloendothelial cells, Hassal's corpuscles and macrophages were detected. From these results, it is clear that chloramphenicol caused damage in the thymus, while administration of black seed oil had a significant improving effect on the histological architecture (Figures 4C and D).

Immunological changes in different groups

A remarkable decrease in the values of both rosette and plaque forming cells were recorded in the rats administered the drug comparing with the control values. It was clear that rats have obtained *N. sativa* in the same time with the drug showed a remarkable increase in the RFC and PFC values. Furthermore, as shown in Figure 5, the continuous administration of *N. sativa* caused an increase in the RFC value. Also, the PFC showed an improvement after administration of *N. sativa* for 30 days compared to the control values. Our results indicated that *N. sativa* oil enhanced both cellular and humoral immunity. The drug-administrated group showed a very weak hemagglutination titer. Significant changes in the values of the hemagglutination titers were obtained in the treated groups after administration of *N. sativa* compared with the drug administrated group (Figure 5). These results revealed that the humoral response was stronger

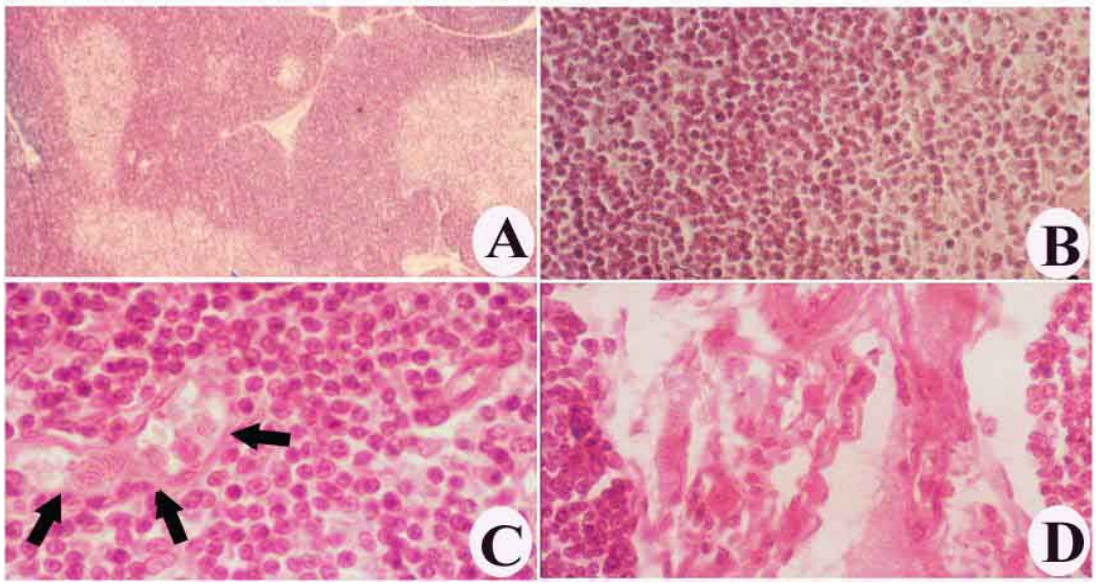

Figure 3. (A) Light micrograph of thymus sections from control rats showing the normal cortical and medullary regions (×100); (B) Magnified sector of the previous section (×200); (C) The cortical region of the drug-treated rats (×400). Dilated blood vessel filled with erythrocytes and depleted number of lymphocytes is revealed; (D) Interlobular hemorrhage in the thymus of the drug-treated rats (×312.5). Sections were stained with hematoxylin and eosin.

