Original Article

The Possible Protective Role of Barley Seeds on the Spleen after Administration of Glucocorticoids in Adult Albino Rats: A Histological and Immunohistochemical Study

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Abstract

Background: Glucocorticoids (GCs) are the main treatment strategy in many autoimmune disease and inflammatory diseases; however, they have immunosuppressive effect on many organs. The barley seeds contain many antioxidant compounds, which may improve the antioxidant status and related physiological functions. Our aim in this work is to evaluate the possible protective role of barley seeds on some immune cells in the spleen against immunosuppressive effect of GCs in adult albino rats. **Materials and Methods:** Forty-five adult albino rats were equally divided into 3 groups. Group I: normal vehicle control (n = 15), Group II: steroid-treated animals (n = 15), and Group III: steroid/barley-treated group (n = 15). Specimens from spleen were processed for light and electron microscopy. **Results:** In steroid-treated group, the histological changes in white and red pulp were in the form of loss of architecture and wide empty spaces among the cells. Most of the cells showed degenerative change, dilatation of blood sinusoids, and deposition of fibrinoid material among the cells of the RP. However, multiple lysosomal bodies were observed in both dendritic and macrophage cells. These changes are improved in steroid/barley-treated group in the form of increasing the number and size of the lymphatic follicles. Most of the splenic cells regained normal structure. Dendritic cell marker CD86 and macrophage marker CD68 expression are increased. **Conclusion:** Barley protects the spleen tissues from steroid-induced structural changes; this could be mediated through its antioxidant effects, so barely is recommended as a healthy diet in patients consuming steroids.

Keywords: Barley, glucocorticoids, spleen, ultrastructural

Introduction

The immune system is a system of biological structures and processes within an organism that protects against disease.[1,2]

The spleen is the largest encapsulated lymphoid organ of human. It represents the most abundant accumulation of lymphoid tissues in the human body. In the spleen, white pulp (WP) is involved in adaptive immunity while the marginal zone is involved in both innate and adaptive immunity, through its specific macrophage populations and marginal zone B-cell.[3]

Glucocorticoids (GCs) are the most commonly used drugs and are widely used for the management of inflammatory diseases.[4,5] The successful therapeutic use of these drugs in a wide range of inflammatory diseases is, however, limited, as they have several adverse effects, especially immunosuppression; GCs exert their immunosuppressive, anti-inflammatory, and antiallergic effects on primary and secondary immune cells, tissues, and organs through genomic and nongenomic mechanisms.[6]

A high intake of dietary fiber is associated with several preventive medical effects in man and animals. Besides fruits and vegetables, whole grain products are the most important sources of dietary fiber. Grains are rich in different types of dietary fibers, most dietary fibers being polysaccharides; for example, beta-glucans (β-glucans), arabinoxylans, cellulose,

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and hemicellulose.[7] The feeding of β-glucans-rich diets resulted in several beneficial physiological effects.[8]

Barley is a rich source of dietary fiber, and possible immunomodulatory effects of barley polysaccharides might explain a potential protective effect.[9]

Different studies suggest that β-glucans are potent immunomodulators with effects on both innate and adaptive immunity.[10-12]

Therefore, this work is carried out to evaluate the protective role of barley seeds on some immune cells in the spleen against immunosuppressive effect that would occur under the influence of GCs in adult albino rats using histological, immunohistochemical, and ultrastructural studies.

Materials and Methods

Materials

Animals

A total number of 45 albino rats were used in this study; these animals were 2 months of age (average weight – 150–200g) and were housed in stainless steel cage under standard condition (light, temperature, and free access to food and water). Animal care and use were in accordance with procedures outlined in the National Institutes of Health Guideline. The experiment was approved by the committee of Medical ethics of Faculty of Medicine, Assiut University.

Animal groups

The animals were divided into 3 equal groups:

1. Group I (control): Animals were fed standard balanced diet
2. Group II (steroid-treated animals): Animals were daily injected with prednisolone in a dose of 20 mg/kg for 2 weeks[13] and fed with balanced standard diet
3. Group III (steroid/barley-treated group): Animals were injected with prednisolone in the same dose and fed with barley mixed with balanced standard diet for 2 weeks.[14] Dose of barley was 53% of available diet for each animal. Each rat had eaten orally 20 g/day, composed of balanced standard diet (50%) nearly 10 g and barley seeds (50%) about 10 g. The amount of barley seeds that had been eaten contains 0.991 proteins, 0.116 total lipids (fat), 7.772 carbohydrate, 1.56 fiber, 0.08 sugars, and 62.263 trace elements/10 g.[15]

Chemicals and reagents

- Prednisolone was purchased from Sigma-Aldrish (St. Louis, MO, USA). The drug was injected intramuscular
- Barley was purchased from the market
- Immunohistochemical kits
  - Primary antibody.
- Dendritic cell (DC) antibody (CD86 [B7-2]): It a mouse monoclonal antibody (Novus Biologicals, USA, catalog number NBP2-25208)
- Macrophage antibody (CD68): It is a mouse monoclonal antibody (Novus Biologicals, USA, catalog number NBP2-2940)
  - Detection system for antibody
- Power-stain™ 1.0 Poly HRP DAB Kit for Mouse + Rabbit (General Biotechnologies, USA, catalog number 94080).

