The effect of *Nigella sativa* on the thymus of young and adult rats: Histological, immunohistochemical, and morphometric study

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Background
The thymic microenvironment plays a central role in T-cell differentiation. Involution of the thymus begins relatively early in life, resulting in decreased immunity. *Nigella sativa* is known for its immunopotentiating effects.

Aim of the work
The aim of the study was to elucidate the effect of *N. sativa* on the structure of the thymus in young and mature adult rats.

Materials and methods
Twenty-eight male Wistar albino rats were divided into two groups: group I and group II. Group I was further divided into subgroup Ia and subgroup Ib. Subgroup Ia included seven 1-month-old young rats weighing 50–60 g that received plain water by gavage for 15 days. Subgroup Ib included seven young rats that received 2 ml/kg body weight of 1% petroleum ether extract of *N. sativa* by gavage for 15 days. Group II was further divided into subgroup IIa and subgroup IIb. Subgroup IIa included seven mature 12-month-old adult rats weighing 600–650 g that received plain water by gavage for 15 days. Subgroup IIb included seven mature adult rats that received 2 ml/kg body weight of 1% petroleum ether extract of *N. sativa* by gavage for 15 days. Thymi from different subgroups were processed for histological, immunohistochemical, and morphometric studies.

Results
In subgroup Ib, the thymus of *N. sativa*-treated rats revealed a significant increase in the number of CD3-positive cells in the cortex. Some CD3-positive cells were seen in the medulla. In subgroup IIa, signs of thymic involution in the form of expansion of perivascular spaces containing adipocytes and stromal cells with a significant decrease in the number of CD3-positive cells were noticed. In addition, CI cells non reticular thymulin secreting cells along the capillaries were detected. In *N. sativa*-treated adult rats (subgroup IIb), restoration of the normal stroma and parenchyma occurred. Moreover, a significant increase in the number of CD3-positive cells in the cortex and medulla with an increase in the secretory activity of reticuloepithelial cells was noticed.

Conclusion
Oral administration of the petroleum ether extract of *N. sativa* for 15 days to young and mature adult rats results in enhanced proliferation of thymocytes and increased activity of reticuloepithelial cells.

Keywords:
CD3, morphometry histology, *Nigella sativa*, thymus, young and adult rats

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Introduction

The thymus is a central immunological gland in which T-cell differentiation and repertoire selection take place. Thymic hormones constitute an important part of the humoral thymus milieu that contributes to T-cell differentiation and selection of T exportable cells to the periphery [1].

A vital role is played by the thymic microenvironment, which includes thymic stromal cells (epithelial cells, macrophages, and dendritic cells), in the process of T-cell differentiation [2]. Thymic reticuloepithelial cells (RECs) have been regarded as the main drivers of thymocyte development and maturation through cell-to-cell contacts and the production of soluble factors (e.g. cytokines, hormones, growth factors, and neurotransmitters) [3]. Moreover, these are involved in the presentation of various antigens to developing lymphocytes and provide growth regulatory signals that may range from stimulatory

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to apoptotic signaling within the thymus [4]. The thymic RECs are functionally specialized on the basis of their location within the thymic microenvironment. Thus, although subcapsular, cortical, and medullary, RECs are derived from a common endodermal origin; their unique location within the gland causes their specialization in terms of their immunophenotypical and physiological properties [4].

The decline in thymic T-cell regenerative capacity begins relatively early in human life, resulting in a limited capacity for T-cell regeneration during young adulthood. As a result, adult humans who experience profound T-cell depletion regenerate T cells primarily through relatively inefficient thymic-independent pathways [5]. In the young, recovery of depleted naive cells is correlated to an increase in thymic cellularity and volume, termed 'thymic rebound', which is evident within the first few months after chemotherapy. Thymic rebound at 6 months after treatment is markedly reduced in young adults when compared with children [6]. Similarly, it was reported that many structural changes in the thymus of aging rats appeared at 6, 12, and 20 months [7].

The delayed immune reconstitution in adults can be attributed to the correlation between increased morbidity and mortality and age [8]. Thus, it is of paramount importance to develop strategies that enhance thymic output and promote immune reconstitution, particularly with advancing age [9]. Data from bone marrow transplantation studies have shown the persistence of some thymopoiesis in adults with a high possibility of reinduction [10]. Therapies are being developed that exploit the thymopoietic potential to promote reconstitution of the thymus and increase the output of appropriately educated naive T cells [9].

The use of alternative plant medicine has increased recently and has attracted the attention of many researchers all over the world [11]. In the past decade, there was widespread belief that green medicine (plants) is healthier than synthetic drugs. One such plant is *Nigella sativa* Linn. (Family Ranunculaceae), commonly known as ajaji, kalika, or black cumin [12]. In old Latin, it is called Panacea, meaning cure all [13]. Chemical analysis of the petroleum ether extract of *N. sativa* seeds revealed the presence of considerable amounts of fatty acids in the oil. Linoleic acid (55.6%) and oleic acid (23.4%) are the major unsaturated fatty acids that together constitute 82.5% of the total fatty acids present in the oil [14]. *N. sativa* is known for its antibacterial [15], antioxidant [16], antidiabetic [17], immunopotentiating [18], and antimetastatic activity in mice [19]. Many of these activities have been attributed to the quinone constituent of the seed [20].

Aim of the work

This study was undertaken to elucidate the immune-enhancing effect of the petroleum ether extract of *N. sativa* on the thymus of young and adult albino rats.

Materials and methods

Animals

A total of 28 male Wistar albino rats were purchased from the animal house of King Fahd Medical Research Center (KFMRC) in King Abdulaziz University (KAU). Fourteen rats were 1 month old, whereas the remaining 14 rats were 12 months old. The rats were caged under environmentally controlled conditions (12-h light/12-h dark cycles). They were fed *ad libitum* with free access to water for 1 week and were treated according to the guidelines from the KFMRC.

Plant material

N. sativa seeds were purchased from a local market in Cairo. The seeds were cleaned, dried, and ground and kept at 4°C. The petroleum ether extract of *N. sativa* was prepared by the Department of Pharmaceutics, Faculty of Pharmacy, Al Azhar University, Cairo.

Three liters of petroleum ether were added to every 1 kg of ground *N. sativa* seeds, and the mixture was left for 3 days. The obtained filtrate was evaporated in a rotary evaporator (Rotavapour R110 Bűchi) under reduced pressure at 45–50°C to remove any excess petroleum ether. The resultant extract was diluted in PBS at the ratio of 1:9. The extract was conserved at 4°C and protected from light and humidity. Thereafter, the petroleum ether extract of *N. sativa* (1%) was used at a dose of 2 ml/kg body weight [21].