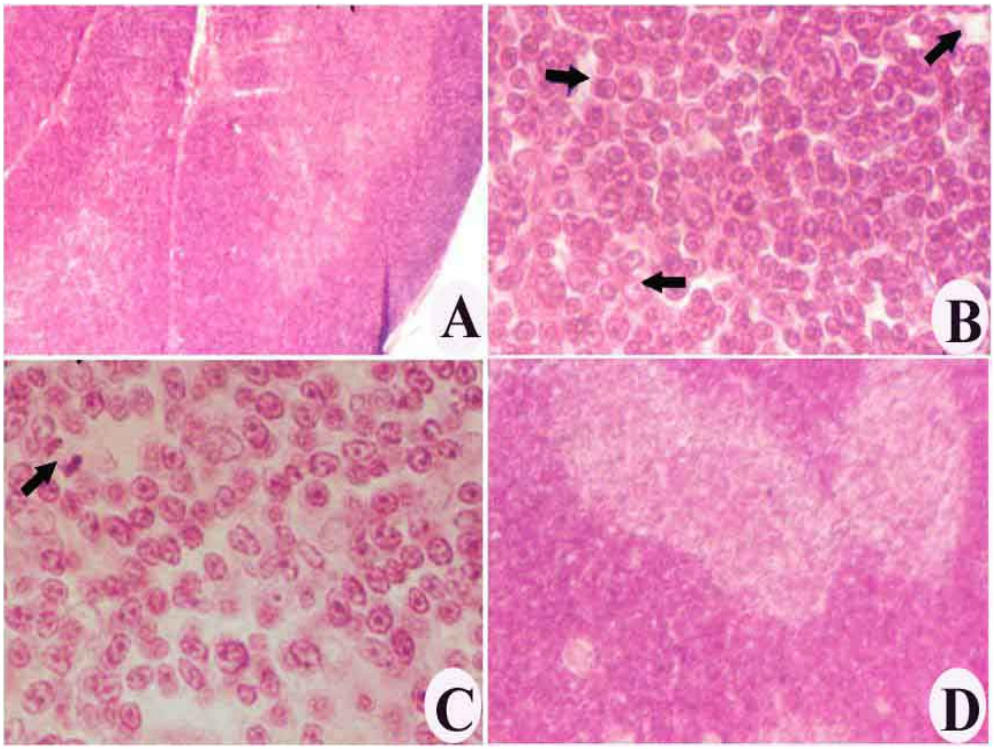

Figure 4. (A) Light micrograph of thymus sections from drug followed by *N. sativa*-treated rats showing the wider medulla (×100); (B) Magnified sector from the previous section (A) showed the focal areas (thick arrows) of the macrophage activities and the reticulo-endothelial cells (thin arrows) (×500); (C) The arrow showed a mitotic figure in thymus from drug+ *N. sativa* followed by *N. sativa*-treated rats (×500); (D) Thymus from drug + *N. sativa* followed by *N. sativa*-treated rats showing a normal histological archeticture (×32). Sections stained with hematoxylin and eosin.

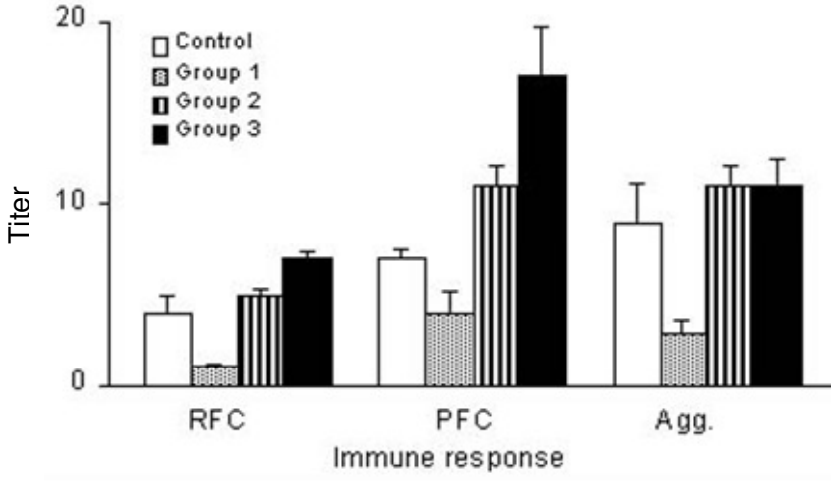

Figure 5. Immunological response (RFC, PFC, and agglutination [Agg]) of the rats treated with chloramphenicol and *N. sativa* in the different groups. Titer was determined as the number of rosettes and plaques in cases of RFC and PFC, respectively, and the highest dilution of the serum in case of agglutination.

than the cellular response.

DISCUSSION

This study is an approach to find whether *N. sativa* oil can protect against harm side effects of chloramphenicol on the peripheral blood and spleen and thymus. A sign of a hemolytic anemia associated with chloramphenicol administration was observed in rats. In addition, the inhibitory action of the drug on the bone marrow resulted in a progressive increase of the immature leukocytes in the peripheral blood. Great hisopathological changes were associated with the treatment of this drug. On the other hand, administration of the *N. sativa* oil showed a time dependent significant repair in the blood picture, spleen and thymus histology, and the cellular and humoral immunity. It was found that *N. sativa* oil and derived thymoquinone have anti-inflammatory (Houghton et al., 1995), antidiabetic (Kanter et al., 2003; 2004; Fararh et al., 2005), anti-tumor (Banerjee et al., 2010) and a hepatoprotective actions (Daba and Abdel-Rahman, 1998).