Methods

For light microscope

Immediately after scarification of the animals, the spleen was dissected and fixed in 10% formalin. After fixation, specimens were dehydrated using a series of ascending grades of alcohols (70%, 90%, 95%, and absolute); then, cleared in methyl benzoate or xylol and finally impregnated and embedded in paraffin. Sections were cut at 3–5 um using a microtome, mounted on slides (all these steps are done in Histology Department, Faculty of Medicine), and subjected to the following techniques:

- Hematoxylin and eosin (H and E) staining for histological assessment
- Immunohistochemical staining for:
  1. Macrophage using antimacrophage antibodies CD68
  2. DC using anti-DC antibodies CD86.[16]

For electron microscopic

Specimens were fixed in 5% cold glutaraldehyde for at least 24 h then washed in 3–4 changes of cacodylate buffer (Ph 7.2) for 20 min in each change and postfixed in cold osmium tetroxide for 2 h. The specimens were washed in four changes 30 min for each. Dehydration was done using ascending grades of alcohol (30, 50, and 70) each for 2 h and then 90% and 100% two changes 30 min each. Embedded samples were kept in incubator at 35°C for 1 day, at 45°C for another day, and for 3 days at 60°C.[17] Then semithin sections (0.5–1 um) were prepared by using LKB ultramicrotome. The sections were stained with toluidine blue, examined in the light microscope, and photographed. Ultrathin sections (50–80 nm) from selected areas of the trimmed blocks were made and collected on copper grids. The ultrathin sections were contrasted with uranyl acetate for 10 min and lead citrate for 5 min.[18] Finally, the sections were examined and photographed in transmission electron microscope JEOL (JEM-100 CX11, Tokyo, Japan) and photographed in 80 KV in Assiut University-Electron Microscope Unit.

Morphometric studies

Computerized image analyzer system software (Leica Q 500 MCO; Leica, Wetzlar, Germany) connected to a camera attached to a Leica universal microscope at the Histology Department, Faculty of Medicine, Assiut University was used to measure the following:

- Area of lymphoid follicle/total area in the examined field in the field of the spleen in different groups was measured in H and E sections at magnification of ×40 times (×4 objective times ×10 ocular magnification
- Number of lymphatic follicles/field was measured using the touch count method in the different groups
- The mean number of dendritic immunopositive cells in the spleen sections stained with CD 86 was measured.

Note, DC/field was measured using the touch count method

- The mean number of macrophage immunopositive cells in the spleen sections stained with CD 68 was measured. Macrophage/field was measured using the touch count method.[19]

Six nonoverlapping fields in ten randomly chosen sections from three different animals for each group were used for each measurement.

Statistical analysis

For statistical analysis, the statistical package for the social sciences (SPSS for windows Version 16; SPSS Inc., Chicago, Illinois, USA) was used. The data were analyzed using one-way analysis of variance test, followed by the *post hoc* test (least significant difference) to compare various groups with each other. Results were expressed as means ± standard deviation. The level of significance was expressed as $P < 0.05$.

Results

Histological results

In Group I (control group), examination of sections of spleen stained with H and E showed that the spleen was surrounded by a capsule composed of dense fibrous tissue and trabeculae extended to the parenchyma, the WP, and the red pulp (RP) [Figure 1a]. The WP is composed of lymphoid follicles; each had an eccentrically located arteriole, which was surrounded by a periarterial lymphatic sheath. A marginal zone demarcated the splenic lymphoid follicle from the RP [Figure 1b]. The cells within the WP are variable in size, shape, and density of the nucleus; small lymphocytes have dense nuclei with thin rim of cytoplasm while large lymphocytes appear lightly stained with vesicular nuclei with the presence of central arteriole [Figure 1c]. The RP contained blood sinuses and splenic cords; megakaryocyte was observed in RP [Figure 1d]. In Group II (steriod-treated group), examination of sections of the spleen of Group II stained with H and E revealed degenerative changes in the form of shrinkage of the lymphatic follicles [Table 1 and Graph 1] and decreased cellularity in the follicles. The number of the lymphatic follicles was markedly decreased in comparison to those of the control group [Table 2 and Graph 2]. The WPs of the spleen showed loss of architecture and wide empty spaces among the cells [Figure 2b]. While in the RP, deposition of fibrinoid material inside the blood sinusoids and among the cells could be seen with marked dilatation of blood sinusoids [Figure 2c]. Hemosiderin deposition was observed in the cytoplasm of many cells [Figure 2d]. In Group III (steroid/barley-treated group), examination of sections of the spleen of Group III stained with H and E revealed preservation of the morphological structure of the spleen either in white or RP compared with that of steroid-treated group. The diameter of lymphatic follicles was more or less similar to that of the control group [Table 2 and Graph 1]. The number of lymphatic follicles in the WP was considerably increased [Table 2 and Graph 2]. The architecture of lymphatic nodules was more or less like normal in the form of increased cellularity with the appearance of germinal center in the nodule. A marginal zone demarcated the WP from the RP [Figure 3b]. Variability in size, shape and density of the nuclei of the cells within the WP with the presence of central arteriole could be detected [Figure 3c].

Examination of the semithin sections of the WP of Group I showed many small lymphocytes, with condensed nuclear

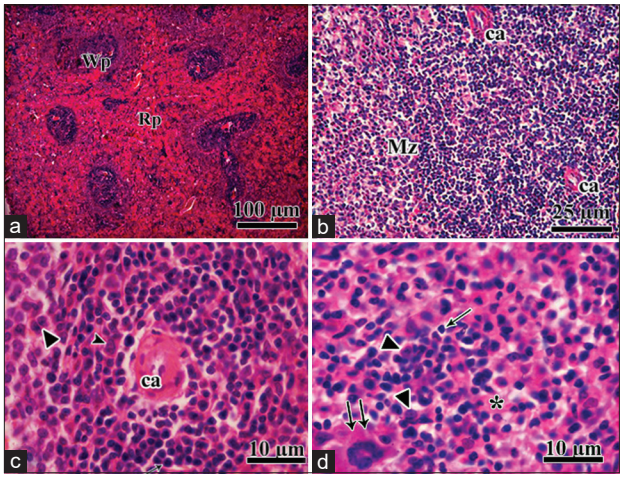


Figure 1: Photomicrographs of Group I stained with H and E: (a) White pulp and red pulp. (b) Lymphatic follicle and central arteriole of the white pulp. A marginal zone. (c) The cells of the white pulp include small lymphocytes with dense nuclei and thin rim of cytoplasm (arrow), large lymphocytes appear lightly stained with vesicular nuclei (triangle), central arteriole. (d) Red pulp. Splenic cords contain small (arrow) and large lymphocytes (triangle) and red blood cell (star). The splenic cords are separated by the blood sinusoids. Large cell with lobulated nuclei is also observed (double arrow)