Experimental protocol

The animals were divided into two major groups of 14 rats each. Rats in group I were 1 month old, whereas those in group II were 12 months old. Plain water and the recommended dose of petroleum ether extract of *N. sativa* were administered daily by gavage for 15 days. The experimental design was as follows:

(1) *Group I:* Fourteen young male rats weighing 50–60 g (1 month old) were used. They were further divided into two subgroups:

(a) *Subgroup Ia:* Seven rats were given plain water at a quantity equal to the dose of *N. sativa* by gavage.

(b) *Subgroup Ib:* Seven rats were given 2 ml/kg body weight of petroleum ether extract of *N. sativa*.

(2) *Group II:* Fourteen male adult rats weighing 600–650 (12 months old) were used. They were further divided into two subgroups:

(a) *Subgroup IIa:* Seven rats were given plain water at a quantity equal to the dose of *N. sativa* by gavage.

(b) *Subgroup IIb:* Seven rats were given 2 ml/kg body weight of petroleum ether extract of *N. sativa*.

At the end of the experiment, animals of all groups were sacrificed under ether anesthetic state to extract their thymus.

Histological study

Hematoxylin and eosin stain [22]

The dissected thymus was cut into small pieces and immersed immediately in 10% neutral buffered

formalin. Specimens were processed and embedded in paraffin wax. Sections (4-μm- thick) were stained with H&E, examined using an Olympus light microscope, (BX51TF; Olympus, Tokyo, Japan), and photographed.

Transmission electron microscopic study

Tissue samples of 1 mm^3 from the thymus were fixed in 5% phosphate-buffered gluteraldehyde for 3 h. Fixed tissue samples were washed with distilled water and then postfixed in 2% osmium tetroxide in 0.1 mol/l cacodylate buffer, dehydrated in graded ethanol, and embedded in Spur epoxy resin. Ultrathin sections (50–60 nm) were cut and stained with uranyl acetate and lead citrate [23]. The stained grids were examined and photographed with a transmission electron microscope (Philips CM 100, Philips/FEI Corporation, Eindhoven, Holland) at 60 kV. Processing was carried out according to the electron microscopic unit protocol of KFMRC at KAU.

Immunostaining [24]

Paraffin sections (4-μm-thick) were mounted on electrostatically charged glass slides. The sections were dried, dewaxed, and rehydrated with water. Heat-mediated antigen retrieval was performed in a Milestone RHS-2 microwave (Sorisole, Italy) at 110°C for 2 min in a 1 mmol/l EDTA buffer with pH 8.0. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in Tris-buffered saline with Tween (TBST) for 10 min. The slides were then washed and incubated for 20 min with 5% normal goat serum (Dako UK Ltd, Ely, UK) in TBST. Excess blocking serum was blown off, and the slides were incubated with PS1, the liquid mouse monoclonal antibody against CD3 (cat. #NCL-CD3-PS1; Novocastra Labs Ltd, Newcastle, UK), diluted to 1:50 in TBST for 60 min. After the washing process, the slides were incubated for 30 min in a mouse-specific EnVision+ System–HRP (Dako UK Ltd) and visualized by incubation in diaminobenzidine (DAB) from the EnVision+ kit for 10 min. The slides were counterstained for 1 min with hematoxylin (Clin-Tech, Guildford, UK). Staining was negatively controlled by substituting the mouse immunoglobulin (Ig) fraction, diluted to the same Ig concentration, for the primary antibody. The NCL-L-CD3-PS1 used was specific for the nonglycosylated epsilon chain of the CD3 molecule, which stains pan T lymphocytes. Positively stained cells had a brown color.

Morphometric study

The number of RECs in the cortex and medulla of the thymus was counted from H&E-stained sections. The developing thymocytes positively stained with CD3 were counted from immunostained sections. For each animal, five nonoverlapping visual fields (×600) in 10 sections were randomly examined. The Image Pro Plus, version 4.5 (Media Cybernetics, Silver Springs, Maryland, USA) software was used.

Statistical study

Statistical analyses were performed using the SPSS program, version 16 (IBM, Armonk, New York, USA). One-way analysis of variance was carried out. When equal variances were assumed, the least significance difference test was applied. Data were presented as mean ± SD. A P value less than 0.05 was considered statistically significant.

Results

Histological and immunohistochemical study

H&E-stained sections of the thymus of 1-month-old rats (subgroup Ia) revealed a well-organized general architecture. A thin loose connective tissue capsule invested the cortex. The outer cortex was thick and characterized by its heavy concentration of the developing T lymphocytes (thymocytes), which contributed to the intense basophilia of this zone. The inner medulla appeared paler with fewer thymocytes (Fig. 1). The thymic parenchyma consisted of a network of RECs surrounding irregularly shaped compartments filled with thymocytes. The thymocytes had centrally located, darkly stained nuclei. Some thymocytes appeared in mitosis. RECs were present in the outer cortical, midcortical, and deep cortical zones. These cells revealed a palely stained eosinophilic cytoplasm and large ovoid pale nuclei with prominent one or two nucleoli. Many cortical blood capillaries were identified between the cells (Fig. 2). In the medulla, the thymocytes were less condensed, and many RECs were observed (Fig. 3). At the ultrastructural level, cortical RECs appeared stellate-shaped with large euchromatic nuclei and a prominent nucleolus. The cells had cytoplasmic processes. The majority of thymocytes had nuclei occupying most of the cell, with the heterochromatin mostly arranged at the periphery along with a rim of cytoplasm. The cytoplasm showed few small vesicles of smooth endoplasmic reticulum and mitochondria. Several thymocytes with pyknotic nuclei were also observed (Fig. 4). The CD3 positively stained developing thymocytes appeared diffuse in the cortex (Fig. 5).

Thymi of rats in subgroup Ib receiving *N. sativa* for 15 days revealed a well-organized structure similar to that in subgroup Ia (Fig. 6). The CD3 positively stained developing thymocytes were distributed in the cortex and some appeared in the medulla (Fig. 7). The ultrastructural study revealed large thymocytes, and some cells appeared in mitosis with two separate centrioles in the field (Fig. 8).

In comparison with group Ia, rats of subgroup IIa showed a disturbed general architecture of the thymus. The subcapsular area was replaced by fibrous connective tissue containing fat cells encroaching deeper into the cortex. Phagocytosed apoptotic bodies were numerous in the cortex (Fig. 9).

Some rounded cells in the cortex with acidophilic granular cytoplasm were located around the capillaries

(Fig. 10). An increase in the number of RECs of the medulla was noticed. Some of them formed rosette-like structures (Fig. 11). Electron microscopic examination revealed that some cortical thymocytes had fragmented nuclei and disrupted mitochondria and cell membranes. Other thymocytes revealed rounded heterochromatic nuclei and a moderate amount of cytoplasm. Some cells showed the presence of many phagosomes and an osmophilic fat droplet inside its cytoplasm (Fig. 12). The CD3 positively stained developing thymocytes were distributed in a scattered patchy manner in the cortex (Fig. 13).