The decreased production of neutrophils were attributed to the failure of hematopoietic tissue in supplying circulation with mature granulocytes due to the inhibitory action of the drug on the protein synthesis. The immunological active role of these cells may be a second decreasing factor since they migrate to the inflammatory sites. This was proved by the large number of such cells in spleen tissue sections. *N. sativa* oil decreases the migration of these cells from peripheral blood because it has an anti-inflammatory action as deduced by Houghton et al. (1995). Thus, a gradual increase of myelocytes and differentiated neutrophil occurred by *N. sativa* in the third and forth group. This confirmed that an increased number of cells in bone marrow which became able to reach advanced developmental stages. Improvement of erythrocyte count after administration of *N. sativa* oil may be explained on the basis of the accelerative effect of *N. sativa* oil on the cellular respiratory mechanism. Thus, protein formation needed cellular events such as mitosis generated in mitochondria which have enzymes involving in biosynthesis of heame, the most important component in erythropoiesis. El-Mahmoudy et al. (2005) and Kanter (2008) found that *N. sativa* may alleviate damage to b-cells in the pancreas because of the significant antioxidant activity of this medicinal herb.

The enlargement of spleen of rat's administrated chloramphenicol, is attributed to its phagocytic role and is due to the increase in the number and size of the reticuloendothelial cells. In the haemolytic anaemia associated with a high number of red blood cells in the spleen as previously detected (Zahran et al., 1996), the spleen is enlarged and there is a marked phagocytosis induced by this increased number of effete erythrocytes. This may also declare that brown pigments detected in the splenic parenchyma are haemosiderin, resulting from destruction of red blood cells in the phagocytosis, actively carried by macrophages and swollen reticuloendothelial cells in the spleen.

Cormak (1987) recorded that, proliferating and differentiating progeny of the activated B-lymphocytes in primary and secondary lymphatic nodules became progressively displaced toward the red pulp, where maturation into antibody secreting plasma cells takes place. This suggestion did not exactly explain the presence of increased number of mitotic figures because of the rare detection of plasma cells in the present studied spleen sections. So, the splenomegaly which had

been recorded in most studied splenic sections presumably due to the engorgement of the spleen with different cellular blood cells in different stages.

According to Junqueira and Carneiro (1980), the white pulp of the spleen produces lymphocytes that migrate to the red pulp and reach the lumen of the sinusoid to retain the decrease of lymphocytes of the entire blood; this may explain the dilation of the sinusoids. Also, the depletion of lymphocytic population in the sinus is directly affected by the periartrial lymphoid sheath of spleen as deduced by Krause and Cutts (1984). Enlargement of the marginal zones may be attributed to the fact that it is the region of the red pulp which receives the incoming arterial blood; thus, it is the site where blood borne cells and particulate matter first contact the splenic parenchyma. These zones, also, trap circulating antigens and are important in the immunological activities of the spleen (Junqueira and Carneiro, 1980; Ebaid, 1996).

Detection of wide and thick capsule and trabeculae filled with fibroblasts in the studied sections may be due to their role in conducting large arteries and veins throughout the spleen and providing major structural support for the fine reticulin network which extends throughout the organ (Wheater et al., 1979).

Because of the spleen is the body's filter against any foreign materials from the blood stream, large number of neutrophils migrates from the peripheral blood into the splenic parenchyma to participate in the humoral immunological responses (Fawcett, 1986; Krücken et al., 2009). This may explain the detection of large number of neutrophils in both marginal zone and red pulps. However, edematous polymorphonuclear cells had been described by Wheater et al. (1979) who explained this phenomenon on the basis of the storage lipid contents in such cells which was elevated due to the increased in corticosteroid hormone librating the lipids from the storage tissue under the effect of the drug.

The drug caused secondary organ damage due to the release of lytic enzymes and oxygen radicals (Shanley et al., 1995). The released free radicals target the phagocyte itself or, on their release, they may attack neighboring cells. However, the slight improvement in the spleen tissue may be due to the antioxidant anticancer properties of *N. sativa* in addition to its role in enhancing the functional capabilities of immune system (Salem et al., 2005; Ali and Blunden, 2003). A study investigated the ability of volatile oils of *N. sativa* to scavenge free radicals generated during aflatoxicosis. Abdel-Wahab and Aly (2005) found that *N. sativa* oil was more effective than *Syzygium aromaticum* oil in restoring the parameters that were altered by aflatoxin in rats.

