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HISTOPATHOLOGICAL APPRAISALS IN THE LYMPH NODE AND SPLEEN OF INDUCED ARTHRITIS RAT MODEL TREATED WITH GINGER AND CURCUMA

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Rheumatoid arthritis (RA) is a chronic, inflammatory, and progressive autoimmune disease characterized by several articular, extra-articular, and systemic effects. Scientists are looking for treatment with herbs (e.g. Ginger and Curcuma) that have anti-inflammatory and fewer side effects properties than ordinary drugs. In the current study, an empirical autoimmune disease (Collagen Induced Arthritis rat model [CIA]) that shares several RA features was used to investigate the histopathological appraisals in lymph node and spleen of the rats as well as the ameliorative anti-arthritic effect after 25 consecutive days of Ginger and Curcuma oral administration. Fifty males of albino rats (6-7 weeks old) were divided into 5 equal groups. Group I; control, groups II, III, IV, and V were arthritic. The arthritic groups III, IV, and V received Ginger, Curcuma, and Ginger/Curcuma mixture orally, respectively. Lymph nodes (LNs) and spleens were collected, processed for histopathological examination. The H&E staining showed that the LN of groups III and IV had histiocytic infiltrates replacing the monocytoid-like cells in group II. Group V showed restoration of the normal LN architecture. The spleen sections showed normal cellular components in groups III, IV, and V as opposed to the histiocytes and edema in group II. The reticulin and collagen fibers' content of both the spleen and LN was significantly decreased in group II than group I. On contrary, both herbs increased the reticular and collagen fibers' content significantly; more obviously when they were introduced in combination (i.e. group V). Therefore, our results suggest that the inflammatory ameliorative effect of the combined two herbs is more than any of them alone.

Keywords: Collagen-Induced Arthritis, Ginger, Curcuma, lymph node, spleen**.**

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, and progressive autoimmune disease marked by several articular, extra-articular, and systemic effects [1, 2]. Joint swelling (edema), stiffness, pain, and redness (erythema) that lead to ankylosis and joint destruction, which may result in movement disability are the inflammatory changes accompanying RA [3]. Rheumatoid nodules, pericarditis, keratoconjunctivitis sicca, vasculitis, rheumatoid lung, and uveitis are involved in the Extra-articular manifestations, whereas, acute-phase protein production, cardiovascular disease (CVD), anemia, fatigue, osteoporosis, and depression are involved in the systemic manifestation [4, 5]. Collagen-Induced arthritis (CIA) is an empirical autoimmune disease for several rodents' strains, which are susceptible to RA. It is induced via immunization with type-II collagen, the major protein component of the articular cartilage, mixed with adjuvant [6]. CIA model emulates the human RA in its clinical, histological, and immunological features as well as its genetic linkage [7].

The ordinary drugs are useful for controlling the inflammatory signs and pain of RA patients despite that they showed little effectiveness with potential toxicity and caused long-term immunodeficiency, and gastrointestinal as well as cardiovascular disorders, in addition to being expensive [8]. Therefore, scientists tried to replace the drugs with herbs that have anti-inflammatory properties and cause minor side effects [9]. Curcuma and Zingiber (Ginger) are South Asia native herbs under the “Zingiberaceae” family and are known for their tasty attractive flavor for using as spices in cooking [10]. Moreover, they were reported for several medicinal applications owing to their anti-inflammatory properties [11, 12]. Ginger has a long history in medicine as it has been reported for treating several ailments, including diarrhea, stomach-aches, gingivitis, asthma, nausea, toothache, respiratory disorders, and arthritis in addition to prevention of motion sickness, and dyspepsia as well as the suppression of inflammations [10, 13]. Traditionally, Curcuma has been used for pain relieving, wound healing, and control tumors and inflammations [14, 15]. Ginger is rich with pungent phenolic compounds (gingerols and shogoals) whilst the active constituents of curcuma are the

phenolic curcuminoids. Lymph nodes (LNs) are encapsulated small immune organs, oval or bean-shaped, distributed throughout the mammals' bodies along the lymphatic vessels. They filter the lymph to remove pathogens or other foreign substances [16]. LNs examination may reflect lesions in organs they drain. The spleen is analog to large LN in structure and function. The current study demonstrated histopathological alteration aspects of the lymph node and spleen in CIA rat model and how Ginger, Curcuma and their mixture can improve them.

