

A Comparative Study between Ginger and Echinacea Possible Effect on the Albino Rat Spleen of Experimentally Induced Diabetes

Original
Article

Hoda M. Said, Hekmat O. Abdelaziz, Nesreen G. Abd Elhaliem, Sherine A. Mohammed

Histology department, Faculty of Medicine, Sohag University, Sohag, Egypt

ABSTRACT

Background: Diabetes is a chronic metabolic serious health problem that affecting different organs in the body as spleen. It led to a decrease in immune function.

Aim: The present study compared between the effect of two herbal extracts ginger and Echinacea on their immunomodulatory role on experimentally induced diabetes.

Materials and Methods: 45 adult male rats were used in the study. They were divided into 2 groups. Control group (group I): included 15 animals and group II included 30 animals and were divided into 3 subgroups 10 animals each. subgroup IIa: diabetic group: received a single intraperitoneal injection of streptozotocin 70 mg/kg dissolved in cold saline solution, subgroup II b: diabetic treated with echinacia daily 100mg/kg for 60 days orally by gastric gavage and subgroup IIc: diabetic treated with ginger 500mg/kg for 60 days orally by gastric gavage. At the end of experiment animals were sacrificed, spleen was dissected and processed for light, electron microscopic study and immunohistochemistry. Morphometric study and statistical analysis were done for the percentage area of collagen fibers and number of positive cleaved caspase 3 cells.

Results: Diabetes led to marked atrophied white pulp and statistically significant increase in the number of cleaved caspase-3 positive cells as well as the area percentage of collagen fibers in the spleen. Echinacea treated group red pulp contain numerous acidophilic cells with vesicular nuclei in between the splenic lymphocytes as well as congested dilated blood sinusoids.. The lymphocytes were more or less similar to those in the control group. Ginger treated group exhibited marked improvement in the splenic architecture. There were significant decrease in both cleaved caspase-3 positive cells and area percentage of collagen fibers that was marked in ginger group.

Conclusion: We concluded that ginger had the most immunostimulatory effect as compared to Echinacea in diabetic experimental model.

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Key Words: Caspase-3, diabetes, echinacea, ginger, spleen.

Corresponding Author: Sherine A. Elsherif, MD, Department of Histology, Faculty of Medicine, Sohag Univirsty, Egypt, **Tel.:** +201120301020, **E-mail:** loua.sherine@yahoo.com

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by a defect in insulin secretion. Chronic hyperglycemia causes degeneration in kidney, heart, muscles, eye and many other organs^[1, 2].

Diabetes decreases immune response due to suppression of immune cell function and atrophy of immune organs^[3]. Many studies have been performed to assess the use of natural products to increase host immune responses^[4, 5]

Echinacea purpurea (E. purpurea, also known as purple coneflower) is one of these natural products which originated in North America . Isobutylamides and polyphenolscaffaic acid derivatives as caftaric acid, chlorogenic acid, cynarin, cichoric acid, and echinacoside represent the active compounds in E. purpurea^[6]. Its Extracts and

dietary supplements exhibited anti-immunosuppressant^[7], antioxidative^[8], anti-inflammatory^[9], antibacterial^[10], antiviral^[11], and anticancer^[12] properties. It has been previously reported that Echinacea compounds improved insulin sensitivity^[13]. The active compounds of E. purpurea have free radical scavenging activity and represent its secondary metabolites. Their fast release and destruction in addition to their low bioavailability, permeation, and solubility limit the use of Echinacea^[14].

Ginger (*Zingiber officinale*) is a widely used spices which originated in Southeast Asia^[15]. Gingerols and shogaol are the active compounds in it^[16]. It has been used as a herbal medicine for gastrointestinal symptoms^[17]. Previous studies had shown protective effects of ginger on β -cells of islets of Langerhans in several animal models with diabetes^[18]. Ginger can affect insulin sensitivity with improvement of the liver, kidneys, nerves and eyes complications associated with diabetes^[19].

MATERIALS AND METHODS

This work has been approved by Sohag Faculty of Medicine ethical committee.

Chemicals

Streptozotocin, echinacea and ginger were purchased from Sigma-Aldarich chemicals co St. Louis, MO.

Animals and experimental design

45 adult male rats were purchased from the animal house of Faculty of Science, Sohag University. Their weights ranged from 200-250 grams. The rats were divided randomly into two main experimental groups.

Group I (15 rats): The normoglycaemic group. It was divided into three subgroups (5 rats for each)

Subgroup Ia: Animals were fed with regular diet for 60 days.

Subgroup Ib: Animals were fed with regular diet and received Echinacea daily 100mg/kg for 60 days orally by gastric gavage.

Subgroup Ic: Animals were fed with regular diet and received ginger daily 500mg/kg for 60 days orally by gastric gavage.

Group II (30 rats): The hyperglycaemic group.

Diabetes mellitus was induced by a single intraperitoneal injection of the diabetogenic agent; streptozotocin 70 mg/kg dissolved in cold saline solution. After 60 days, tail blood samples were collected from each rat, and the blood glucose concentration was measured using diagnostic's kits. Streptozotocin treated rats which had blood glucose below 300 mg/dl were excluded from the study. Then the diabetic rats were divided into three subgroups (10 rats for each):

Subgroup IIa: Animals were fed with regular diet for 60 days.

Subgroup IIb: Animals fed with regular diet and received Echinacea daily 100mg/kg for 60 days orally by gastric gavage.

Subgroup IIc: Animals fed with regular diet and received ginger daily 500mg/kg for 60 days orally by gastric gavage.

Methods

At the end of the experiments, the rats were anesthetized by inhalation of diethyl ether. The animals were sacrificed and their spleens were dissected out and processed for histological and immunohistochemical study.

Light microscopic study

EFormalin fixed splenic tissues were processed and prepared for serial paraffin sections of 5µm thickness which stained with:

- **H&E staining** for general histological structure.

- **Masson' trichrome staining** for detection of collagen fibers.

- **Immunohistochemical staining** for cleaved caspase-3 (anti caspase-3 ab-4, rabbit polyclonal antibody, RB-1197-PO, Lot: 1197P10100; NeoMarkers, Fremont, California, USA) was used for detection of apoptotic cells. The reaction appeared as cytoplasmic brownish staining. The immunohistochemical staining was performed using the avidin biotin peroxidase technique using a rabbit polyclonal antibody. Sections were counterstained with Mayer's hematoxylin, then dehydrated, cleared, and mounted. Negative control experiments were made by omitting the primary antibody. Palatine tonsil specimens were the positive control^[20].

Transmission electron microscopic study

Some specimens were fixed in 2.5% glutaraldehyde and then processed for obtaining semithin section (0.5-1µm) and stained by toluidine blue. The ultrathin sections (500-800 nm) were contrasted by the uranyl acetate for 10 min and lead citrate for 5 min, then examined and photographed using a JEOL JEM 1010 electron microscope (JEOL Ltd, Tokyo, Japan) at the Electron Microscope Research Laboratory of the Histology and Cell Biology Department, Faculty of Medicine, Assuit University, Egypt.

Morphometric study and statistical analysis

The light microscope Leica ICC50 Wetzlar (Germany) at the Histology Department, Faculty of Medicine, Sohag University was used, ten high power fields (x400) for each section in all groups were taken, and analysis of each field was done using Image J 1.51n software (National institutes of health USA Java 1.8.0_66 (32-bit) as follow:

1-The percentage areas of collagen fibers^[21].

2-The number of cleaved caspase-3 positive cells; labeling index (LI) was calculated as: (the number of positive cells/number of total cells counted in the field) x100^[22].