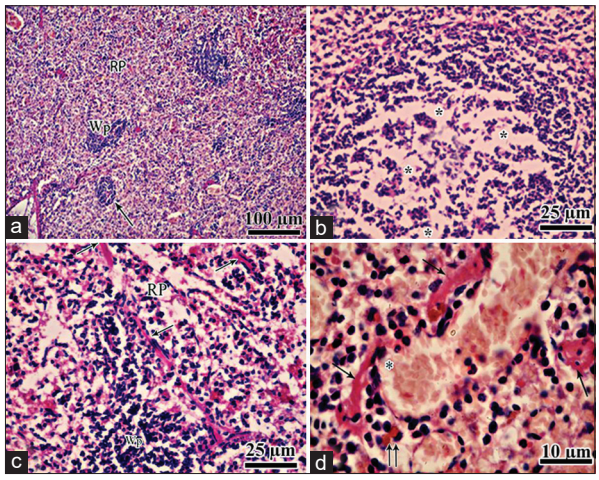
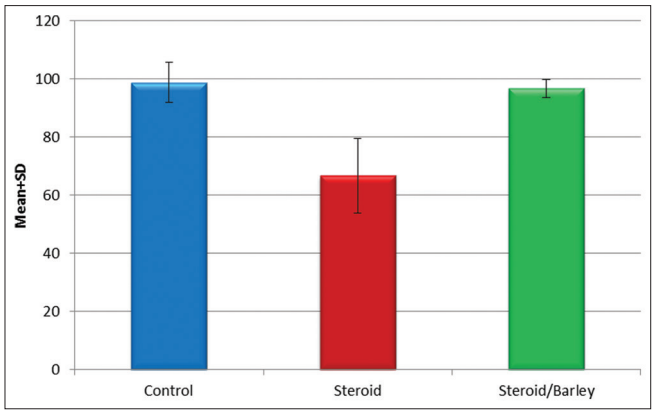
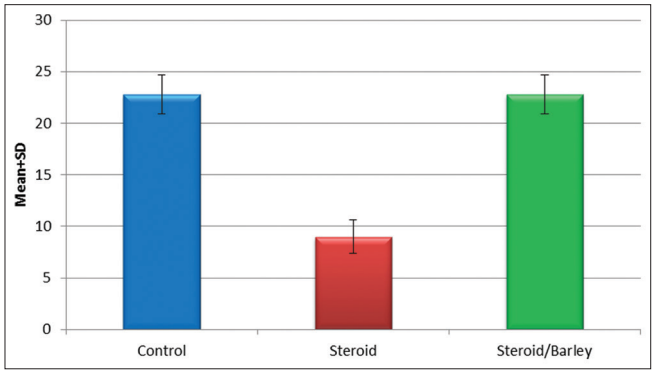


Figure 2: Photomicrographs, of Group II stained with H and E: (a) decrease number and size of lymphatic follicles (arrow). (b) Loss of architecture of lymphatic follicles. Note: wide empty spaces cells in the core of white pulp (star). (c) Loss of architecture of red pulp. Note: the presence of fibrinoid material inside the blood sinusoids and among the cells of the red pulp (arrow). (d) dilatation of blood sinusoids and fibrin deposition adjacent to the blood sinusoid (arrow) note deposition of hemosiderin pigments loaded cells (double arrow)



Graph 1: The mean value + standard deviation of diameter of lymphatic follicles of the spleen



Graph 2: Mean value + standard deviation of the number of lymphatic follicles of the spleen per total area examined

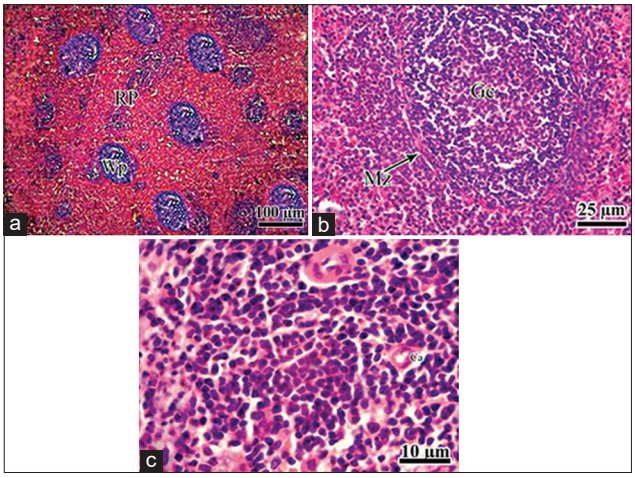


Figure 3: Photomicrographs of Group III stained with H and E: (a) the parenchyma of the spleen (white pulp + red pulp). Note: average number of lymphatic nodules. (b) increase cellularity with appearance of germinal center in the nodule. A marginal zone demarcated the white pulp from the red pulp. (c) variability in size, shape and density of the nuclei of the cells within the white pulp. Note: the presence of central arteriole

chromatin, surrounded by thin rim of cytoplasm. Large lymphocytes with a large euchromatic (pale) nuclei and central arteriole with its prominent internal elastic lamina were observed [Figure 4a]. Semithin section of WP of Group II showed many apoptotic bodies. Some cells show dense irregular nuclei. Empty wide spaces were present among the cells [Figure 4b]. In Group III, the splenic architecture was more or less similar to those of control in the form of appearance of small lymphocytes with dense nuclei and thin rim of cytoplasm. Large lymphocytes lightly stained with vesicular nucleus were observed; the central arteriole with its prominent internal elastic lamina could be observed [Figure 4c].