In subgroup IIb (given *N. sativa* for 15 days), thymocytes at different stages of mitosis were observed in sections stained with H&E among many palely stained nuclei of RECs (Fig. 14). Deeper areas of the cortex revealed dark RECs with electron-dense cytoplasm and irregular nuclei with coarse clumps of heterochromatin. These cells possessed long, thin, tightly fitting cytoplasmic processes rich in organelles and secretory vesicles filled with electron-dense or translucent material and mitochondria. The processes surrounded thymocytes of variable sizes that had abundant cytoplasm and heterochromatic nuclei. Other large thymocytes had more euchromatic nuclei (Fig. 15). In some areas RECs with open euchromatic nuclei and a moderate number of membrane-bound cystic vesicles of different sizes containing granular material and electron-dense bodies were observed (Fig. 16). Immunohistochemically stained sections of the same group revealed groups of CD3 positively stained developing T cells in the cortex (Fig. 17); however, they were numerous and mainly present in the medulla (Fig. 18).

Morphometric study

The number of cortical and medullary RECs in the thymus of rats of subgroup Ib was significantly increased in comparison with that of subgroup Ia. Adult rats in subgroup IIa revealed a highly significant increase in the number of cells when compared with subgroup Ia. Adult rats receiving *N. sativa* for 15 days (subgroup IIb) showed a highly significant increase in the number of RECs when compared with subgroups Ia, Ib, and IIa (Table 1). The number of thymocytes positively stained for CD3 was highly significantly decreased in adult rats (subgroup IIa) compared with young ones (subgroup Ia). Moreover, sections of the thymus of rats in subgroup IIb receiving *N. sativa* for 15 days showed a highly significant increase in the number of CD3 positively stained thymocytes when compared with groups Ia, Ib, and IIa (Table 1).

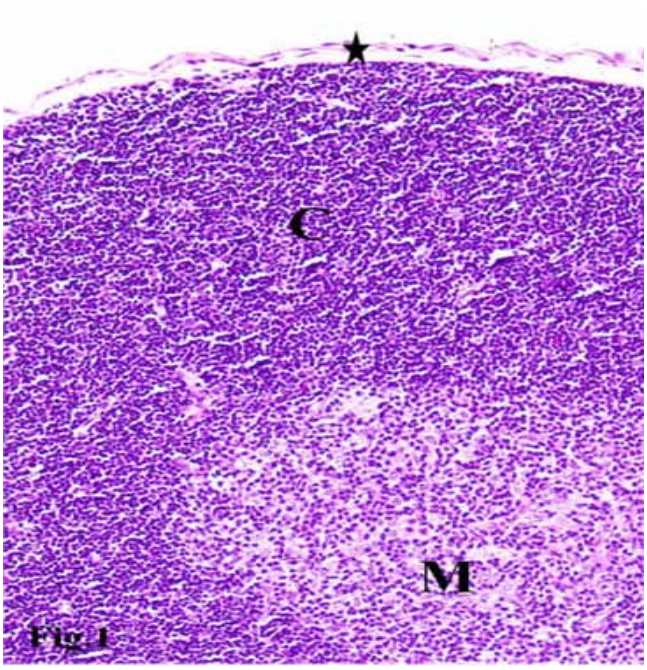

Figure 1. A photomicrograph of a section of the thymus of a rat (subgroup Ia) showing a well-organized general architecture. The cortex (C) is thick and characterized by heavy thymocyte concentration. The medulla (M) appears paler with fewer thymocytes than in the cortex. Notice the thin connective tissue capsule investing the cortex (*).
H&E, × 200.

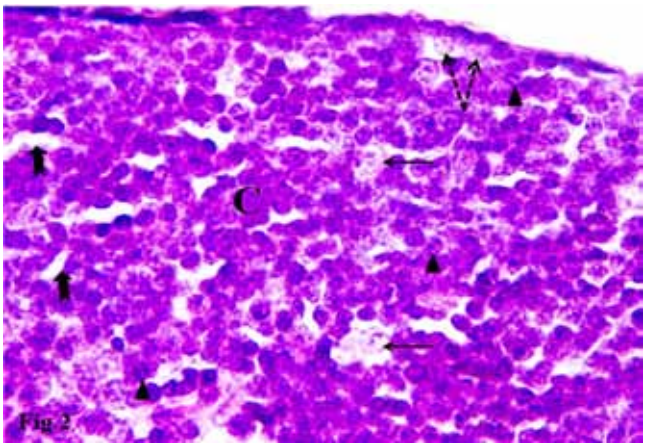

Figure 2. A photomicrograph of a section of the thymus of a rat (subgroup Ia) showing the cortex (C) formed of thymocytes and reticuloepithelial cells (RECs). Large palely stained nuclei of RECs are present in the outer cortical (dashed arrows), midcortical, and deep cortical layers (thin arrows). Some thymocytes are in mitosis (▲). Notice the cortical blood capillaries (notched arrows).
H&E, × 1000.

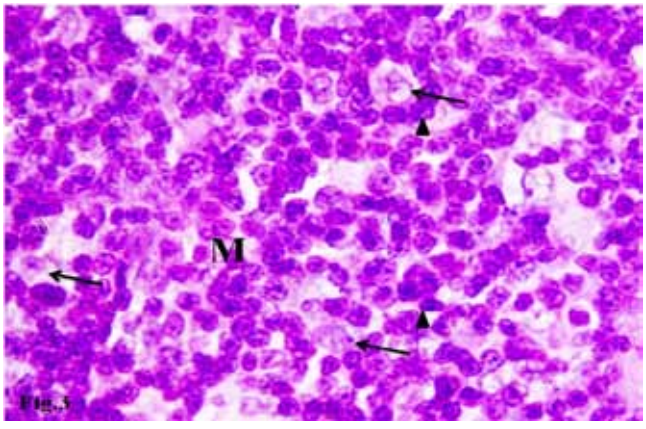

Figure 3. A photomicrograph of a section of the thymus of a rat (subgroup Ia) showing a pale medulla with less condensed thymocytes (▲) and many reticuloepithelial cells (arrows). M, medulla.
H&E, × 1000.