RESULTS

Examination of all subgroups in group 1 revealed the same histological findings so we considered all as a control group. H & E stained control spleen sections, consisted of separated lymphoid follicles (white pulp) and surrounded by highly vascular matrix (red pulp). The white pulp was formed of Peri-Arteriolar Lymphatic Sheath (PALS) and marginal zones. The PALS was represented the dark area around the central arteriole. Some follicles had germinal centers (Figure 1a). The germinal center contains acidophilic cells with vesicular nuclei. The red pulp was consisted of network of blood cells cords and numerous venous sinuses. Hemosiderin-laden macrophages were

also seen (Figure 1b). On the other hand, diabetic group IIa sections examination, showed disturbed splenic architecture with atrophied lymphoid follicles. There was no germinal center and marginal zone in most of the follicles. Marked expanded red pulps containing multiple haemosiderin laden macrophages and dilated congested venous sinuses were depicted (Figure 2a). Cellular depletion in the atrophied follicle and most of cells had pyknotic nuclei (Figure 2b). Echinacea treated group IIb exhibited mild improvement in the diabetic histological changes. The white pulp appeared with well defined architecture as in the control group (Figure 3a). However it showed numerous acidophilic cells with vesicular nuclei in between the splenic lymphocytes. The lymphocytes were more or less similar to those in the control group. There was marked congestion of red pulp and distended venous sinuses. Red pulp contained areas of hemorrhage and numerous large cells with acidophilic cytoplasm and dense nuclei (Figure 3b). Examination of ginger treated group IIc observed marked improvement in the degenerative changes found in the diabetic group. Large number of proliferating lymphocytes around the central arteriole in the white pulp was seen. Most of the follicles exhibited germinal center. There were well-defined marginal zone and red pulp (Figure 4 a and b).

There was fine collagen fiber around blood vessels as well as the matrix of the splenic tissue of the control group (Figure 5). The diabetic group IIa exhibited marked increase in the collagen fibers as compared with the control group (Figure 6). Examination of the Echinacea treated group IIb observed mild to moderate increase in the collagen fibers (Figure 7). However, in ginger treated group IIc collagen fibers appeared more or less similar to control (Figure 8).

Cleaved caspase-3 immunostained sections of control group exhibited few positive cells in both white and red pulp. The positive reaction observed as cytoplasmic brownish coloration (Figure 9). On the other hand diabetic group IIa exhibited numerous positive cells in both white and red pulp (Figure 10). Echinacea treated group IIb showed some positive cells less than those in group IIa but still more than those in the control group (Figure 11). Ginger treated group IIc sections were more or less similar to control (Figure 12).

Electron microscopic examination of the control spleen white pulp contained closely packed small lymphocytes. Their nuclei showed condensed chromatin pattern and surrounded by a thin rim of cytoplasm. Some lymphoblast with large euchromatic nucleus and prominent nucleolus were seen (Figure 13). Number of lymphoblast, plasma cells and macrophages are noticed in the germinal center.

Lymphoblast exhibited large euchromatic nucleus and prominent nucleolus. Plasma cells had eccentric rounded nuclei with central rounded nucleoli and dilated RER. Macrophages contained euchromatic irregular nuclei, mitochondria and lysosomes (Figure 14). Red pulp contained lymphocytes; macrophage and rarely seen neutrophils that contained segmented lobed nuclei with characteristic granules (Figure 15).

On the other hand diabetic group IIa exhibited degenerated lymphocytes with irregular heterochromatic nuclei with dilated perinuclear cisternae and mitochondria with destructed cristae (Figure 16). Red pulp contained numerous degenerated erythrocytes. Neutrophils had heterochromatic segmented nucleus and variable sized vacuoles. Macrophages with destructed mitochondria, vacuoles and ingested erythrocyte were seen (Figure 17).

Echinacea treated group IIb showing numerous macrophages in the marginal zone. They have intended euchromatic nuclei, lysosomes, mitochondria and variable sized vacuoles (Figure 18). Germinal center contained plasma cells engorged with dilated RER cisternae and macrophages rich of lysosomes (Figure 19). Red pulp showed numerous erythrocytes and hemosiderin laden giant cells. Some degenerated lymphocytes were seen (Figure 20).

Examination of ginger treated group IIc observed most of the cells were more or less similar to the control. Lymphoblast contained euchromatic nuclei and prominent nucleoli (Figure 21). Numerous plasma cells with dilated RER cisternae were seen in the red pulp. Macrophage had eccentric euchromatic nucleus and the cytoplasm contained hemosiderin particles, mitochondria and lysosomes (Figure 22).

Statistical results

The area percentage of collagen fibers in the spleen of the diabetic group and Echinacea treated group significantly increased as compared with the control group. However both ginger and Echinacea treated group showed significant decrease as compared with diabetic group. On the other hand, ginger treated group exhibited a significant decrease and a non significant difference as compared with Echinacea treated group and control group (Table 1 and Figure 23).

There was a significant increase in area percentage of cleaved caspase-3 positive cells and labeling index in diabetic and Echinacea treated group as compared with control group. However ginger treated group exhibited significant decrease as compared with diabetic and Echinacea treated group while there was non significant change in control group (Table 1 and Figure 23).

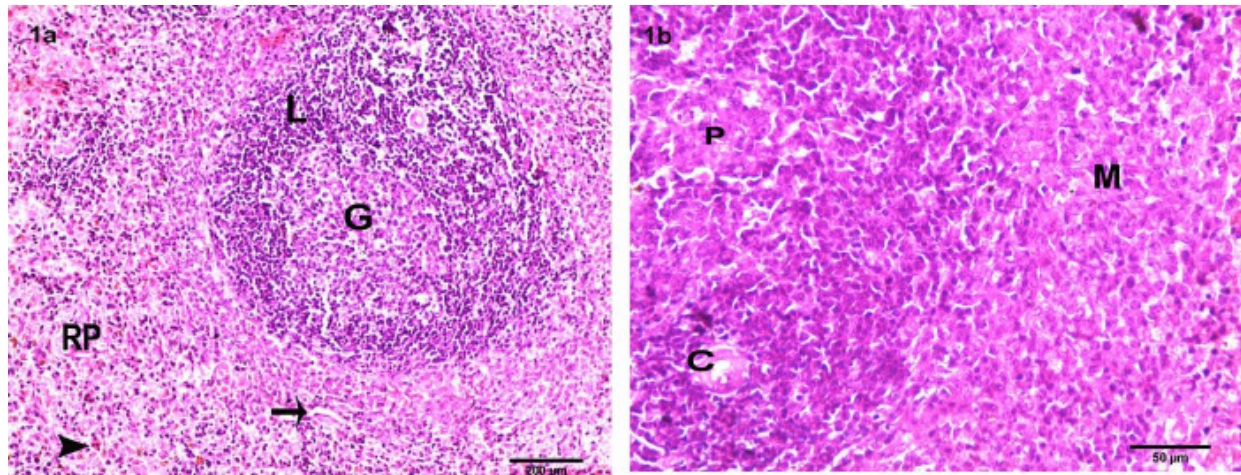


Fig. 1: Photomicrographs of control spleen showing 1a) lymphoid follicles (L) of the white pulp embedded in a highly vascular matrix red pulp (RP) with numerous venous sinuses (arrow) and some follicles have germinal center (G). The white pulp consists of peri-arteriolar lymphatic sheath around the central arteriole (arrow head). Red pulp (RP). 1b) Hemosiderin-laden macrophages (arrow head) . peri-arteriolar lymphatic sheath around the central arteriole (C), germinal center and well distinct marginal zone (M). Germinal center contains acidophilic cells with vesicular nuclei (P). H&E (X100,X400),scale bar (200um, 50um).