2. MATERIALS AND METHODS

2.1. Animal care conditions

Males of Charles River albino rats' strains (Rattus rattus), six to seven weeks age (180 ± 30 g), were obtained from Assuit University's animal house, Egypt. The rats were transferred to the animal house of the Faculty of Medicine, Sohag University where the experiment has proceeded. They were kept in cages under controlled conditions of temperature (24 - 26˚ C), humidity (55 - 60%), and photoperiod (12:12 h) dark-light cycle. The animals were acclimated to the laboratory conditions for two weeks. The feed and water were provided ad labitum. The infection-free status of the animals was ensured at the onset of the experiment. The human care of animals was according to the institutional guidelines.

2.2. Experimental design

Fifty (50) rats were assigned to our trial and classified into five groups (10 rats/cage, in duplicate). Group I was the control that received no injection. Group II was an arthritic (CIA) untreated group. Groups III, IV, and V were arthritic and received, orally (by gavage), 200 mg/kg ginger (Sigma Aldrich, Egypt) suspended in 1ml distilled water (ginger-treated group), 200 mg/kg curcuma (Sigma Aldrich, Egypt) suspended in 1ml distilled water (curcuma-treated group), and 400 mg/kg ginger/ curcuma mixture (1:1) (200 mg/kg ginger+200 mg/kg curcuma) suspended in 1ml distilled water (ginger/ curcuma- treated group), respectively, and daily from the day of arthritic induction for 20 consecutive days [12].

2.3. CIA induction

For CIA induction, the rats were immunized twice with two subsequent immunizations (two weeks interval), Freund's Complete Adjuvant (FCA) mixed with alum precipitated collagen, and Freund's Incomplete Adjuvant (FIA), respectively. Freund's Complete Adjuvant (FCA) was prepared as described by Stils [17] via well mixing of 8.5 ml paraffin oil (Sigma Aldrich, Egypt), 1.5 ml Arlacel A as an emulsifying agent (Sigma Aldrich, Egypt), and 50 mg lipopolysaccharides (LPS). The mixture was autoclaved (121˚C, 15 min) before use. FIA was prepared by the same method without LPS. Two additional rats were kept for collagen preparation according to the methods described by Habermehi [18] from their tail tendons. The obtained collagen was precipitated using potassium alum according to Henry and Ibraheem [19, 20] and kept at 4˚C until use. Purified *E. coli* bacterial isolate was used for preparing bacterial lipopolysaccharide (LPS) following the method of Al-Hendy and Apicella [21, 22]. The bacterial isolate has been brought from the Bacteriology Department, Animal Health Research Institute, Sohag branch, Egypt.

The rats were immunized firstly by collagen/FCA emulsion prepared prior to use, 10 ml of the alum precipitated collagen emulsified with 1.5 mL of (FCA) [23, 24]. One ml of collagen/FCA emulsion was distributed throughout the body of each arthritic rat; 0.1 ml was injected intraperitoneal, and 0.9 ml intradermal (0.1 ml at each palmar and plantar surface, and 0.5 ml at the dorsal surface of the tail) [19, 25]. A booster dose of 1ml collagen/FIA emulsion was injected after two weeks intervals into the rats in the same manner as the first immunization.

2.4. Sampling and tissue collection

On the 21st day of the experimental onset, all the animals were sacrificed. The axillary LNs and spleens were extracted and examined for

macroscopic in shape and size. Samples of both organs were washed in saline solution and fixed in neutral buffered formalin (10%).

2.5. Histological preparations

The fixed samples of LNs and spleen were run through the ordinary technique of histology [26]. Sections were cut at 5 µm. Haematoxylin and Eosin (H&E), reticulin, and Masson trichrome stains were applied. The stained sections were mounted using DPX and investigated under a monocular microscope (XSZ-109 B, China). H&E-stained sections were examined for tissue histopathological signs. Photographs of the stained sections were captured by an industrial digital camera (LCMOS05100KPA, China) at Zoology Department Central Lab, Faculty of Science, Assuit University.