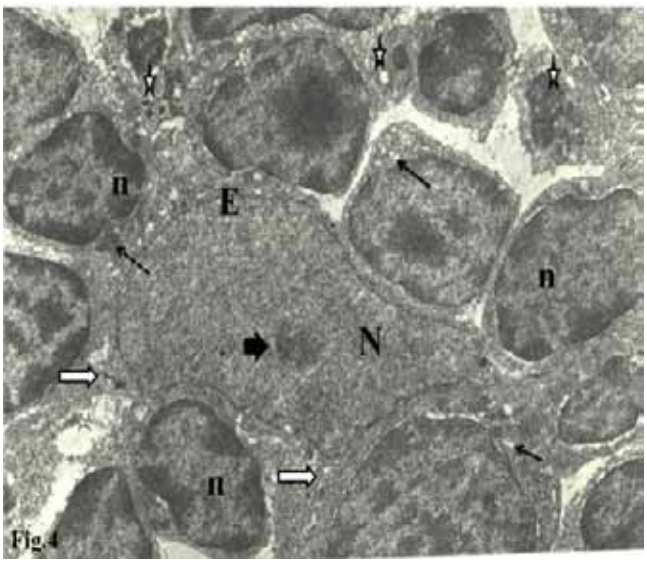

Figure 4. An electron micrograph of the thymic cortex of a rat (subgroup Ia) showing a stellate-shaped reticuloepithelial cell (E) with cytoplasmic processes (white arrows) having a large euchromatic nucleus (N) and prominent nucleolus (thick black arrow). The majority of thymocytes have nuclei (n) occupying most of the cell with the heterochromatin arranged mostly at the periphery. The cytoplasm shows few small vesicles of SER (thin black arrows) and mitochondria (dashed arrow). Notice the thymocytes with pyknotic nuclei (*).

× 3000.

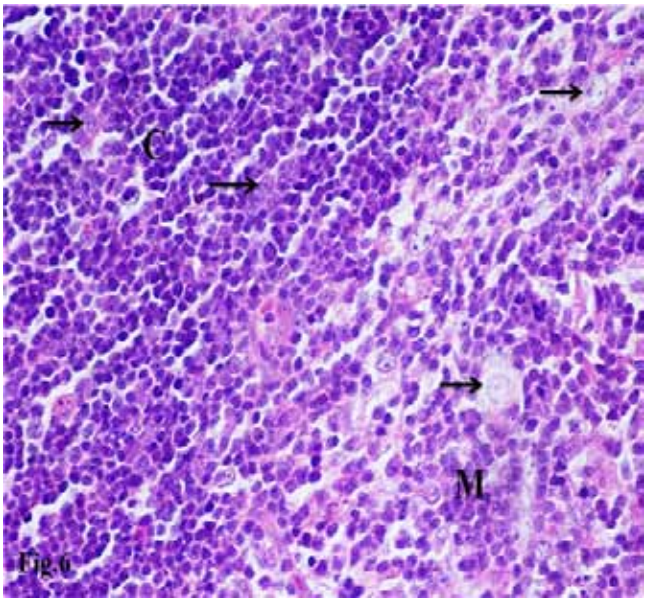

Figure 6. A photomicrograph of a section of the thymus of a rat (subgroup Ib) showing a well-organized structure. Notice the reticuloepithelial cells (arrows) in the cortex (C) and medulla (M).

H&E, × 600.

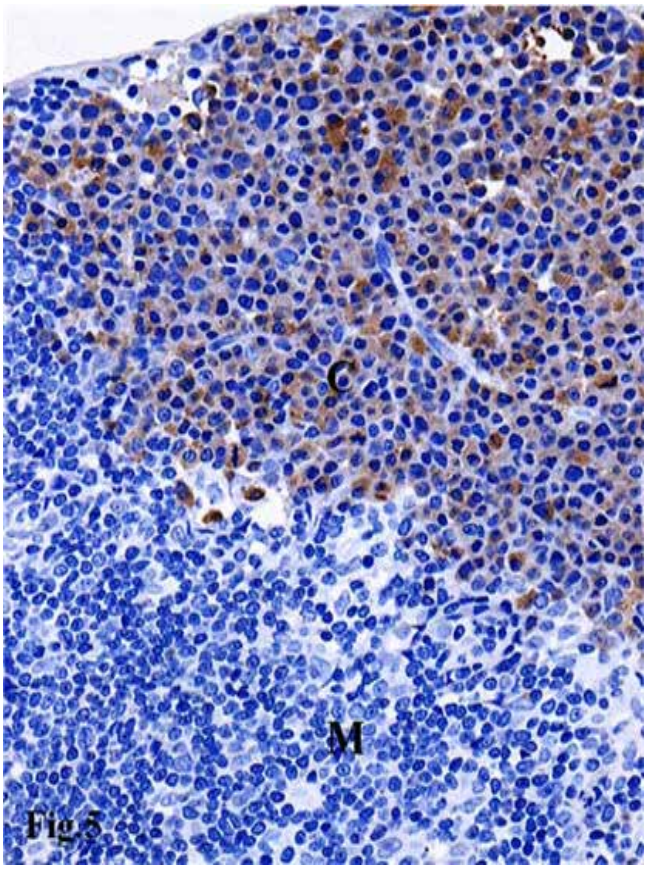

Figure 5. A photomicrograph of a section of the thymus of a rat (subgroup Ia) showing diffuse distribution of CD3 positively stained developing thymocytes (brown) in the cortex (C). M, medulla.

Immunostaining, × 400.

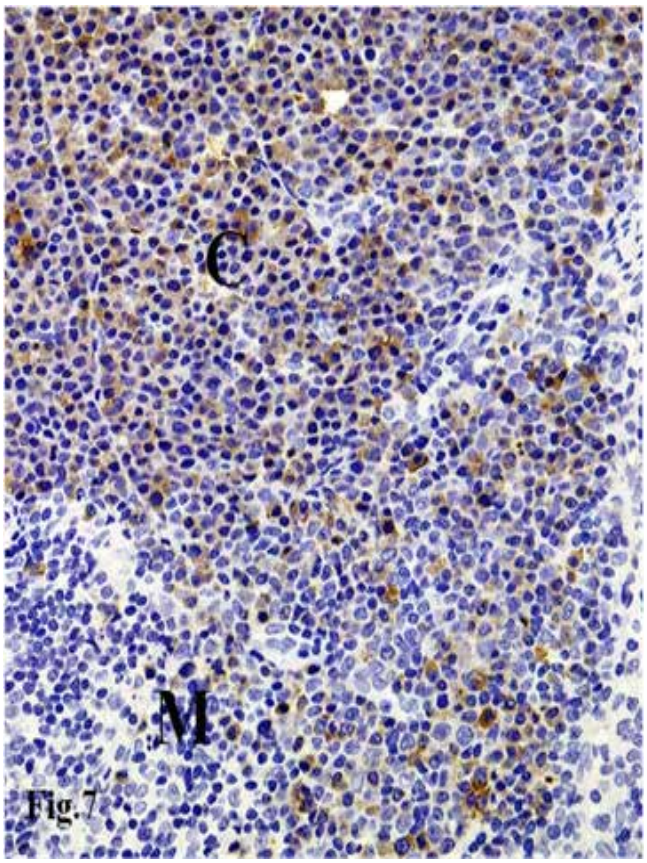

Figure 7. A photomicrograph of a section of the thymus of a rat (subgroup Ib) showing diffuse distribution of CD3 positively stained developing thymocytes (brown) in the cortex (C) and only some in the medulla (M).

Immunostaining, × 400.