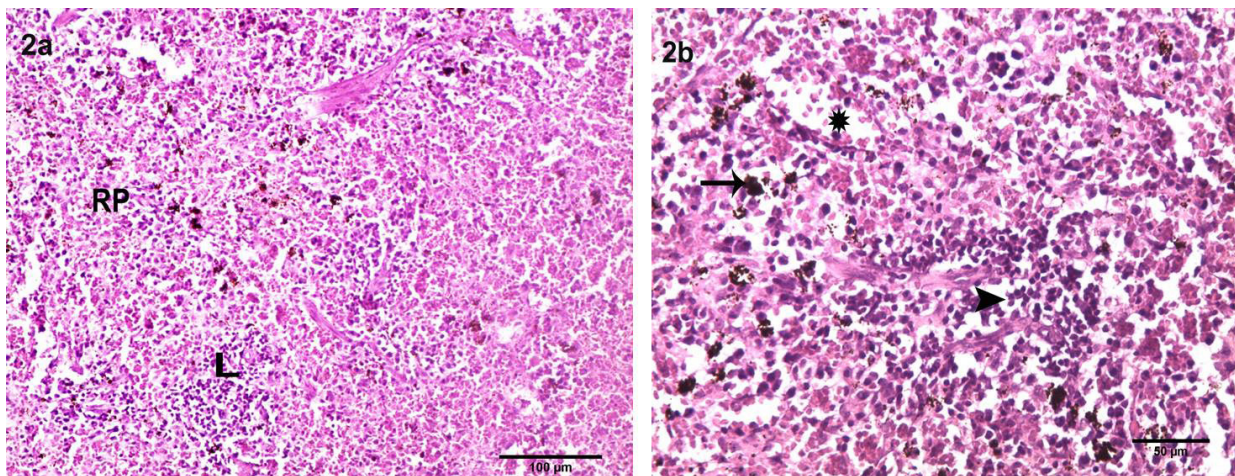


Fig. 2: Photomicrographs of diabetic spleen showing 2a) disturbed architecture with atrophied lymphoid follicles (L) and highly expanded red pulps(RP) 2b) Dilated congested venous sinuses (*) and multiple haemosiderin laden macrophages (arrow) are seen in red pulp. Splenic lymphocytes in the atrophied follicle have pyknotic nuclei (arrowhead). H&E (X100,X400), scale bar(100 um, 50 um).

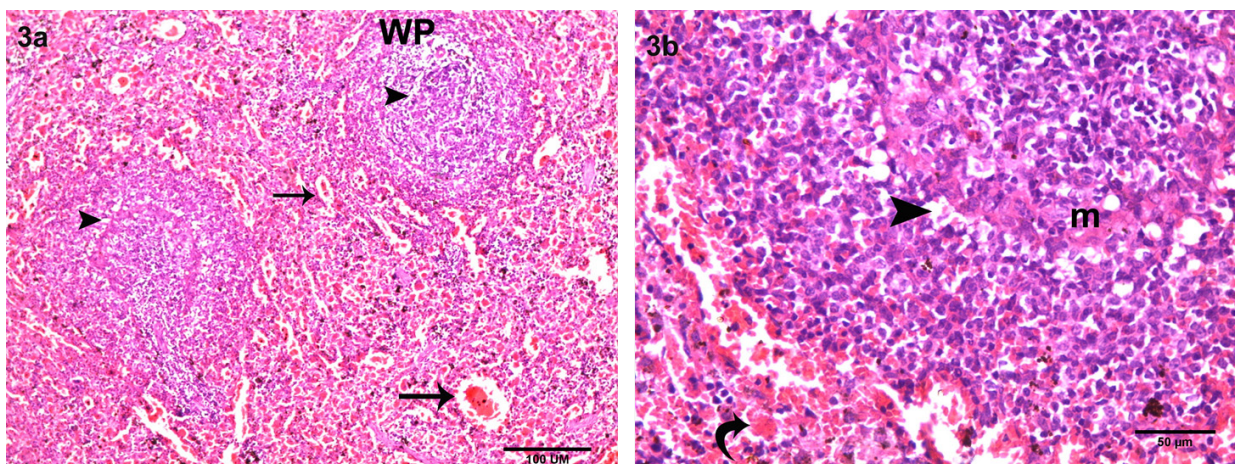


Fig. 3: Photomicrographs of splenic sections of group IIb showing 3a) marked congestion of red pulp and distended venous sinuses (arrow). White pulp (WP) appear more or less similar to control apart from dilated blood vessels (arrowhead). 3b) magnified part of 3a photograph shows some lymphoid follicles in the white pulp contain numerous acidophilic cells with vesicular nuclei (m) and dilated blood vessels (arrowhead) around the periarterial sheath. Areas of congested blood vessels and extravasation of RBCs (curved arrow) are frequently seen in the red pulp. H&E (X100,X400), scale bar(100 um, 50 um).

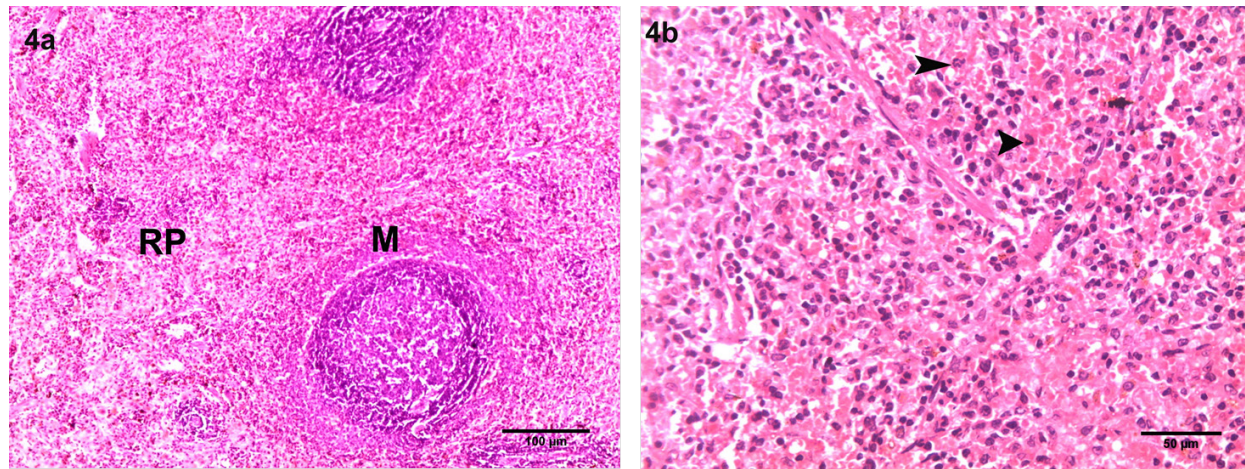


Fig. 4: Photomicrographs of spleen of group IIc showing 4a) a lymphoid follicle with well defined germinal center around the central arteriole in the white pulp. Note, well-defined marginal zone (M) and (R) red pulp. 4b) Red pulp contains numerous neutrophils (arrow head). H&E (X100,X400), scale bar (100 um, 50 um).

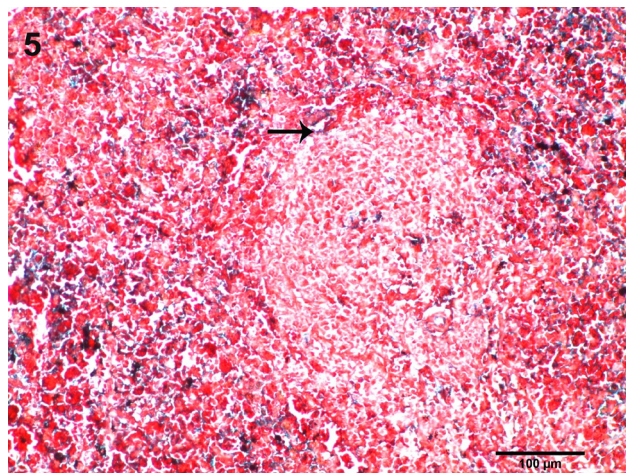


Fig. 5: A photomicrograph of spleen of control group showing fine collagen fibers around the blood capillaries and sinusoids (arrow). Masson' trichrome stain (X200) , scale bar (100 um).