Immunohistochemical results

The expression of the DC antibody (CD86) in Group I revealed positive reaction in the cytoplasm of the DC in the form of dark brown granules. Their number per mm^2 ranged from 4 to 5.5 [Figure 5a, Table 3 and Graph 3]. While in Group II, there was decreased intensity of the reaction in the cytoplasm of DC compared to that of the control group; and their number per mm^2 significantly decreased compared to Group I, i.e., it ranged from 1 to 2.6 [Figure 5b, Table 3 and Graph 3]. In Group III, the expression of the CD86 antibody was increased in the cytoplasm of DC and number per mm^2 significantly increased compared to group, i.e., it ranged from 3.4 to 5 [Figure 5c, Table 3 and Graph 3].

Macrophage antibody (CD68) was highly expressed in the cytoplasm of macrophage cells in Group I in the form of dark brown granules. Their number per mm^2 ranged from 4 to 4.9 [Figure 6a, Table 4 and Graph 4] while in Group II, the expression was weak, and their number per mm^2 significantly decreased compared to Group I, i.e., it ranged from 1 to 2.9 [Figure 6b, Table 4 and Graph 4]. The intensity of the reaction was increased in the cytoplasm of macrophage cells in Group III, their number per mm^2 significantly increased compared to Group I, and it ranged from 3.3 to 5 [Figure 6b, Table 4 and Graph 4].

Electron microscopic results

The ultrastructure of Group I revealed small lymphocytes with condensed chromatin pattern nuclei, surrounded by a thin rim of cytoplasm. Large lymphocytes with large euchromatic nucleus, cytoplasm contained rough endoplasmic reticulum (RER) and smallrounded mitochondria, free ribosomes, and RER could be seen [Figure 7a]. Plasma cells with heterochromatic eccentric nuclei, numerous RER, and mitochondria were observed [Figure 7b]. Dendritic cell with electronlucent cytoplasm studied with electrondense granules, RER as well numerous mitochondria with characteristic many cytoplasmic processes could be noticed [Figure 7c]. Macrophage with irregular-shaped eccentric oval nucleus and numerous Golgi complex, lysosomal bodies, and mitochondria in their cytoplasm was observed [Figure 7d]. Megakaryocyte had voluminous cytoplasm which contained internal membrane system, numerous small dense granules, Golgi complex, and numerous mitochondria. The nucleus was lobulated, euchromatic with few clumps of heterochromatin [Figure 7e].

The ultrastructural examination of WP of Group II demonstrated many small lymphocytes with heterochromatic nuclei, dark

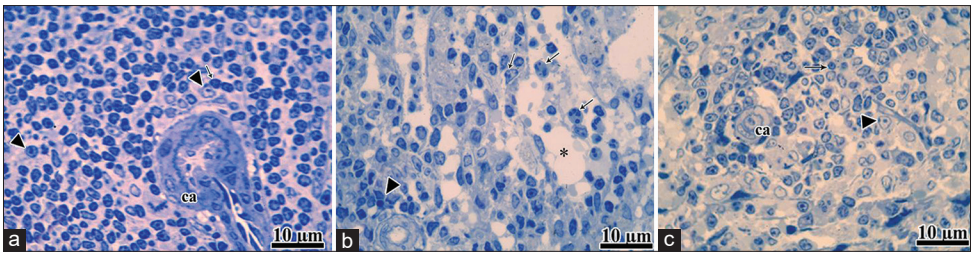


Figure 4: Semithin sections. Group I (a) white pulp stained with toluidine blue reveals small lymphocytes (arrow) and large lymphocytes (triangle) and central arteriole with its prominent internal elastic lamina. Group II (b) many apoptotic bodies (arrow). Some cells show dense irregular nuclei (triangle). Empty wide spaces were present among the cells (star). Group III (c) small lymphocytes with dense nuclei and thin rim of cytoplasm (arrow). Large lymphocytes appear lightly stained with vesicular nucleus (triangle). Central arteriole with its prominent internal elastic lamina

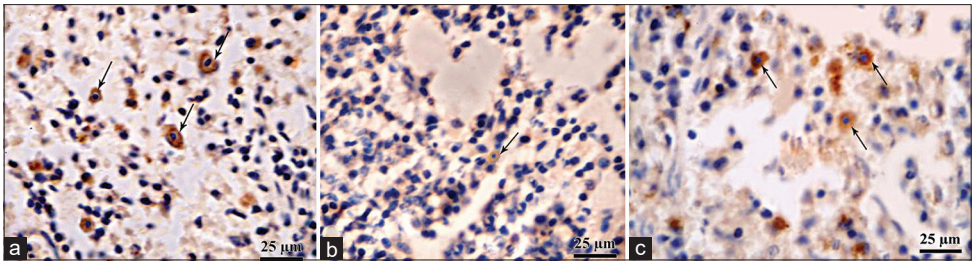


Figure 5: Photomicrographs, CD86 immunostained sections. Group I (a) positive reaction in the cytoplasm of the dendritic cell (arrow) in the form of dark brown granules. Group II (b) decrease in the intensity of the reaction in the cytoplasm of dendritic cell (arrow). Group III (c) positive reaction in the form of dark brown granules (arrow)