According to Rappaport (1966), the presence of mitotic figures may be presumed to extramedullary hematopoietic role of spleen in an attempt to recover the bone marrow failure, which resulted from drug use (Zahran et al., 1996), in supplying the blood with normal cells. This may explain many normally appeared mitotic figures which were detected in the forth group after administration of *N. sativa*. These mitotic figures may be induced by *N. sativa* since it play an important role in regulating vital cellular functions, including cell proliferation and differentiation (Salem et al., 2005; Ali and Blunden, 2003). Spleen cells were used to investigate the humoral and the cellular immune responses. Results showed significant changes after administration of *N. sativa*. This confirmed the improvement of the spleen tissue though it was still lower than that of the thymus. Furthermore, the immunological studies showed that the humoral response was stronger than the cellular response. This is because of the spleen tissue damage, since such damage is considered the first factor affecting the cellular response (Ellis, 1981). The increase in cellular and humoral levels may be due to the activation reactivity of the *N. sativa* on some immunological functions (Barkarda et al., 1983).

Concerning the histopathological changes in the thymus in the present study, the drug treatment resulted in a marked depletion in coritical lymphocytes, disturbed lobular pattern, increased reticuloepithelial cells and dilated blood vessels. These pathological findings indicated thymic involution which is attributed to its sensitivity to any stress as declared by Junqueira et al. (1989). Thymic involution occurs in response to a wide variety of stimuli including release of high level of adrenocorticotrophic hormone in the body (Fawcett, 1986). In the present work, hormones may be released by drug. Lymphocytes are destroyed by macrophages since many focal areas were detected (Ebaid, 1996).

The recorded amerolative effect of *N. sativa* upon the pathological alterations induced by the drug could be explained by its role in regulating vital cellular functions, including cell proliferation and differentiation (Salem et al., 2005; Ali and Blunden, 2003) as mentioned above. Furthermore, El-Saleh et al. (2004) have found that the active antioxidant components of the *N. sativa* plant protected against the development of methionine-induced hyperhomocysteinemia and its associated state of oxidative stress. It had been found that treatment with *N. sativa* oil produced a dose-dependent amelioration of the gentamicin nephrotoxicity (Ali, 2004) and CCl_4-induced liver damage (Al-Ghamedi, 2003). On the other hand, Ali (2004) found that treatments of rats with *N. sativa* did not cause any overt toxicity and enhanced growth in the renal cortex. However, in this work, the amelioration effect of *N. sativa* on the histological and immunological indices was a time-dependant one.

ACKNOWLEDGMENT

This work was gratefully supported by El-Minia University and the Centre of Excellence for Biodiversity Research, College of Science, King Saud University, Riyadh, Saudi Arabia.

REFERENCES

Abdel-Wahab MA, Aly SE (2005). Antioxidant property of *Nigella sativa* (black cumin) and *Syzygium aromaticum* (clove) in rats during aflatoxicosis. J. Appl. Toxicol., 25: 218-223

Alenzi FQ, El-Bolkiny YS, Salem ML (2010). Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. Br. J. Biomed. Sci., 67: 20-28.

Al-Ghamdi MS (2003). Protective effect of *Nigella sativa* seeds against carbon tetrachloride-induced liver damage. Am. J. Chin. Med., 31: 721-728.

Ali BH (2004). The effect of *Nigella sativa* oil on gentamicin nephrotoxicity in rats. Am. J. Chin. Med., 32: 49-55.

Ali BH, Blunden G (2003). Pharmacological and toxicological properties of *Nigella sativa.* Phytother Res., 17: 299-305.

Banerjee S, Azmi AS, Padhye S, Singh MW, Baruah JB, Philip PA, Sarkar FH, Mohammad RM (2010). Structure-activity studies on therapeutic potential of Thymoquinone analogs in pancreatic cancer. Pharm. Res., 27: 1146-1158.

Barakada B, Bouffard H, Derman V (1983). The effect of Coumarin derivative on the immunological system of man. Agents and actions, 31: 50-52.

Cormak DH (1987). Ham's Histology. 9th ed. Lippinocott JB. Philadephia. Junqueira LC. and Carneiro J. (1980) Basic Histology. 3rd ed. Lange Medical Publication, Los Altos, California.

Daba MH, Abdel-Rahman MS (1998). Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol. Lett., 95: 23-29.

Ebaid H (1996). The anti-carcinogenic effect of the black seed (*Nigella sativa*) on leukemia. MSc. thesis, Faculty of Science. El-Minia University, Egypt.