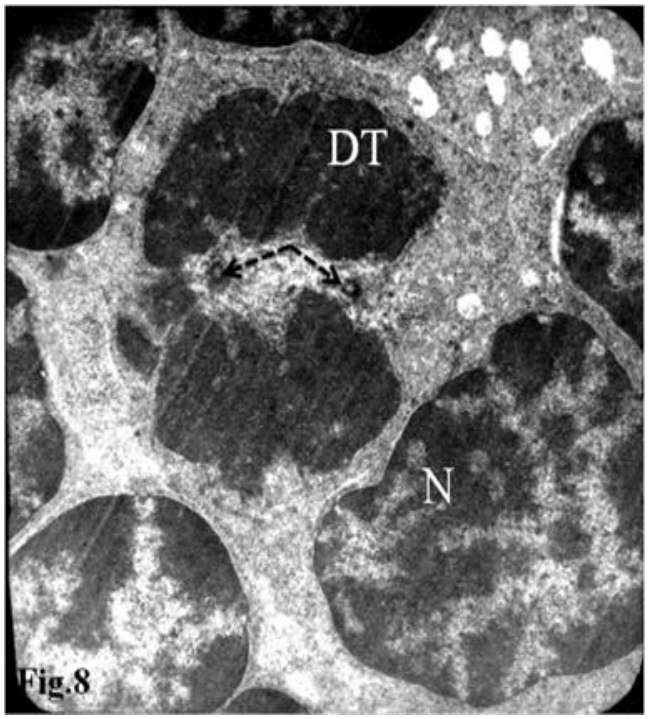

Figure 8. An electron micrograph of the thymus of a rat (subgroup Ib) showing a dividing thymocyte (DT) with two separate centrioles in the field (dashed arrows). Notice the large thymocytes (N).

× 6000.

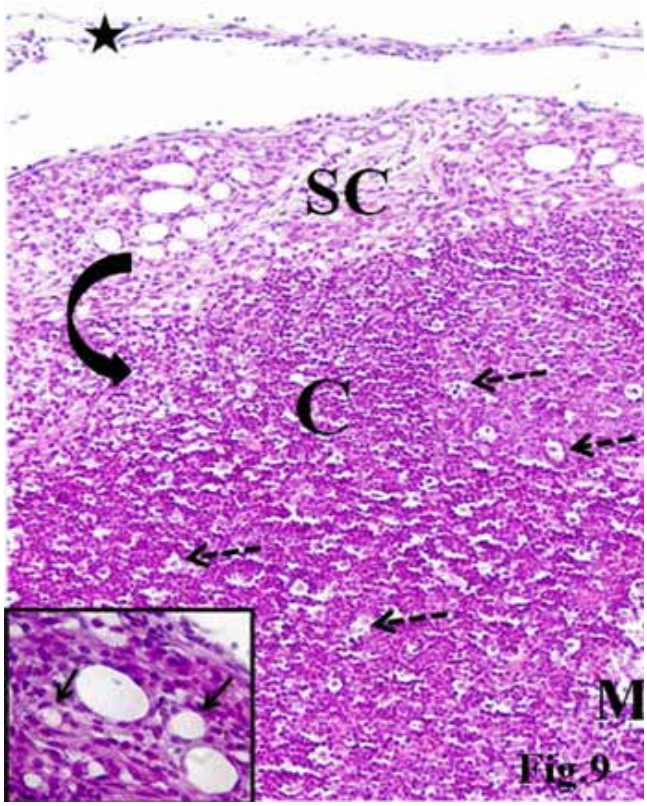

Figure 9. A photomicrograph of a section of the thymus of a rat (subgroup IIa) showing disturbed general architecture of the thymus. The subcapsular area (SC) is replaced by fibrous connective tissue containing fat cells encroaching deeper into the cortex (C) (curved arrow). Phagocytosed apoptotic bodies are seen dispersed in the cortex (dashed arrows). Connective tissue capsule (*); M, medulla. Inset: a higher magnification of the subcapsular area containing fat cells (arrows).

H&E, × 200; inset, × 1000.

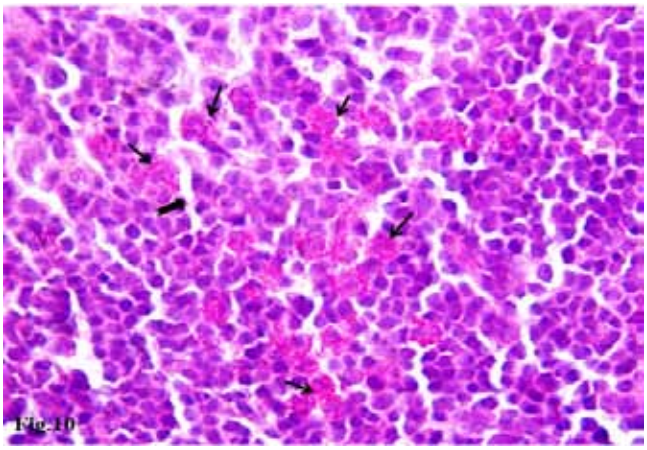

Figure 10. A photomicrograph of a section of the thymic cortex of a rat (subgroup IIa) showing rounded cells with acidophilic granular cytoplasm (thin arrows) located around the blood capillaries (notched arrow).

H&E, × 1000.

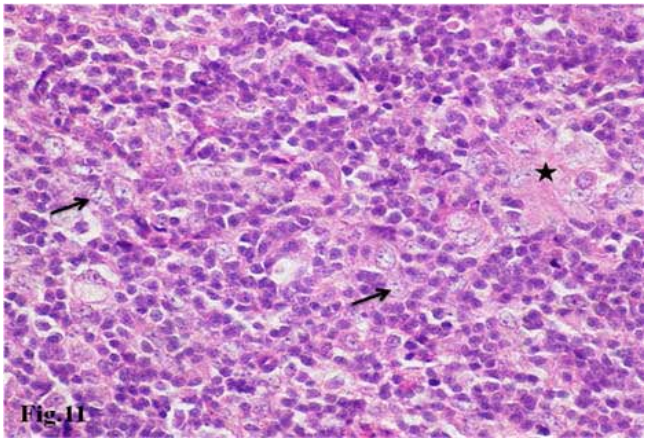

Figure 11. A photomicrograph of a section of the thymic medulla of a rat (subgroup IIa) showing numerous reticuloepithelial cells (arrows). Some of them form rosette-like structures (*).

H&E, × 600.