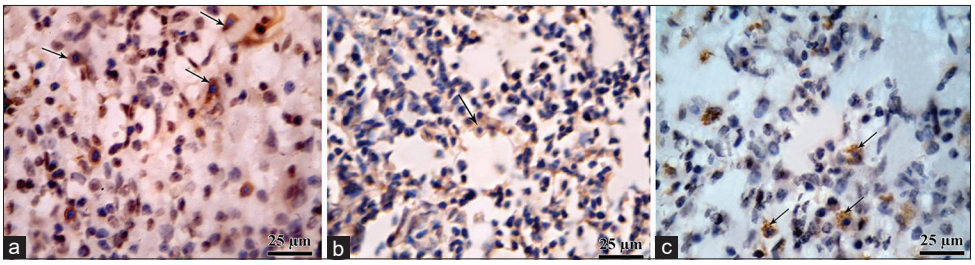
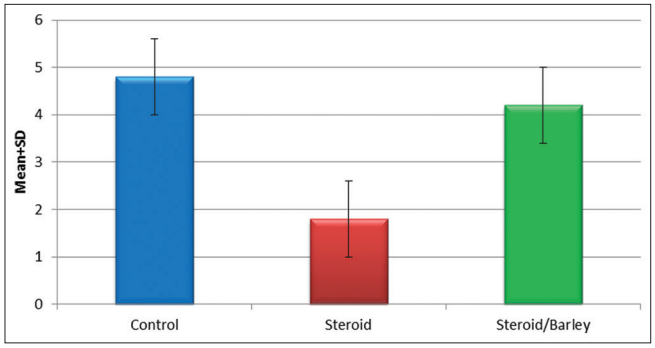


Figure 6: Photomicrographs, CD68 immunostained sections. Group I (a) positive reaction in the cytoplasm of macrophage (arrow) in the form of dark brown granules. Group II (b) decrease in the intensity of the reaction in the cytoplasm of macrophage (arrow). Group III (c) positive reaction in the form of dark granules (arrow)



Graph 3: Mean value + standard deviation of the number of dendritic cell in the spleen using CD86 per total area examined

cytoplasm, and dilated perinuclear cisternae. Empty spaces among the cells were observed [Figure 8a]. Plasma cells with eccentrically located nuclei and their cytoplasm were engorged with dilated RER [Figure 8b]. DC showed degenerative change in the form of numerous irregular vacuoles and slightly dilated RER [Figure 8c]. Macrophage with multiple lysosomal bodies with heterogeneous contents, many irregular vacuoles could be observed [Figure 8d]. Megakaryocyte had a large irregular nucleus with dilated perinuclear cisternae. Its cytoplasm contained many vacuoles and swollen destructed mitochondria [Figure 8e].

Electron microscopical examination of WP of Group III showed few empty spaces among the cells [Figure 9a]. Most of the plasma cells retained their normal structure [Figure 9b]. DC had a large nucleus with dense rim of heterochromatin. Circular Golgi region and abundant ribosomes can be noticed [Figure 9c]. Macrophage with eccentric nucleus and lysosomal bodies was observed [Figure 9d]. Megakaryocyte with lobulated nucleus, the cytoplasm was studied with huge number of granules and multiple demarcation channels could be seen. [Figure 9e].

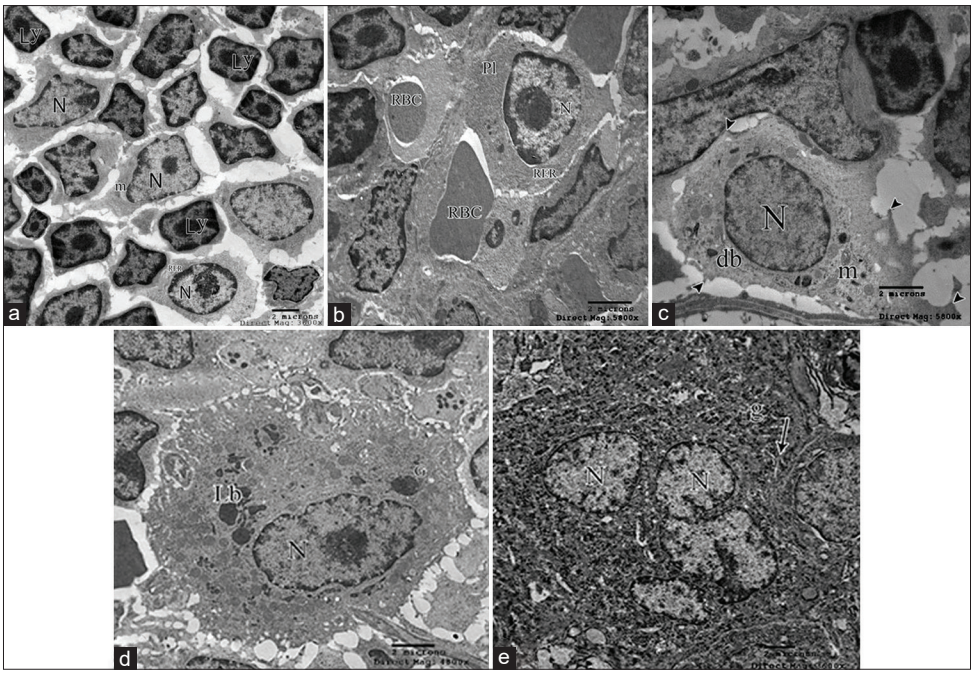


Figure 7: Electron micrographs, Group I. (a) White pulp. Small lymphocytes (Ly), their nuclei showed a condensed chromatin pattern, while large lymphocytes with large euchromatic nucleus (N), Note: Rough endoplasmic reticulum and mitochondria (m). (b) Red pulp. Red blood cell and lymphocytes. Plasma cell (Pl) nucleus (N) with rough endoplasmic reticulum and Golgi body (g). (c) Dendritic cell cytoplasmic processes (head arrow), mitochondria (m) and electron dense bodies. (d) Macrophage, oval eccentric nucleus (N), Golgi complex (g) and lysosomal bodies. (e) Megakaryocyte with internal membrane system (arrow) and numerous small dense granules (g). The nucleus (N)