Ellis AE (1981). Stress and modulation of defense mechanism in fish. In: Stress and Fish (Pickering AD. Ed.) Academic Press, 147: 170.

El-Mahmoudy A, Shimizu Y, Shiina T, Matsuyama H, El-Sayed M, Takewaki T (2005). Successful abrogation by thymoquinone against induction of *Diabetes mellitus* with streptozotocin via nitric oxide inhibitory mechanism. Int. Immunopharmacol., 5: 195-207.

El-Saleh SC, Al-Sagair OA, Al-Khalaf MI (2004). Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats. Int. J. Cardiol., 93: 19-23.

Fararh KM, Shimizu Y, Shiina T, Nikami H, Ghanem MM, Takewaki T (2005). Thymoquinone reduces hepatic glucose production in diabetic hamsters. Res. Vet. Sci., 79: 219-223.

Fawcett MD (1986). A textbook of Histology. 11th ed. Saunders WB. Company, Igaku-Shoin, Sounders.

Hajhashemi V, Ghannadi A, Jafarabadi H (2004) Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. Phytother. Res., 18: 195-199.

Hamdy NM, Taha RA (2209). Effects of *Nigella sativa* oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. Pharmacology, 84: 127-134.

Houghton PJ, Zarka R, Heras BD, Hoult JR (1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Medica, 61: 33-36.

Kalus U, Pruss A, Bystron J, Jurecka M, Smekalova A, Lichius JJ, Kiesewetter H (2003). Effect of *Nigella sativa* (black seed) on subjective feeling in patients with allergic diseases. Phytother. Res., 17: 1209-1214.

Kanter M (2008). Effects of *Nigella sativa* and its major constituent, thymoquinone on sciatic nerves in experimental diabetic neuropathy. Neurochem. Res., 33: 87-96.

Kanter M, Meral I, Yener Z, Ozbek H, Demir H (2003). Partial regeneration/ proliferation of the b-cells in the islets of Langerhans by *Nigella sativa* L. in streptozocin-induced diabetic rats. Tohoku J. Exp. Med., 20: 213-219.

Kapoor S (2009). Emerging clinical and therapeutic applications of *Nigella sativa* in gastroenterology. World J. Gastroenterol., 15: 2170-2171.

Krause JW, Cutts JH (1984). Concise text of Histology. 4th ed. Williams and Wilkins. Baltimore, London.

Krücken J, Delić D, Pauen H, Wojtalla A, El-Khadragy M, Dkhil MA, Mossmann H, Wunderlich F, 2009. Augmented particle trapping and attenuated inflammation in the liver by protective vaccination against Plasmodium chabaudi malaria. Malar. J., 8: 54.

Li CH, Cheng YW, Liao PL, Yang YT, Kang JJ (2010). Chloramphenicol causes mitochondrial stress, decreases ATP biosynthesis, induces matrix metalloproteinase-13 expression, and solid-tumor cell invasion. Toxicol. Sci., 116: 140-50.

McGarey DJ, Allerd DR (1994). Characterization of hemagglutinating components on the *Anaplasma marginale* initial body surface and identification of possible adhesions. Infec. Immun., 62: 4587-4593

Onoé K, Fernandes G, Good RA. (1980). Humoral and cell-mediated immune responses in fully allogeneic bone marrow chimera in mice. J. Exp. Med., 151: 115-32.

Rappaport H (1966). Tumors of the hematopoietic system. Atlas of Tumor Pathology. Sec. III, Fasc. 8, Washington, DC, Armed Forces Institute of Pathology, p. 241.

Salem ML (2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int. Immunopharmacol., 5: 1749-1770.

Salomi MJ, Nair SC, Satish C, Pankkar KR (1991). Inhibitory effects of *Nigella sativa* and saffron (*Crocus sativa*) on chemical carcinogenesis in mice. Nut. Cancer., 16: 67-72.

Shanley TP, Warner RL, Ward PA (1995). The role of cytokines and adhesion molecules in the development of inflammatory injury. Mol. Med. Today, 1: 40-45.

Wheater PR, Burkitt HG, Daniels VJ (1979). Functional histology. The English language Book Cociety and Churchill Livingstones.

Zahran WM, El-Feki MA, Gabri MS, Ebaid H (1996). The anti-carcinogenic effect of the black seeds (*Nigella sativa*) on leukemia.1-Bone marrow and blood picture. El-Minia Sci. Bul., 9: 1-19.