2.6. Quantitative and statistical analysis of reticular and collagen fibers

Image J software (version 1.8) was used for measuring the area fraction (% Area) of reticular, and collagen fibers of the reticulin, and Masson trichrome stained sections in triplicate photomicrographs per slide per group. The statistical analysis for the obtained data was performed using Microsoft excel 2013 software. To analyze the differences between groups, the one-way ANOVA test of variance and Least Significant Difference (*LSD)* calculation was used for paired comparison of means. Statistical significance was accepted at $p < 0.05$. The results are reported as mean ± SD.

3. RESULTS

3.1. Macroscopic examination

Macroscopically, lymph nodes of the arthritic groups were generally larger in size (60-70 mg) than the normal control (20-40 mg) (Fig.1). No differences were observed in the spleen size and morphology in all studied groups.

3.2. Microscopic examination of axillary lymph nodes

3.2.1. Histopathological findings

H&E stained axillary LNs cross-sections of the control animals (group I) showed normal preserved architecture which consists mainly of an outer cortex, inner medulla, and para-cortex in between (fig.2a_1). The LN is enclosed in a capsule, a thin layer of dense fibrous connective tissue. The sub-capsular sinuses, which are located beneath the capsule are of normal appearance and contain few lymphocytes. Lymphocytes with different sizes represent the LN parenchyma; they have small spherical nuclei with abundant dark staining condensed chromatin and pale staining cytoplasm. They are aggregated as lymphoid nodule in the cortex or lymph cords in the medulla. Histiocytes/ Macrophages are present in the cortical sinuses; they have eccentric round or bean-shaped nucleus and have lightly stained lacy cytoplasm (Fig. 2a_2).

Arthritic-untreated rats (group II) showed marked sub-capsular distention with monocytoid cells versus those of controls. These cells are similar in shape with monocytes which are large in size and have large eccentric bland-looking irregular nuclei and more abundant cytoplasm than lymphocytes (fig. 2b). The administration of ginger (group III) showed partial effacement of architecture with sub-capsular histiocytic infiltrate. Also, interstitial fibroblasts were noticed (fig. 2c). Giving of curcuma (group IV) showed the same histopathological changes of group III but fat cells appeared in the cortex, and there is a decrease in the sub-capsular sinuses distension than those in group II (fig. 2d). Administration of both ginger and curcuma together (group V) showed nearly restoration of normal LN with para-cortical infiltration by red eosinophilic cells (fig. 2e).

3.2.2. Reticular fibers contents

Axillary LN sections of the control group showing deep grey- to black-stained reticular meshwork in the cortex. Sub-capsular sinuses showed narrow reticular fibers immediately below the black-stained capsules. More deeply, a network of reticular fibers trapping lymphoid cells in their spaces (fig. 3a). An obvious depletion of the reticular fibers' deposition was observed in group II compared to group I. The fibers

appeared in the peripheral capsules as dark grey thin strands. In addition, they were dispersed around the lymphoid follicles as few fine strands (fig. 3b). Groups III, IV, &V showed a slight increase in the contents of the stained reticular fibers under the capsule than in group II. A marked increase in the reticular fibers strands in the ginger/curcuma-treated group than those of rats treated with ginger or curcuma alone (fig. 3c, d, & e).

Quantitative results by Image analysis of reticulin-stained sections of LN showed that the reticular fibers significantly decreased in group II (7.894%) in comparison to group I (28.891%). They also significantly increased in groups III, IV, and V (13.851%, 13.775%, and 23.763%, respectively) comparing to group II (7.894%). No significant difference between groups III, and IV While a significance difference between group V and both groups III, and IV were noticed (fig.4). Least Significant Difference value (LSD) = 5.608

3.2.3. Collagen fibers contents

Axillary lymph node sections of the control group showed normal content and distribution of collagen fibers, which take the blue color, in the capsule and ground substance of the cortex (fig. 5a). Arthritic animals showed a marked decrease in collagen fibers content and distribution in comparing with controls (fig. 5b). Groups III, and IV showed a slight increase in the collagen fibers content and distribution than group II (fig. 5c, & d). Combination of ginger and curcuma showed an obvious increase in the collagen fibers content and distribution than in groups III and IV (fig. 5e).