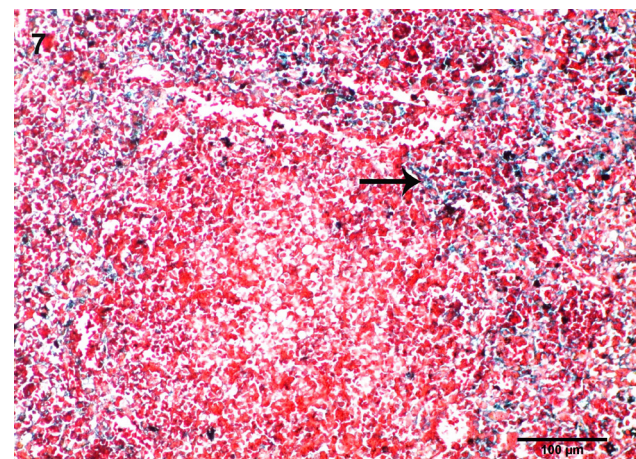


Fig. 7: A photomicrograph of spleen of groupIIb showing marked decrease in the collagen fibers (arrow) as compared to group II. Masson' trichrome stain (X200) , scale bar (100 um).

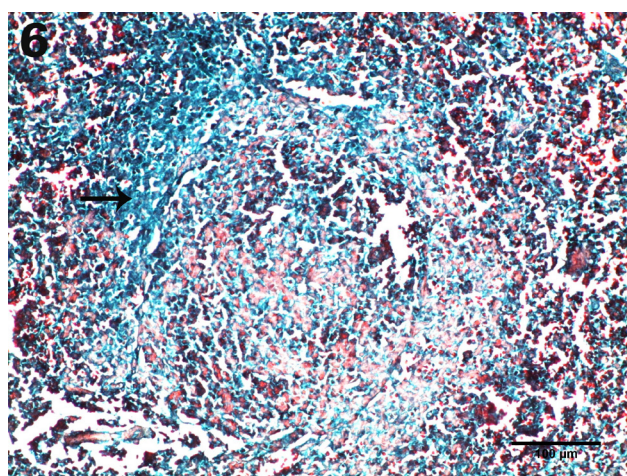


Fig. 6: A photomicrograph of spleen of group IIa showing numerous collagen fibers in the parenchyma of the splenic tissue mainly in the red pulp (arrow) as compared to control group. Masson' trichrome stain (X200) , scale bar (100 um).

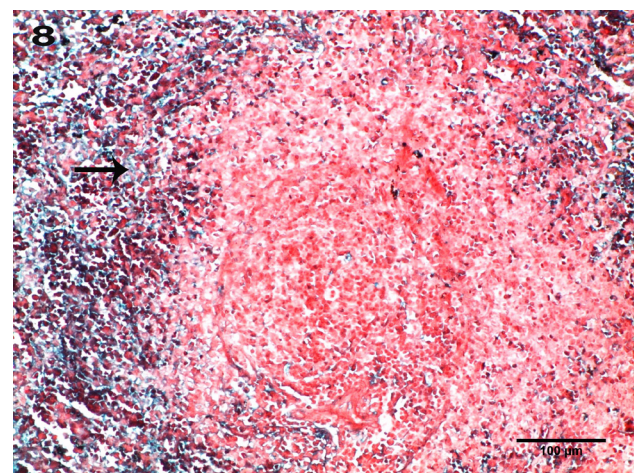


Fig. 8: A photomicrograph of spleen of group IIc showing marked decrease in the collagen fibers (arrow)as compared to group IIa and they are more or less similar to control. Masson' trichrome stain (X200) , scale bar(100 um).

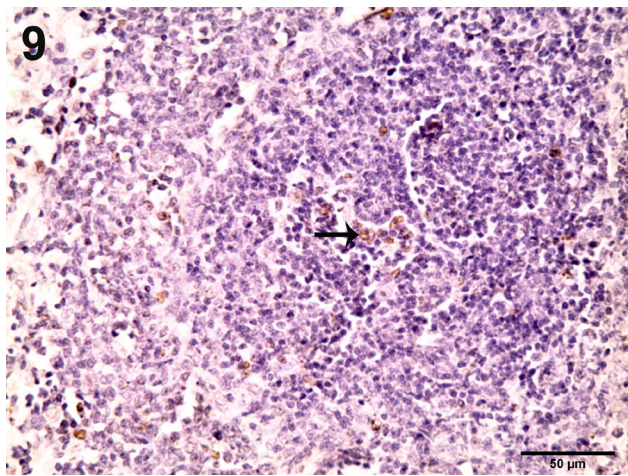


Fig. 9: A photomicrograph of control spleen group I immunostained with caspase-3 antibody showing few positive stained cells (arrow) as cytoplasmic brownish coloration. X400 , scale bar (50 μm).

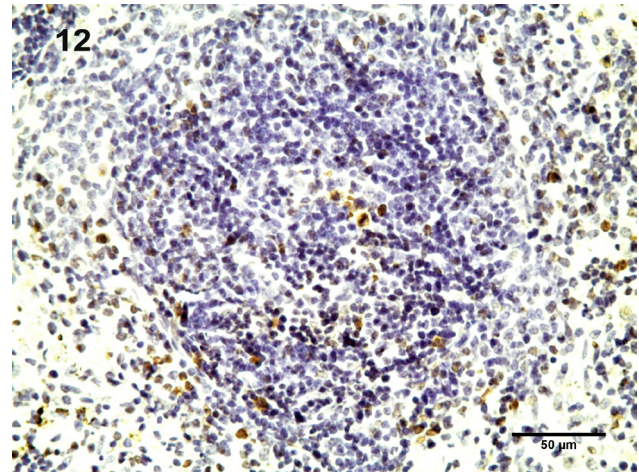


Fig. 12: A photomicrograph of spleen group IIc immunostained with caspase-3 antibody showing few positive cells more or less similar to the control group. X400 , scale bar (50 μm).

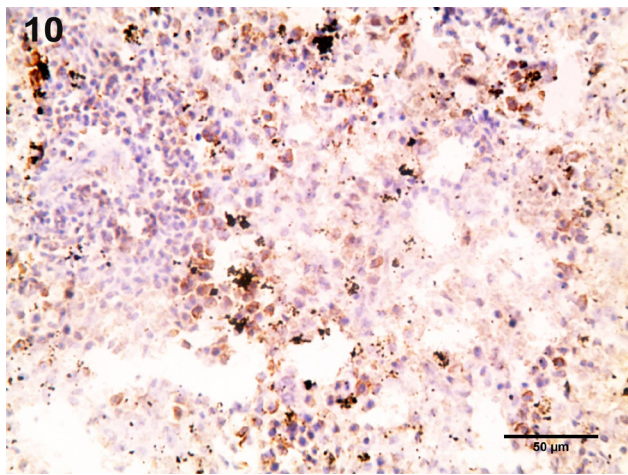


Fig. 10: A photomicrograph of diabetic spleen group IIa immunostained with caspase-3 antibody showing numerous positive cells in both white and red pulp. X400 , scale bar (50 μm).