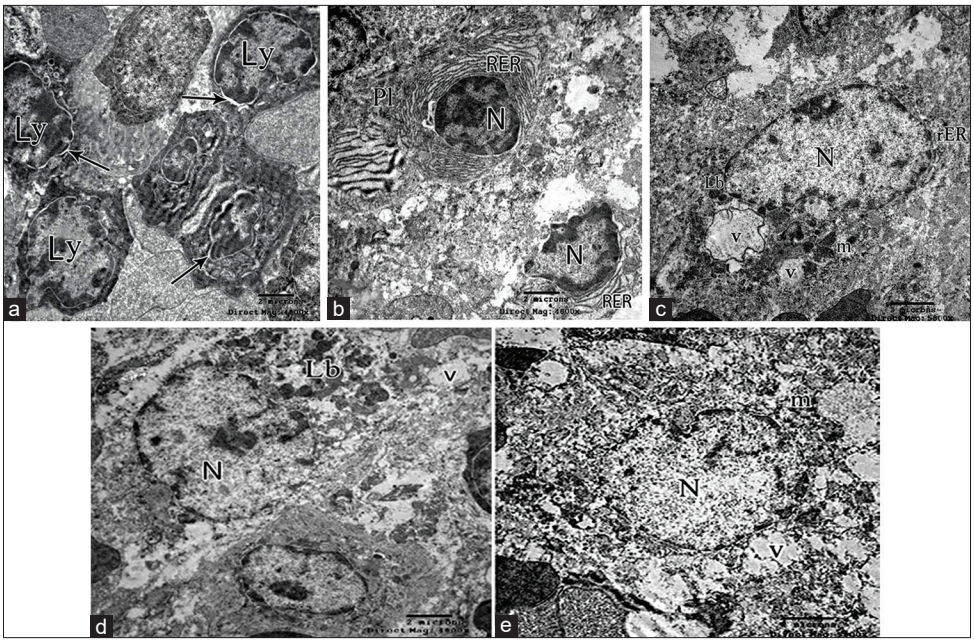


Figure 8: Electron micrographs of Group II. (a) Lymphocytes (Ly), their nuclei are surrounded by dilated perinuclear cisternae (arrow). (b) Plasma cells (Pl), with eccentric nuclei (N), and marked dilatation of rough endoplasmic reticulum. (c) Dendritic cell. Numerous irregular vacuoles (v) in the cytoplasm, slightly dilated rough endoplasmic reticulum and numerous lysosomal bodies. (d) Macrophage with ill-defined plasma membrane, large euchromatic nucleus (N), and multiple lysosomal bodies, vacuoles are also observed (v). (e) Megakaryocyte with large irregular nucleus (N) with dilated perinuclear cisternae, vacuoles and swollen destructed mitochondria (m)

Discussion

The present study demonstrated that prednisolone (one of GCs) induced marked degenerative changes in the spleen of the rats in favor of GC apoptotic effects. In accordance with our finding, Thomas and Bell[20] stated that GC induced cell size changes and nuclear fragility in rat thymocytes. It induces lymphocytolysis, with progressive appearance of pyknotic

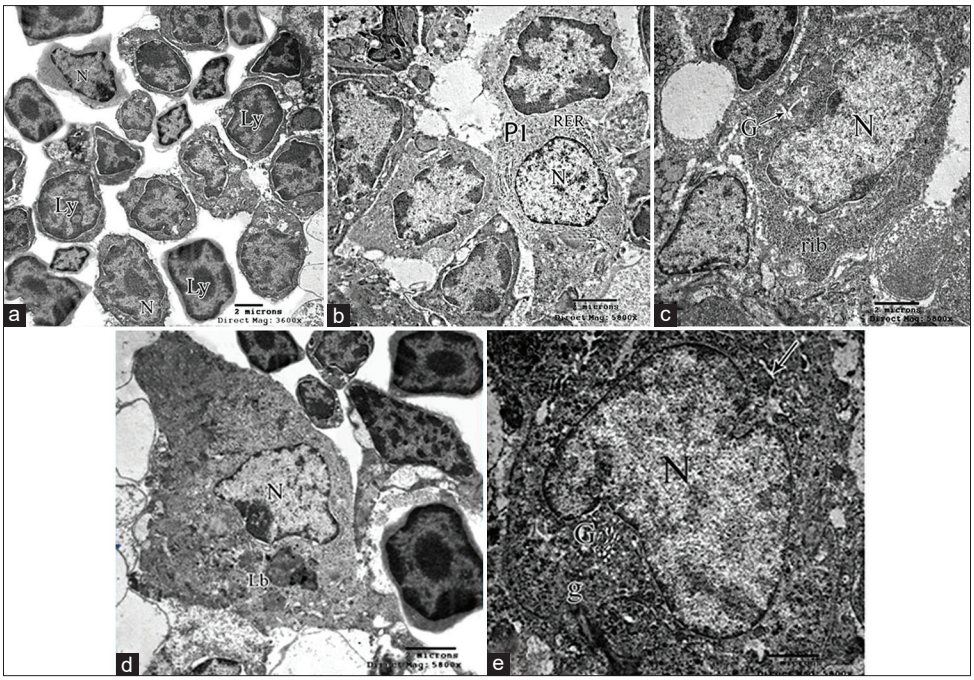
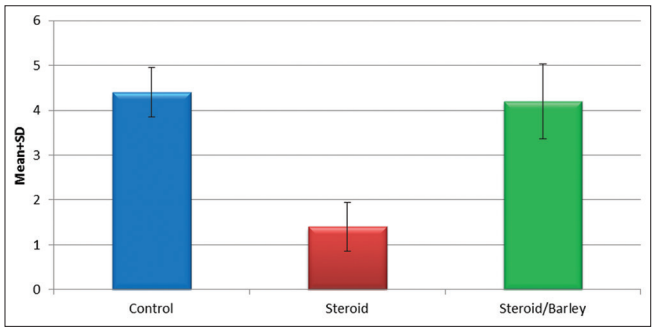