Quantitative results by Image analysis of collagen-stained sections of LN showed that the collagen fibers significantly decreased in group II (22.381%) in comparison to group I (39.185%). They also significantly increased in groups III, IV, and V (27.549%, 26.975%, and 36.551%, respectively) comparing to group II (22.3813%). No significant difference between groups III, and IV (27.549%, 26.9746%). There is a significance difference between group V and both groups (III, and IV) were noticed (fig.6). LSD = 2.757

3.3. Microscopic examination of Spleen

3.3.1. Histopathological findings

Group I (control rats) showed normal appearance of red pulp and white pulp (fig. $7a_1$). The splenic follicle of white pulp appears normal (fig. $7a_2$). Group II (arthritic animals) showed marked edema and distention of the splenic follicle with many histiocytes (fig. 7b). Groups III, and IV (ginger and curcuma treated rats, respectively) showed nearly normal cellular components of splenic follicles with few histiocytes in group IV (fig. 7c & d). Group V (rats given mixture of ginger and curcuma) showed normal appearance of splenic follicle (fig. 7e).

3.3.2. Reticular fibers

Spleen sections of group I showed deeply stained dark grey to black reticular meshwork in the dense connective tissue capsule surrounding the spleen and in the stroma (fig. 8a). Group II showed an obvious decrease in the reticular fibers content of the capsule and the ground substance with an obvious increase in background cellularity essentially histiocytes when compared with group I (fig. 8b). Groups III, IV, & V showed thicker capsule and slight increase in the content and recurrence of the reticular fibers than group II. A marked increase in the reticular fibers content and recurrence was detected in group V than the previous two groups (III, and IV) as shown in (fig. 8c, d, &e).

Quantitative results by Image analysis of reticulin-stained sections of the spleen showed that the reticular fibers significantly decreased in group II (6.3463) in comparison to the group I (31.679). They also significantly increased in groups III, IV, and V (13.2953, 12.993, and 28.605, respectively) compared to group II (6.346). No significant difference between groups III, and IV. While a significant difference between group V and both groups III, and IV was observed (fig.9). LSD = 6.484

3.3.3. Collagen fibers

Spleen sections of control group showed normal content and distribution of the blue collagen fibers in the capsule and in the stroma (fig. 10a). Group II showed an obvious decrease and faint staining collagen fibers (fig. 10b). Groups III and IV showed a slight increase in the collagen fibers content than group II (fig. 10c & d). Group V showed a marked increase in collagen fibers content than groups III and IV (fig. 10e).

Quantitative results by Image analysis of collagen-stained sections of spleen showed that the collagen fibers significantly decreased in group II (22.833) in comparison to group I (41.13). They also significantly increased in groups III, IV, and V (26.334, 25.899, and 38.609, respectively) comparing to group II (22.833). No significant difference between groups III, and IV (26.334, and 25.899). There is a significant difference between group V and both groups (III, and IV) were noticed (fig.11). LSD = 2.536.