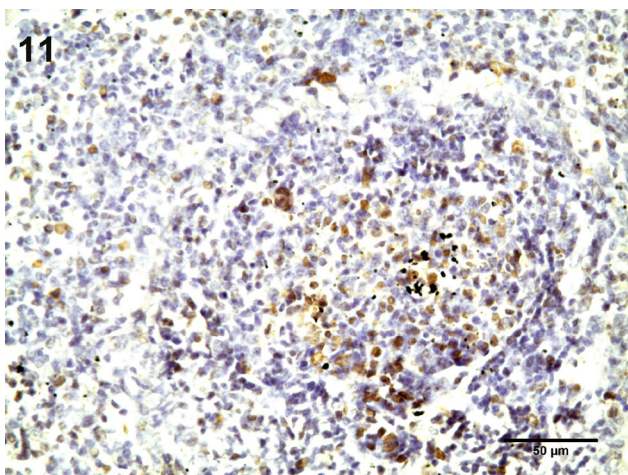
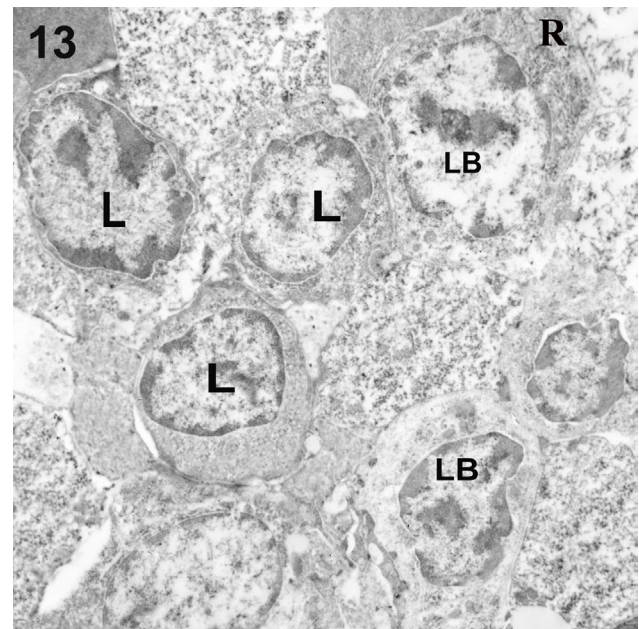


Fig. 11: A photomicrograph of ginger treated spleen group IIb immunostained with caspase-3 antibody showing some positive cells. X400 , scale bar (50 μm).



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Fig. 13: An electron micrograph of control rat splenic white pulp showing closely packed small lymphocytes (L). Their nuclei have a condensed chromatin pattern with small nucleoli and surrounded by a thin rim of cytoplasm. Lymphoblasts (LB) have large euchromatic nucleus with prominent nucleolus and cytoplasm containing RER cisternae (R) X4800

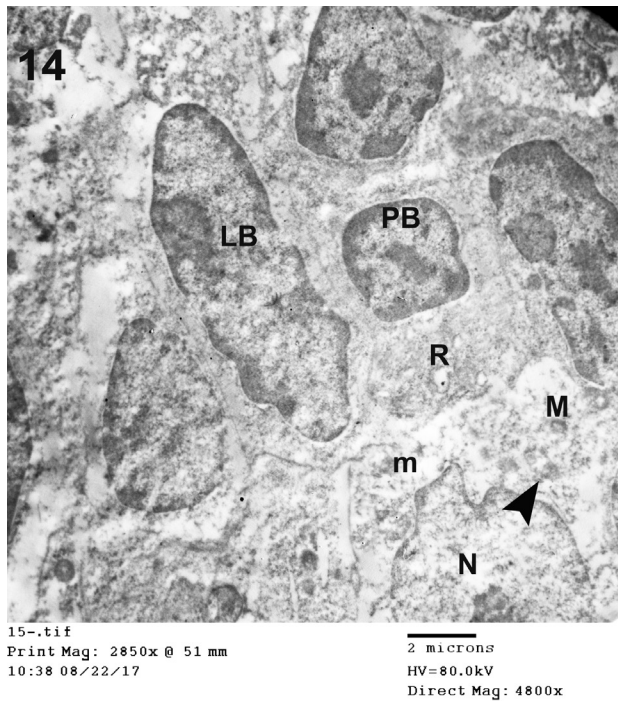


Fig. 13: An electron micrograph of control rat splenic white pulp showing closely packed small lymphocytes (L). Their nuclei have a condensed chromatin pattern with small nucleoli and surrounded by a thin rim of cytoplasm. Lymphoblasts (LB) have large euchromatic nucleus with prominent nucleolus and cytoplasm containing RER cisternae (R). X4800.

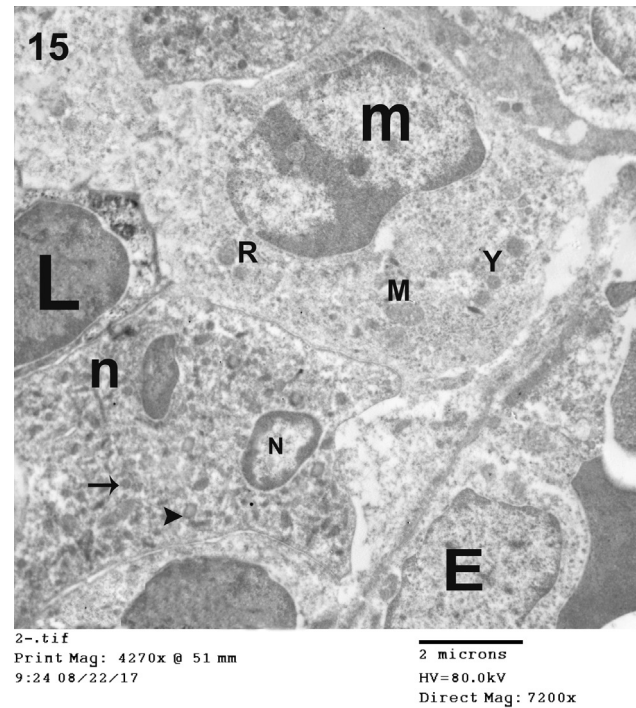


Fig. 15: An electron micrograph of control rat spleen showing red pulp containing lymphocytes (L) with heterochromatic nucleus and thin rim cytoplasm. Macrophage (m) has euchromatic nucleus, mitochondria (M), RER cisternae (R) and lysosomes (Y). Neutrophils (n) contains segmented nucleus (N) with variable electron dense (arrow) and lucent (arrow head) granules. Note: endothelial cell (E) lining of blood sinusoid X7200.

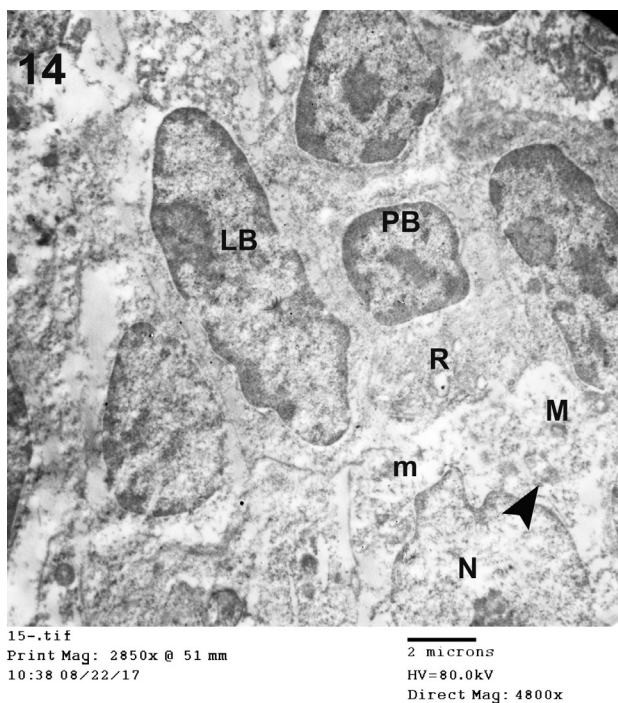


Fig. 14: An electron micrograph of control rat splenic germinal center showing number of lymphoblast (LB) and plasmoblast (PB) have euchromatic nucleus with prominent nucleolus and mitochondria (M) Plasmoblast(PB) has dilated cisternae of RER (R). Some cells may be macrophage (m) contains euchromatic irregular nucleus (N), mitochondria (M) and lysosomes (arrow head). X4800.