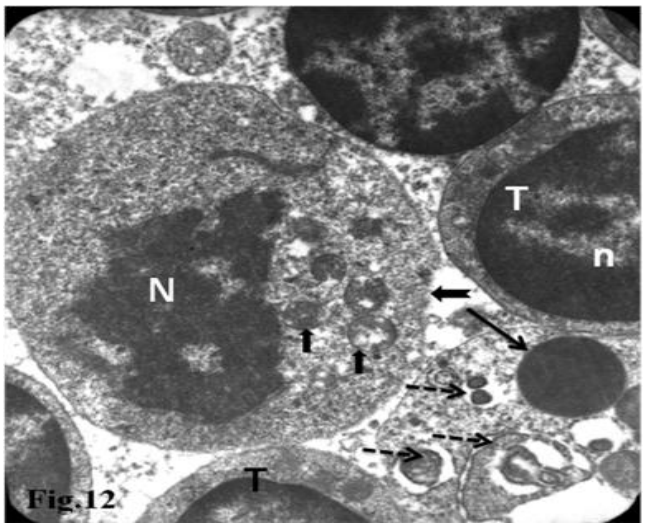

Figure 12. An electron micrograph of the thymic cortex of a rat (subgroup IIa) showing an apoptotic thymocyte with a fragmented nucleus (N) and disrupted mitochondria (arrows) and cell membrane (notched arrow). Other thymocytes (T) have rounded heterochromatic nuclei (n) and moderate amount of cytoplasm. Another cell shows many phagosomes (dashed arrows) and a osmophilic fat droplet (thin black arrow) inside its cytoplasm.

× 6000.

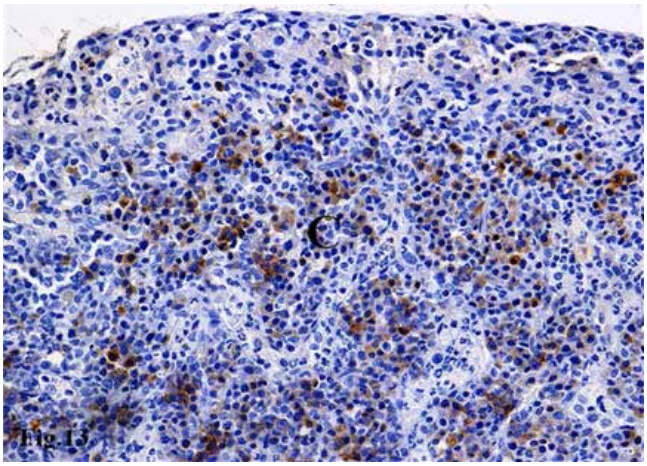

Figure 13. A photomicrograph of a section of the thymus of a rat (subgroup IIa) showing scattered patchy distribution of CD3 positively stained thymocytes (brown) mainly in the cortex (C).
Immunostaining, × 400.

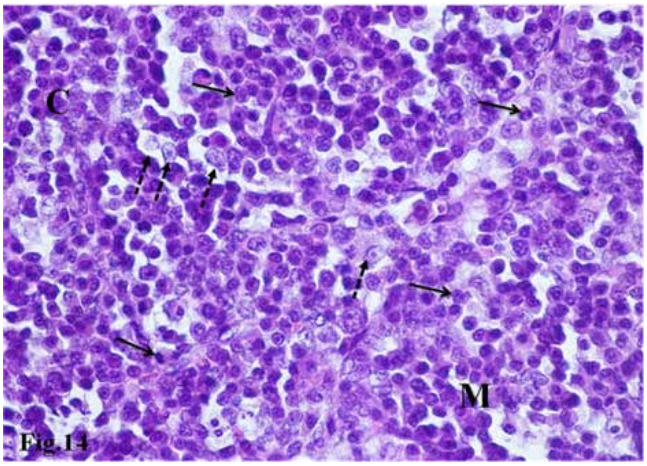

Figure 14. A photomicrograph of a section of the thymus of a rat (subgroup IIb) showing thymocytes at different stages of mitosis (arrows). Notice the many palely stained reticuloepithelial cells (dashed arrows). C, cortex; M, medulla.
H&E, × 600.

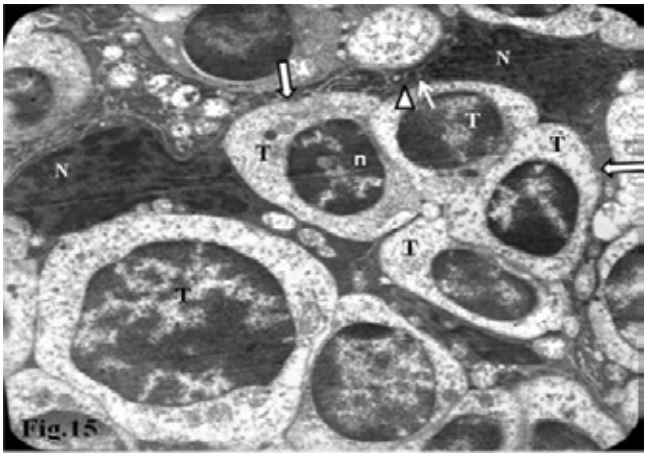

Figure 15. An electron micrograph of the thymic deep cortex of a rat (subgroup IIb) showing dark reticuloepithelial cellss having electron-dense cytoplasm and irregular nuclei with coarse clumps of heterochromatin (N). They possess long, thin, tightly fitting cytoplasmic processes (thick white arrows) rich in numerous secretory vesicles filled with electron-dense or translucent material (thin white arrow and arrowhead, respectively). The processes surround a group of thymocytes (T) with heterochromatic nuclei (n) and abundant cytoplasm.
× 4000.

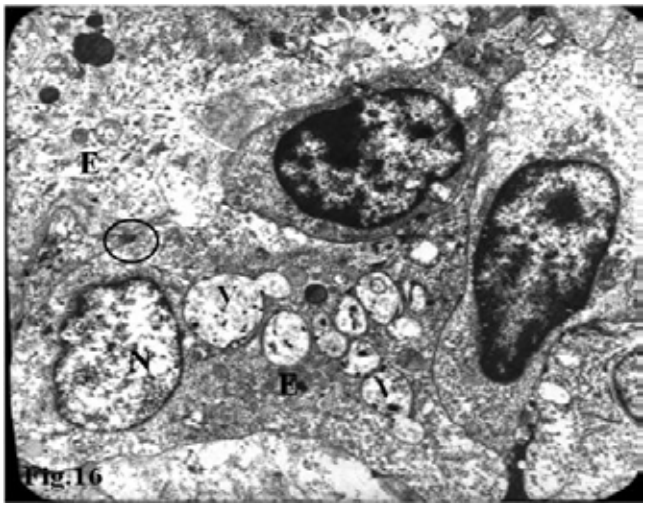

Figure 16. An electron micrograph of the thymic medulla of a rat (subgroup IIb) showing reticuloepithelial cells (E) joined by desmosomes (*) The cytoplasm shows a euchromatic nucleus (N) and many vesicles containing granular material and electron-dense bodies (V).
× 4000.