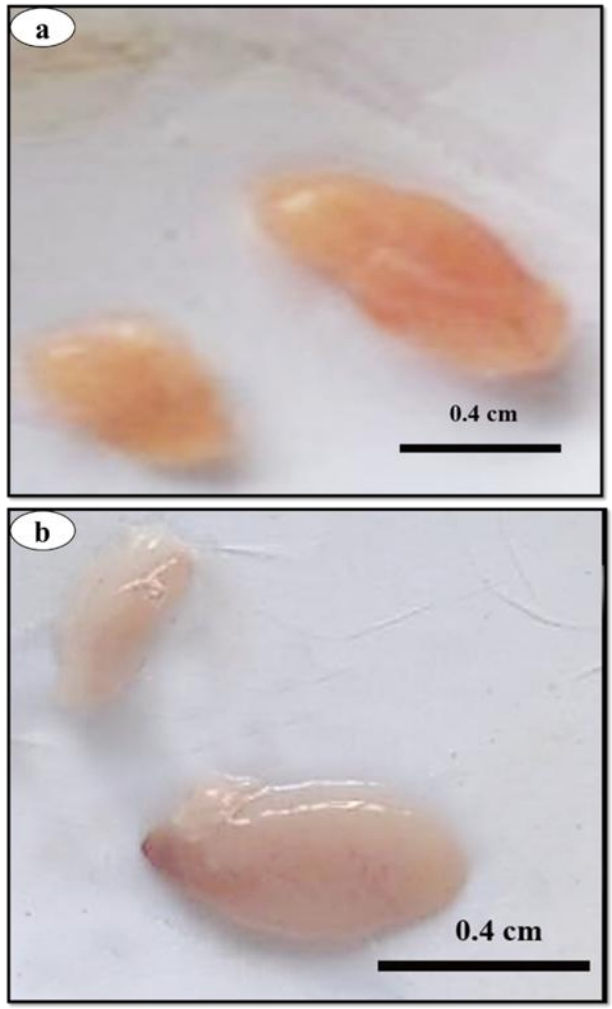

Figure 1. Macroscopic appearance of axillary lymph node (LN). (a) Normal axillary LN of the control group. (b) Enlarged LN of the arthritic group.

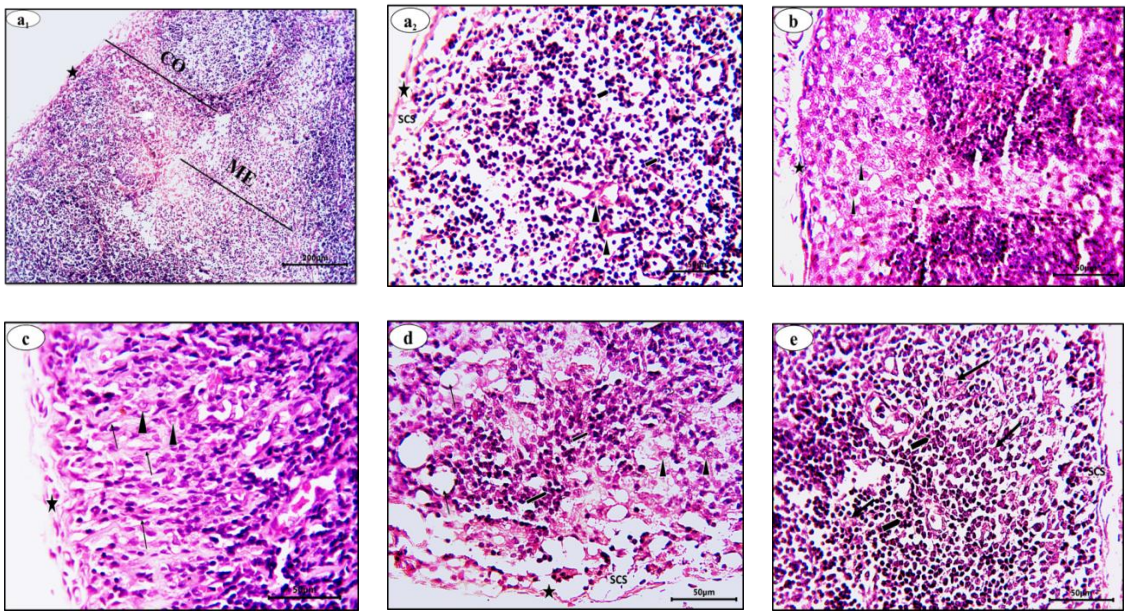

Figure 2. (a_1) Axillary LN section of control group I showing normal lymph node is enclosed in a connective tissue capsule (star) under which the cortex (CO), and medulla (ME), H&E, X10. (a_2) High magnification of a_1 showing the sub-capsular sinuses (SCS) and the cortex with normal different sized lymphocytes (pentagon) and more or less histiocytic distribution (triangle) in between, lymphocytes have small spherical nuclei with abundant dark staining condensed chromatin and pale staining cytoplasm, histiocytes are undifferentiated in shape with lightly stained lacy cytoplasm and eccentric round or bean-shaped nucleus. (b) Group II showing marked sub-capsular distention with medium-sized monocytoid cells (triangle), which are similar in shape to monocytes and contain more abundant light-staining cytoplasm than lymphocytes, and bland-looking irregular nuclei (triangle). (c) Group III showing partial effacement of architecture with sub-capsular histiocytic infiltrate (triangle) replacing the monocytoid like cells in group II and interstitial fibroblasts (arrows) was noticed. (d) Group IV showing the same histopathological changes of the group (III); partial effacement of architecture with histiocytic infiltration (triangle), numerous fat cells (arrows) are observed in the cortex, a decrease in sub-capsular sinuses (SCS) distension than those in group II. (e) Group V showing nearly restoration of normal LN architecture and paracortical infiltration by red eosinophilic cells (arrows). H&E, X40