Figure 9: Electron micrographs of Group III. (a) Small lymphocytes (Ly) with nuclei a thin rim of cytoplasm. The Large lymphocytes with a large euchromatic nucleus (N). Few empty spaces could be seen among the cells. (b) Many plasma cell (Pl), with large eccentric nucleus (N) and well-developed rough endoplasmic reticulum. (c) Dendritic cell. The cytoplasm contains circular Golgi body (g) and abundant ribosomes (rib), large nucleus (N). (d) Macrophage with eccentric nucleus (N), and multiple lysosomal bodies. (e) Megakaryocyte with lobulated nucleus (N), granules (g), Golgi complex (g) and multiple demarcation channels (arrow)



Graph 4: Mean value + standard deviation of the number of macrophages in spleen using CD68\per total area examined

cells accompanied by increased nuclear fragility; it was dose dependent. The observed decreased cellularity of the follicles might be resulting from death of cells or sequestration of these cells as confirmed from apoptotic changes in lymphocytes in the present work; however, other cells were observed entrapped in blood vessels. These suggestions are raised after the work of some authors who found that acute effects of GC on lymphoid cells in man are probably caused by sequestration of the cells rather than by cell lysis although there is evidence that certain types of activated T lymphocytes are susceptible to GC-induced apoptosis.[21] Similar observation was reported by Rungruang *et al.*[22] who found that dexamethasone (Dex) injection induced apoptosis of cells in the spleen of mice. Others reported that GCs reduce spleen weight up to 31%.[23] Another study done in asthma mouse models used GC (either systemic or localized) treatment-induced thymic atrophy, also they demonstrate that GCs decrease T regulatory cells numbers and activity indifferent asthma mouse models, probably by reducing thymic production of T Cells.[24]

Other authors supported our finding and mentioned that long-term use of Dex could affect B-cell transformation by suppressing growth.[22] They reported that long term use of Dex without infection also causes defective spleen due to B-cell defects. The defective function of B lymphocytes might be reflected also on the plasma cell as observed in the present study, from the engorged RER cisternae in the cytoplasm of plasma cells due to defective and accumulated secretion as well as heterochromatic nuclei thus might be resulting in impairment of protein synthesis and antibody secretion by these cells due to degenerative effect of GCs on immune cells. These suggestions are supported by the work of McKay and Cidlowski[21] who mentioned that high doses of GCs might kill B-cells resulting in inhibition of immunoglobulin synthesis as well as decreased production of components of the complement system. However, Fauci[25] reported that GCs exposure induced differential degrees of humoral and cell-mediated immunity suppression in experimental animals.

Nevertheless, the observed dilated RER cisternae in plasma cells in the present work might be explained by excessive production and accumulation of protein; these changes might occur secondary to defective secretion. Cupps *et al.*[26] supported these suggestions and stated that the GCs favor antibody production by promoting the generation of immunoglobulin-secreting plasma cells. Plasma cells are

Table 1: Area of lymphoid follicles/total area in field examined

	Area of lymphoid follicle/total area in field examined	*P*
Control	98.8±6.9	A<0.001**
Steroid	76.7±12.9	B<0.001**
Steroid/barley	96.7±3.1	C=1.0 (NS)
		D<0.001**

**Statistically significant difference (*P*<0.01). A: Comparison between all groups, B: Comparison between control and steroid groups, C: Comparison between control and steroid/barley groups, D: Comparison between steroid and steroid/barley groups, Ns: No statistically significant difference (*P*>0.05)

Table 2: Number of lymphatic follicles of the spleen\ total area in field examined

	Number of lymphatic follicles of the spleen	*P*
Control	22.8±1.9	A<0.001**
Steroid	9.0±1.6	B<0.001**
Steroid/barley	20.4±1.9	C=1.0 (NS)
		D<0.001**

**Statistically significant difference (*P*<0.01). A: Comparison between all groups, B: Comparison between control and steroid groups, C: Comparison between control and steroid/barley groups, D: Comparison between steroid and steroid/barley groups, NS: No statistically significant difference (*P*>0.05)

Table 3: Number of dendritic cells in the spleen using CD86\per total area examined

	Number of DC in the spleen using CD86	*P*
Control	4.8±0.8	A<0.001**
Steroid	1.8±0.8	B<0.001**
Steroid/barley	4.2±0.8	C=0.279 (NS)
		D=0.001**

**Statistically significant difference (*P*<0.01). A: Comparison between all groups, B: Comparison between control and steroid groups, C: Comparison between control and steroid/barley groups, D: Comparison between steroid and steroid/barley groups, NS: No statistically significant difference (*P*>0.05), DC: Dendritic cell

Table 4: Number of macrophages in spleen using CD68

	Number of macrophages in spleen using CD68	*P*
Control	4.4±0.55	A<0.001**
Steroid	1.4±0.55	B<0.001**
Steroid/barley	4.2±0.84	C=0.640 (NS)
		D<0.001**