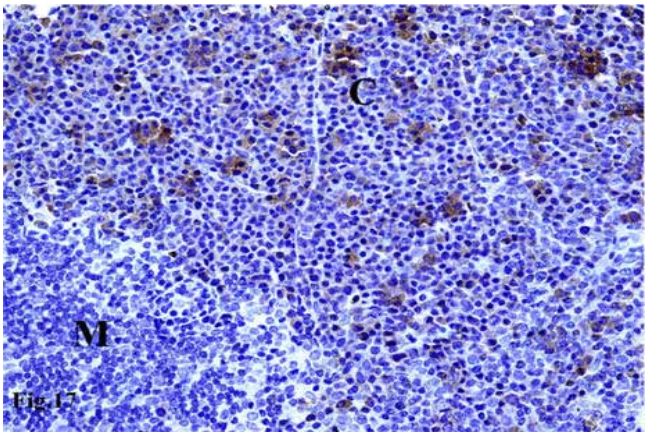

Figure 17. A photomicrograph of a section of the thymus (group IIb) showing groups of CD3-positive thymocytes in the cortex (C). M, medulla.
Immunostaining, × 400.

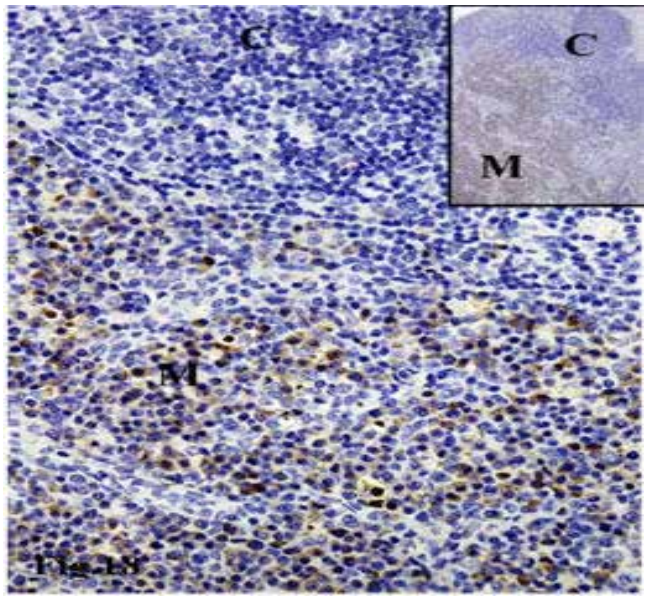

Figure 18. A photomicrograph of a section of the thymus (group IIb) showing CD3-positive developed T cells mainly in the medulla (M). C, cortex. Inset: a lower magnification.
Immunostaining, × 400; inset, × 100.

Table 1. The number of cortical and medullary reticuloepithelial cells and CD3 positively stained thymocytes in the four groups

	Subgroup Ia	Subgroup Ib	Subgroup IIa	Subgroup IIb	*P* value
Cortical reticuloepithelial cells	16.00 ± 3.71	21.40 ± 3.43^{*a}	24.30 ± 2.71^{**a}	38.30 ± 5.12^{**a,b,c}	0.001
Medullary reticuloepithelial cells	14.50 ± 2.75	19.40 ± 2.75^{*a}	25.40 ± 3.94^{**a}	43.50 ± 6.46^{**a,b,c}	0.001
CD3 positively stained thymocytes	211.90 ± 6.70	351.20 ± 13.24^{**a}	154.50 ± 7.82^{**a}	398.70 ± 13.97^{**a,b,c}	0.001

Statistical analysis is carried out using analysis of variance test.
Values are expressed as mean ± SD.
[a]In comparison with group Ia.
[b]In comparison with group Ib.
[c]In comparison with group IIa.
[*]Significant, $P<0.05$.
[**]Highly significant, $P<0.001$.
NS, P>0.05.

Discussion

The duration of the experiment was determined on the basis of the hypothesis of other investigators that mature single positive T lymphocytes spend ~12 days in the medulla before being exported from the thymus [25].

Chronic thymus involution resulted in less efficient T-cell development and decreased immigration of naive T cells to the periphery. The thymic tissue was plastic, and the involution process might be therapeutically reversed [9].

The present study revealed that the thymus of subgroup Ia was formed of a dark outer cortex that was densely populated with thymocytes and an inner paler medulla that was less densely populated. Some cells were in the process of mitotic division, whereas other cells showed apoptotic changes. This was in agreement with the results of previous studies. The researchers proved that the thymus did not grow in size or cell number, despite the disparity between the number of T cells generated and leaving the thymus daily. This was because ~98% of the thymocytes that develop in the thymus also die within the thymus. No damage was seen, indicating that death mostly happened by apoptosis [26,27].

Moreover, the thymus contained six types of REC widely distributed in the cortex and medulla [28]. In the H&E sections in the present study, RECs appeared with large pale nuclei and acidophilic cytoplasm. In electron microscopic sections, some RECs had pale nuclei with granular chromatin and a nucleolus. The smooth endoplasmic reticulum observed was reported to be important in the process of steroid hormone synthesis and calcium sequestration, which regulate T-cell function [39]. These cells also had cytoplasmic processes. At the corticomedullary junction, the REC had a nucleus with many clumps of heterochromatin. Their bodies and cytoplasmic processes were electron dense.

In subgroup Ia, the upper cortical RECs involved in the thymocyte repertoire appeared stellate-shaped with cytoplasmic processes. It was reported that thymic cell education is characterized by the expression and deletion of specific surface CD antigens in both the cortex and medulla. During this process of maturation, T cells express T-cell receptors (TCR), CD3, CD4, and CD8 [28]. Thymocytes that express TCRs undergo positive selection in the thymic cortex. It involves a contact between the receptors (TCR) of developing T cells and cortical RECs expressing both of the major histocompatibility complex (MHC) molecules MHCI and MHCII [30].

In subgroup Ib of the present study, *N. sativa*-treated thymi showed a significant increase in the number of CD3-positive cells and RECs, indicating hyperactivity of the gland. Moreover, dividing thymocytes were seen in EM sections.

In addition, an increase in lymphocytic count and cell-mediated immunity was recorded in cases of rabbit hemorrhagic disease virus treated with *N. sativa* [31].

In subgroup IIa of the present study, signs of thymic involution appeared in the form of disturbed architecture with the presence of fibrous tissue containing fat cells in the cortex. Moreover, a significant decrease in the number of CD3-positive cells was detected. Previous findings showed that the involution of the thymus in humans is not linear and that the initial stages characterized by lymphocyte depletion began in the cortex [32].

In addition, the apparent deposition of fat cells observed in the cortex was in accordance with other results in which gradual deposition of fat/adipose tissue between the lobules of the thymus was observed with advancing age [33]. Moreover, it was reported that there were dual components to the thymus: the true thymic epithelial spaces in which thymopoiesis occurs and the nonepithelial, nonthymopoietic perivascular spaces (adipocytes and stroma) that did not harbor thymopoiesis. The expansion of adipocytes and stroma with age resulted in a decrease in the output of T cells to the periphery. In addition, RECs and adipocytes produce IL6, which resulted in rapid gland involution [9].