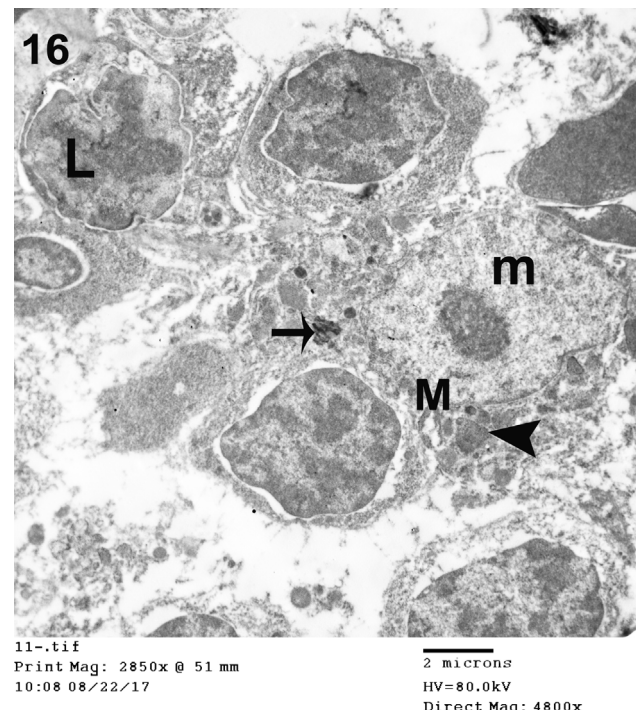
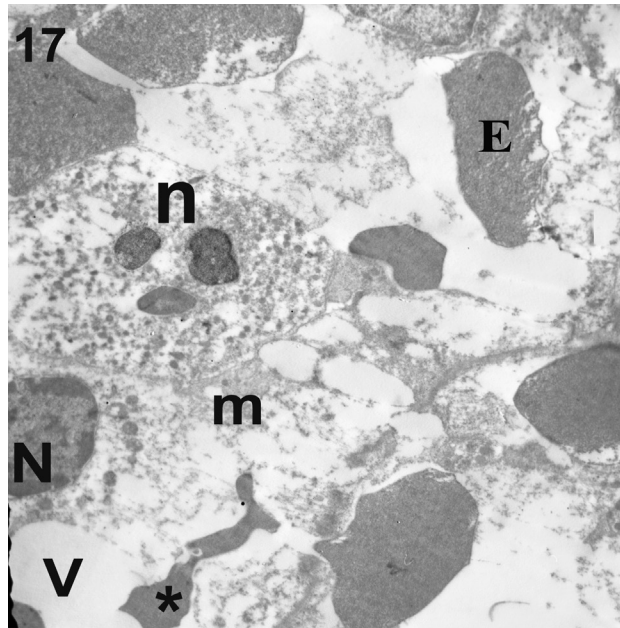


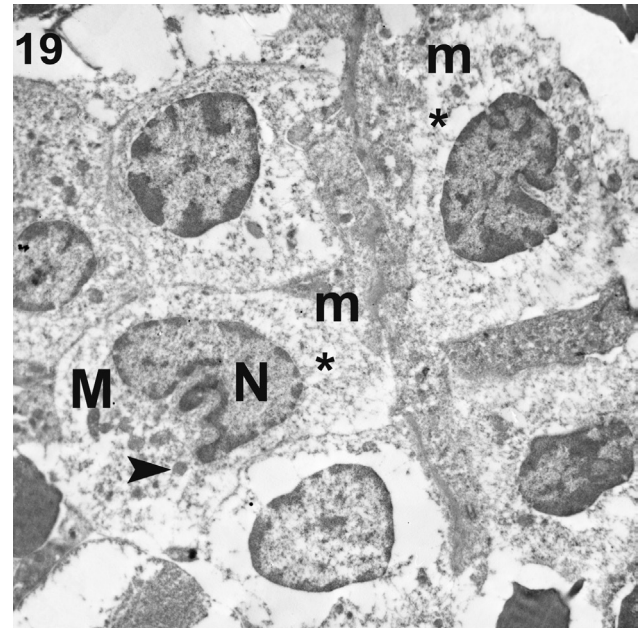
Fig. 16: An electron micrograph of diabetic rat spleen group IIa showing degenerated lymphocytes (L) with irregular heterochromatic nuclei with dilated perinuclear cisternae. Macrophage (m) cytoplasm contains mitochondria with destructed cristae (M), phagosome with hemosiderin particles (arrow) and lysosomes (arrow head). X4800



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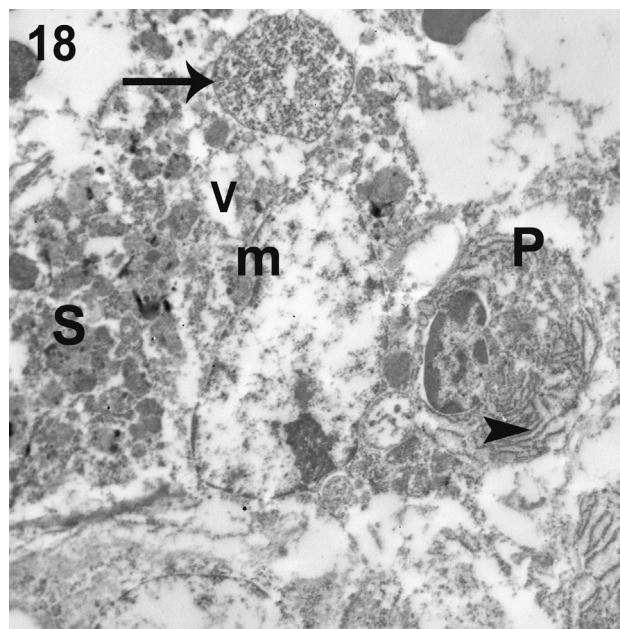
Fig. 17: An electron micrograph of diabetic rat spleen group IIa showing degenerated erythrocytes (E). Neutrophils (N) degenerated neutrophils with pyknotic segmented nuclei segmented nucleus and vacuolated cytoplasm. Macrophage (m) contains heterochromatic nucleus (N), mitochondria, vacuoles (V) and ingested erythrocyte (*). X4800.