**Statistically significant difference (*P*<0.01). A: Comparison between all groups, B: Comparison between control and steroid groups, C: Comparison between control and steroid/Barley groups, D: Comparison between steroid and steroid/Barley groups, NS: No statistically significant difference (*P*>0.05)

responsible for humoral immunity in form of secretion of antibodies which are specific globulins produced in response to penetration by antigens. These antibodies are synthesized in RER.[27,28]

The observed destructed mitochondria in the present work might reflect GCs-induced oxidative stress and decreased function of most cell population of the spleen that might indicate a strong relationship between GC-induced apoptosis and mitochondrial function.[29]

The present study demonstrated that the number of macrophages was deceased. Thus, macrophages might be considered a target for the immunosuppressive effects of GC. In accordance with our finding, Russo-Marie[30] stated that resident tissue macrophages, which play a critical role in the immune responses, have been proposed as a target for the immunosuppressive effects of GC in rats. Most importantly, the observed GCs lymphocytic effects might be also secondary to those observed in macrophage; the later cells might exert regulatory effects on T lymphocytes and inhibit their mitogenic potential through cytokine secretion.

In the present work, in addition to the reduction of macrophages, DCs number was also significantly decreased in steroid-treated animals. In addition to the numerical affection of DCs, structural changes were also observed, in the form of degenerative changes and vacuolations. The observed low expression in CD 86 associated with more endocytic vacuoles in the present work suggesting DCs retarded antigen presenting capability but still have higher endocytic activity. In accordance with our result, Piemonti *et al*.[31] stated that GCs may act at the first step of the immune response by modulating DC differentiation, maturation, and function. Furthermore, Dex-DC showed a higher endocytic activity, a lower APC function, and a lower capacity to secrete cytokines than untreated cells. The observed lymphocytes degenerative changes in the present work might be secondary to detrimental effects of DC cells on lymphocytes; these suggestions revealed a new immunosuppressive mechanism of GC action, from inhibition of T cell-mediated terminal maturation by DC.

In our work, Pink fibrinoid material scattered among the cells of splenic cords of the RP and dilated blood sinusoids were observed. The observed dilatation of blood sinusoids in steroid-treated animals attributed to fibrin deposition in their walls and/or accumulation of cells and can obstruct arterial supply which could result in growth inhibition and spleen ischemia.[22] These materials might occur secondary to degenerative changes in histiocytes; similar observations were reported after Dex treatment in animals, thus resulting in lymphoid cells fragmentation (Lino Businco *et al*., 1961).

The observed orange pigment in the present work is most probably a hemosiderin pigment engulfed by macrophage. In accordance with this finding, Losco[32] reported that iron pigments (i.e., hemosiderin and ferritin) are the most common pigments in the macrophage of the RP. Moreover, Suttie[33] stated that hemosiderin pigment could be increased in hemolytic anemia or methemoglobinemia.

It is clear from the present study that megakaryocyte might be observed in normal animals which is documented in previous studies.[34,35] However, it could be also considered as an extramedullary hemopoeisis that occurring compensatory to the extensive degeneration occurring in the hemopoeisis organs after administration of steroid. These suggestions were in agreement with those reported in rats after myctoxin (immunosuppressed agent) treatments; thus, an extramedullary hemopoeisis might be resulting as a compensatory to the extensive degeneration occurring in the hemopoeisis organs.[36]

With concomitant barley administration, most of the splenic cells preserved their normal structure and the splenic follicles regained their regular architecture, except mild dilatation of blood sinusoids, and the appearance of specific and nonspecific granules in some granular leukocytic series. As well as, moderate dilatation of the RER of plasma cells which indicating recovery of the activity of the protein synthesis. Modulation of the immune response might play a significant role in maintaining a disease-free state.

The observed preservation with barley administration in the present study might be exerted through a complex polysaccharides known as B-glucan, which is one of the active compounds responsible for the immune effects of herbal products.[37] The antioxidant protective effect of glucan against H_2O_2 is responsible for DNA fragmentation. Immunomodulatory effects of barley also might involve in the amelioration of atrophic changes in the present work, either at the level of lymphoid series or antigen presenting cell as well as macrophage system; these suggestions are in accordance with those recorded after supplementation with β-glucan in the chicken, where improvement was observed in ultrastructural changes in macrophage and DCs as well as increased DCs and macrophage immune positive cells (Al-Terehi *et al*., 2012); Goodridge *et al.*[38] supported these suggestions and added that several receptors were expressed on the surface of immune cells, neutrophils, macrophages, and DCs; these receptors are capable of recognizing β-glucan in its various forms. This enhanced immune function is the result of increased pro-inflammatory cytokine production, oxidative burst, and chemokine production. The Bglucan immunomodulatory effects on DCs maturation may be the most important immunostimulant effects ,because mature DCs might induce either immunogenic Tcells or immunotolerogenic regulatory T cells (Tregs).[39]

Barley-extracted β-glucan might enhance macrophage in the present work; Weng *et al*., 2006 supported these opinions and added that ß-glucan has enhanced phagocytosis and bactericidal capability and upregulated the mRNA expression of dectin and survivin which may imply an expanded defense to the infection; barley β-glucans have also been shown to alter immune function of weanling pigs by increasing blood lymphocytes and the proportion of naïve T-cells.[39,40]

β-glucan markedly increased the cytokine production of DCs and surface expression of DC markers. In addition, DCs treated with β-glucan showed a higher capacity to stimulate allogeneic spleen cell proliferation compared to those treated with medium alone.[41] In accordance to our results, cell viability assay solution demonstrated that β-glucan significantly increased the viability of DCs; thus, β-glucan may enhance the survival of DCs and also helps maintain normal cell size.[42-44]

Conclusion

Barley can protect spleen tissue from steroid-induced structural changes, and this could be mediated through its antioxidant effects, so barely is recommended as a healthy diet in patients consuming steroids.

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Conflicts of interest

There are no conflicts of interest.

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