In the present study apoptosis of thymocytes was evident in group IIa by light and electron microscopy. Apoptotic bodies, electron-dense granules, and lysosome-like bodies were seen in the cytoplasm of some cells. Some investigators observed the presence of vacuoles and cystic inclusions inside the cytoplasm of some RECs. They suggested that these vacuoles might contain degenerated material, which was the result of thymocytes being destroyed by reaction with self-antigens as a result of some factors released from RECs [34]. In contrast, researchers reported the presence of apoptotic changes

in thymocytes and apoptotic bodies inside macrophages described as tingible body macrophages [26,27].

The same group of researchers also revealed a significant increase in the number of RECs. This was similar to the studies of other investigators who declared that there was an age-related alteration in RECs in the form of hypertrophy and hyperplasia. The RECs were sometimes arranged in cords or ribbons and this was considered characteristic of a structural and functional adaptation [26,35].

The present work revealed the presence of large rounded cells with acidophilic granules along the capillaries of involuted thymi in subgroup IIa. Other investigators designated them as CI cells and added that these cells were not RECs because of a negative reactivity to anti-keratin antibodies. These cells contained thymulin granules that had an enhancing effect on thymocytes. Their number increased in the involuted thymi [1].

The renovation of the thymus in response to the *N. sativa* was obvious in group IIb. *N. sativa* treatment resulted in a significant increase in the total number of CD3-positive cells in both the cortex and the medulla; however, such cells were mainly located in the medulla. Similarly, oral administration of *N. sativa* to rats subjected to irradiation produced significant regeneration of the depleted thymus [36].

The CD3 antibody marks lymphocytes that carry TCR in their membranes. Other researchers explained that the CD3 complex is integral for relaying TCR signals; thus, a decrease in the number of CD3 molecules would affect the ability of T cells to respond to such TCR-dependent signals and hence impair thymopoiesis [37]. Therefore, it appeared that *N. sativa* intake assisted the progression of T cells to final maturation [31].

The number of RECs in subgroup IIb was significantly increased, and the medullary cells showed multivesicular vacuoles that were considered by some authors as evidence of hormonal secretion [4]. In addition, it was confirmed that distinct populations of RECs differ in their capacity to synthesize thymic hormones thymulin, thymosin, thymopoietin, macrophage colony-stimulating factor, stem cell factor, IL1, IL3, IL6, IL7, and humoral factors that regulate thymopoiesis and involution [38]. Moreover, it was reported that *N. sativa* contained thymoquinone and dithymoquinone, which had immunomodulator effects on macrophages and T cells [39].

Furthermore, it was suggested that medullary RECs are major antigen-presenting cells that perform negative selection. This process takes place to remove self-reactive thymocytes bearing TCRs that strongly react with abundant thymic self-antigens [40].

It was reported by other investigators that RECs express thyroglobulin, insulin, and rennin [41]. The majority of rats that were treated with *N. sativa* oil for 4 weeks showed a 55% increase in the CD4 to CD8 T-cell ratio and a 30% increase in natural killer cell function. They also produced IL1 and IL8 [42].

Conclusion

The results of this study indicate that thymi of mature adult rats presenting with signs of involution were activated by oral administration of a petroleum ether extract of *N. sativa* for 15 days. It appeared that the 15-day duration influenced and activated the thymocytes and RECs involved in the positive and negative selection of developing thymocytes.

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Conflicts of interest

There is no conflict of interest to declare.

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الملخص العربى

تأثير حبة البركة على الغدة السعترية في الجرذ الصغيرو الجرذ البالغ: دراسة هستولوجية و هستوكيميائية مناعية ومورفومترية

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المقدمة: إن البيئة المحيطة بالغدة السعترية تلعب دورا مركزيا في تمايزالخلايا السعترية. و يبدأ ضمور الغدة في وقت مبكر نسبيا في الحياة، مما يؤدي إلى نقص المناعة. تعرف حبة البركة بتأثيرها المنشط للجهاز المناعي.

الهدف: كان الهدف من هذه الدراسة هو إلقاء الضوء على تأثير حبة البركة على تركيب الغدة السعترية في الجرذان الصغيرة والبالغة.

المواد والطرق: تم تقسيم 28 من ذكور الجرذان البيضاء إلى مجموعتين. و قد قسمت المجموعة الأولى الي مجموعتين فرعيتين أ و ب و اشتملت المجموعة الفرعيةالاولي أ: سبعة جرذان صغيرة تزن من 50-60جم (عمرها شهر واحد) و قد اعطيت الماء العادي. و اشتملت المجموعة الفرعيةالاولي ب علي سبعة جرذان من نفس العمر و الوزن و تم اعطائها 2 مل لكل كيلو جرام من ٪1 من مستخلص اثير البترول من حبة البركة عن طريق الفم لمدة أسبوعين. و قد قسمت المجموعة الثانية الي مجموعتين فرعيتين أ و ب و اشتملت كل مجموعة فرعية علي سبعة جرذان بالغة ناضجة تزن من 600-650جم (عمرها12 شهرا). و قد تم اعطاءالمجموعة الفرعيةالثانية أ الماء العادي بينما أعطيت المجموعة الفرعية الثانية ب2 مل لكل كيلو جرام من ٪1 من مستخلص اثير البترول من حبة البركة عن طريق الفم لمدة أسبوعين تم أخذ عينات من الغدة السعترية من كل المجاميع للدراسة الهستولوجية و هستوكيميائية مناعية والمورفومترية.

النتائج: أظهرت نتائج المجموعة الفرعية (1 ب) أن اعطاء حبة البركة للجرذان يؤدي الي زيادة ذات دلالة في عدد الخلايا الايجابية التفاعل ل CD3 في القشرة التي امتدت الي اللب و في المجموعة الفرعية (2 أ) تظهر علامات الانكماش في الغدة في شكل تمدد النسيج الضام حول الشعيرات الدموية و يحتوي هذ ا النسيج علي خلايا دهنية مع حدوث نقص ذو دلالة في عدد الخلايا الايجابية التفاعل مع CD3 كما لوحظ وجود خلايا (س أ) في الغدة الضامرة حول الشعيرات الدموية.أما في حيوانات المجموعة الفرعية (2 ب) التي أعطيت حبة البركة فقد ظهر النسيج الضام و خلايا القشرة و اللب في صورة طبيعية مع زيادة الخلايا الايجابية التفاعل مع CD3 و زاد النشاط الافرازي للخلايا الظهارية الشبكية.

الخلاصة: إن تناول مستخلص اثير البترول من حبة البركة عن طريق الفم فى الجرذان البالغين الناضجين قد عزز من نشاط الخلايا الظهارية الشبكية و التوتية فى الغدة السعترية المنتكسة وخاصة بعد مرور 15 يوما.