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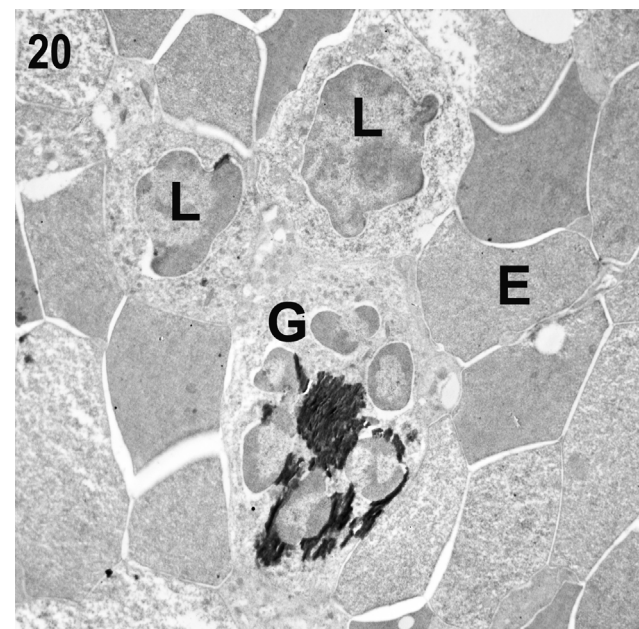
Fig. 19: An electron micrograph of Echinacea treated rat spleen group IIb showing numerous macrophages (m). They have indented euchromatic nuclei (N), lysosomes (arrowhead), mitochondria (M) and variable sized vacuoles (*).X4800..



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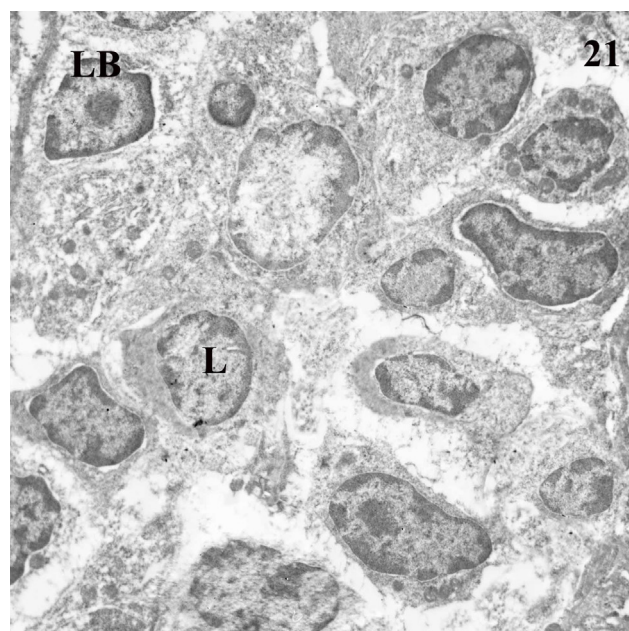
Fig. 18: An electron micrograph of Echinacea treated rat group IIb splenic lymphoid follicle showing plasma cell (P) with eccentric euchromatic nucleus and cytoplasm engorged with dilated RER cisternae (arrowhead). Macrophage (m) has euchromatic nucleus, multiple lysosomes (S), vacuoles (V) and phagosome (arrow). X4800.



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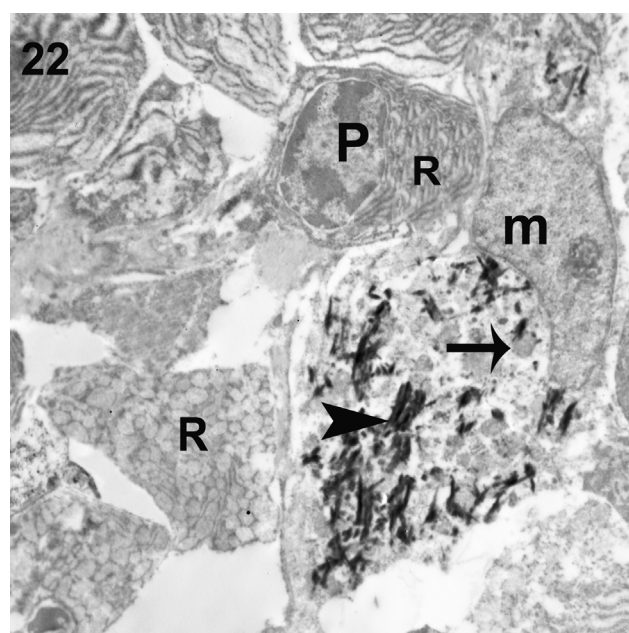
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Fig. 20: An electron micrograph of diabetic Echinacea treated rat spleen group IIb showing multinucleated giant cell (G) with hemosiderin deposition and numerous erythrocytes (E) in the red pulp. Note: degenerated lymphocytes (L) with irregular heterochromatic nuclei. X4800.



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Fig. 21: An electron micrograph of ginger treated rat group IIc splenic white pulp showing numerous lymphoblasts (LB) with euchromatic nuclei and prominent nucleoli and lymphocytes (L) which are more or less similar to control. X4800.



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Fig. 22: An electron micrograph of ginger treated rat group IIc splenic red pulp showing numerous plasma cells with dilated RER cisternae (R). Macrophage (m) has eccentric euchromatic nucleus and the cytoplasm contains phagosome with hemosiderin particles (arrow head) and lysosomes (arrow). X4800.

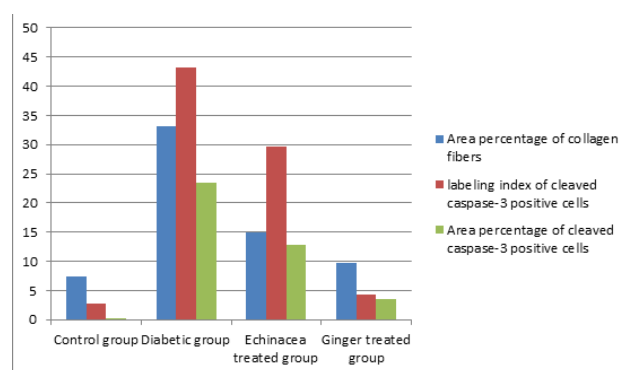


Fig. 23: The mean of area percentage of collagen fibers, area percentage of cleaved caspase-3 positive cells and labeling index in different groups..

	Area percentage of collagen fibers	labeling index of cleaved caspase-3 positive cells	Area percentage of cleaved caspase-3 positive cells
Control group	7,39± 0.38	2.79± 0.43	0.25± 1.35
Diabetic group	33.07± 0.62*	43.26± 0.98*	23.48± 0.87*
Echinacea treated group	14.99± 0.40* #	29.59± 1.21* #	12.77± 1.64* #
Ginger treated group	9.72± 0.51# =	4.32± 0.26# =	3.56± 0.43# =

SE (stander error)

*P significant as compared with the control

P significant as compared with the diabetic group

=P significant as compared with the Echinacea treated group

DISCUSSION

Diabetes mellitus is a chronic metabolic disease characterized by degenerative complications in many organs^[2]; including suppression of immune cell function^[23].

In this study, diabetes mellitus in male albino rat was induced by a single intraperitoneal injection of the diabetogenic agent streptozotocin 70 mg/kg dissolved in cold saline solution^[24]. After 60 days of diabetes induction, histological studies revealed disturbed splenic architecture and increased collagen fibers Previous studies showed similar results with splenic fibrosis and thickened capsule and trabeculae in diabetic models^[25].

In the present study, the red pulps were expanded with multiple haemosiderin deposits and dilated congested splenic sinuses. Previous studies reported same results in induced cirrhosis models. They observed dilated congested splenic sinuses which caused expansion in red pulp area^[26]. In cirrhosis the congestion was due to portal hypertension, but in this study the congestion might be

due to cellular degeneration and splenic fibrosis caused by diabetes. Mechanical compression due to fibers deposition in the sinusoidal wall induced the observed congestion and dilatation.

In our study, most follicles were atrophied with pyknotic nuclei in most of their cells. No germinal centers were seen in most of them. Previous studies on diabetic spleen showed similar results^[24]. In contrast, other studies reported an increase in the white pulp representation with activation of germinal centers after one month of diabetes induction^[27]; and this might be due to the short duration of the disease in their study.

This follicular atrophy could be attributed to cell death caused by apoptosis that was confirmed by immunohistochemical studies in our work. There was significant increase in the expression of the apoptotic marker cleaved caspase-3. Similar results in previous studies was reported as well as decreased expression of the antiapoptotic marker Bcl-2^[20]. Reactive oxygen species (ROS) in many target organs that are produced secondary to diabetes can lead to considerable cellular damage and apoptosis when insufficient cytoprotective molecules are available as previously studied as in kidney and liver^[28]. Apoptosis result in the release of cytochrome c from mitochondria, then cleavage and activation of the cytoplasmic enzymes; Caspases^[29]. Moreover, high glucose concentration induces cytokine mediated apoptosis as revealed in other studies in rat islet cell in vitro^[30].

Electron microscopic examination confirmed these findings. Morphological features of cellular degeneration and apoptosis as irregular heterochromatic nuclei and mitochondria with destructed cristae were seen both in lymphocytes and macrophages. Similar finding of apoptotic splenic cells were revealed in previous studies of splenic toxicity after cadmium administration with nuclear deformation, marginalization of chromatin, and pyknosis^[31]. Other studies on diabetic patients showed lymphocytic nuclear changes in peripheral blood in the form of irregular contour and more euchromatin. They correlated these changes with the level of blood glucose^[32].

Echinacea is one of the medical important herbs with a great role in activation of immune system. It increases T-cell production and activity, and stimulates cytokine production and cellular respiration^[33]. Studies reported that Echinacea compounds improved insulin sensitivity^[13].

In the current study, Echinacea treated group showed mild improvement in the diabetic histological changes. Numerous acidophilic cells with vesicular nuclei were seen. These cells could be considered lymphoblasts as seen with electron microscope. Numerous macrophages and plasma cells were seen by electron microscope. This indicated activation of lymphocytes. Similar results were reported by previous studies and they explained

that Echinacea attenuated the inflammatory response of macrophages to immune stimulus^[34]. Previous studies reported improvement of histological changes in the diabetic rat testis after administration of Echinacea^[35]. In contrast, other studies proved that Echinacea is potentially effective in stimulating an in vivo, non-specific immune response with an increase in inflammatory cytokines release in normal rats^[36].

On the other hand, it was found that congestion in splenic sinuses was still observed due to fibrosis.

A significant decrease in apoptosis was found with Echinacea administration compared to the diabetic group. In contrast, previous reports observed that Echinacea had a strong growth-inhibitory effect against colon cancer cells with induction of apoptosis^[12]. The antiapoptotic effect in this study could be attributed to its antioxidant role^[37], or its role in improvement of hyperglycemia^[35].

Ginger is one of the spices which have shown protective effects on β -cells; it restored the level of insulin in several animal models of diabetes^[18] and in clinical trials^[2]. In the current study, ginger administration improved the histological and ultrastructural degenerative changes found in the diabetic group. This improvement was significantly more than that caused by Echinacea. A large number of proliferating lymphocytes in the white pulp was seen. Most of the follicles exhibited germinal center with well-defined marginal zone. Similar results were reported in previous studies. Ginger maintains the balance of the immune system. It had been found that ginger suppress the Th2-mediated immune response a mouse model of asthma^[38]. It also has a protective role against the harmful effects of xenobiotics^[39]. In contrast, other studies observed a deleterious effect of high dose ginger on spleen and they explained that ginger affects spleen and immune balance in a dose dependent manner^[40].

In the current work, attenuation of splenic fibrosis and eventually sinuses congestion in red pulp were observed. This antifibrotic effect was markedly seen compared to that in Echinacea. Similar antifibrotic effects of ginger were observed in previous studies investigated its effect on rat model of liver fibrosis induced by CCl₄^[41].

In our study, it was found that ginger had a significant antiapoptotic role. In line with our results, the antiapoptotic effect of ginger was reported in previous studies of hepatotoxicity^[42] and testicular toxicity^[43] due to its antioxidant effects which prevent DNA damage and lipid peroxidation^[44].

Finally, we concluded that ginger had the most immunostimulatory effect as compared to Echinacea in diabetic experimental model. Also, ginger had an antifibrotic effect. It can be used as an adjuvant treatment for splenic disorders and immune function disturbance associated with diabetes.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربي

دراسة مقارنة بين التأثير المحتمل للزنجبيل والإشنسا على طحال الجرذ الأبيض لمرض السكري المحدث

هدى محمد السيد , حكمت عصمان عبدالعزيز، نسرين جمال الدين عبدالحليم، شيرين أحمد محمد

قسم الهستولوجي - كلية الطب - جامعة سوهاج

المقدمة: يعتبر مرض السكري مشكلة صحية خطيرة في التمثيل الغذائي المزمن تؤثر على الأعضاء المختلفة في الجسم كالطحال. ويؤدي إلى انخفاض في وظيفة الجهاز المناعي.

الهدف: قارنت الدراسة الحالية بين تأثير اثنين من مستخلص الأعشاب من الزنجبيل وإشنسا على دورهما المناعي في مرض السكري المحدث.

مواد وطرق البحث: تم استخدام ٤٥ من الجرذان الذكور البالغين في الدراسة وتم تقسيمهم إلى مجموعتين. المجموعة الأولى (المجموعة الضابطة): شملت ١٥ حيواناً والمجموعة الثانية شملت ٣٠ حيواناً ، وتم تقسيمها إلى ٣ مجموعات فرعية ١٠ حيوانات لكل منها. المجموعة الفرعية الأولى (مجموعة السكري): تلقت حقنة واحدة داخل الصفاق من الستربتوزوتوسين ٧٠ ميلي غرام لكل كيلوغرام مذاب في محلول ملحي بارد ، المجموعة الفرعية الثانية: مرضى السكري يعالجون بالإشنسا يومياً ١٠٠ ميلي غرام لكل كيلوغرام لمدة ٦٠ يوماً عن طريق الفم بواسطة التزقيم المعدي والمجموعة الفرعية الثالثة: السكري يعالج بالزنجبيل ٥٠٠ ملغ / كغ لمدة ٦٠ يوماً عن طريق الفم بواسطة التزقيم المعدي. في نهاية التجربة تم تشريح الحيوانات، استخراج الطحال وتحضيره للفحص بالميكروسكوب الضوئي والإلكتروني والصبغة الكيميائية المناعية أيضاً. تم عمل دراسة قياسية لمساحة ألياف الكولاجين وعدد الخلايا الالتهابية المنشقة للكابيس ٣ و تحليل احصائي للنتائج.

النتائج: أدى مرض السكري إلى ضمور ملحوظ في اللب الأبيض وزيادة معتد بها إحصائياً في عدد خلايا كاسباس ٣- الإيجابية المشقوقة وكذلك نسبة مساحة ألياف الكولاجين في الطحال. يحتوي اللب الأحمر في مجموعة إشنسا المعالجة على العديد من الخلايا الحمضية مع نوى حويصلية بين الخلايا الليمفاوية الطحالية وكذلك الأوعية الدموية المتوسعة المزدهمة. وكانت الخلايا الليمفاوية مماثلة أو أكثر مماثلة لتلك الموجودة في المجموعة الضابطة. أظهرت مجموعة الزنجبيل المعالجة تحسناً ملحوظاً في تركيب الطحال. كان هناك انخفاض كبير في كل من الخلايا المشقوقة كاسباس ٣- الإيجابية ونسبة مساحة ألياف الكولاجين في مجموعة الزنجبيل.

الخلاصة: خلص هذا العمل إلى أن الزنجبيل كان له أكثر تأثير منبه للمناعة بالمقارنة مع إشنسا في النموذج التجريبي لمرض السكري.