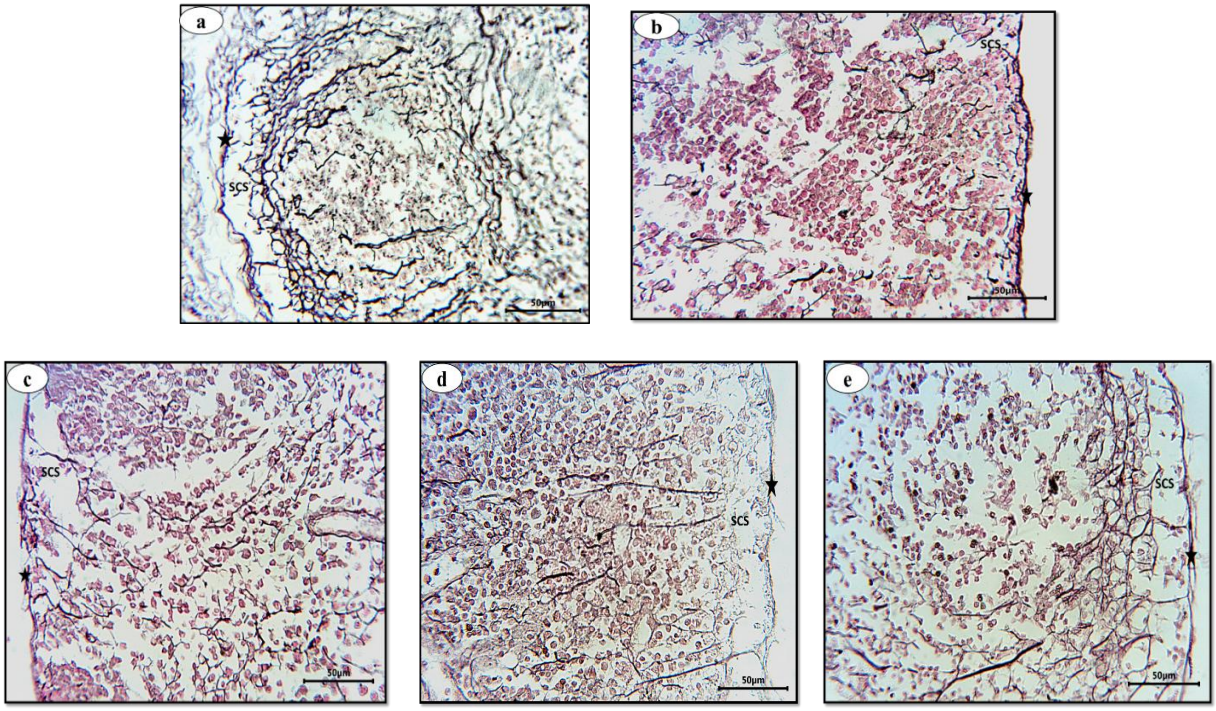

Figure 3. (a) Axillary LN section of control group I showing deeply stained grey to black reticular meshwork in the LN cortex. The capsule (star) is black, immediately below, the few reticular fibers spaces or the sub-capsular sinuses (SCS). More deeply, a network of reticular tissue in whose spaces the cortex lymphoid cells are situated. (b) Group II showing an obvious depletion of reticular fibers deposition when compared with group I. They appeared as dark grey thin fibers in the peripheral capsule and few fine fibers strands dispersed around lymphoid follicle and lighter in both color and recurrence than group I. (c, d & e) Groups III, IV, &V showing a slight increase in the content and recurrence of the stained reticular fibers under the capsule (star) than in group II. A marked increase in the reticular fibers strands in the last group than both two previous groups. Reticulin stain. X40

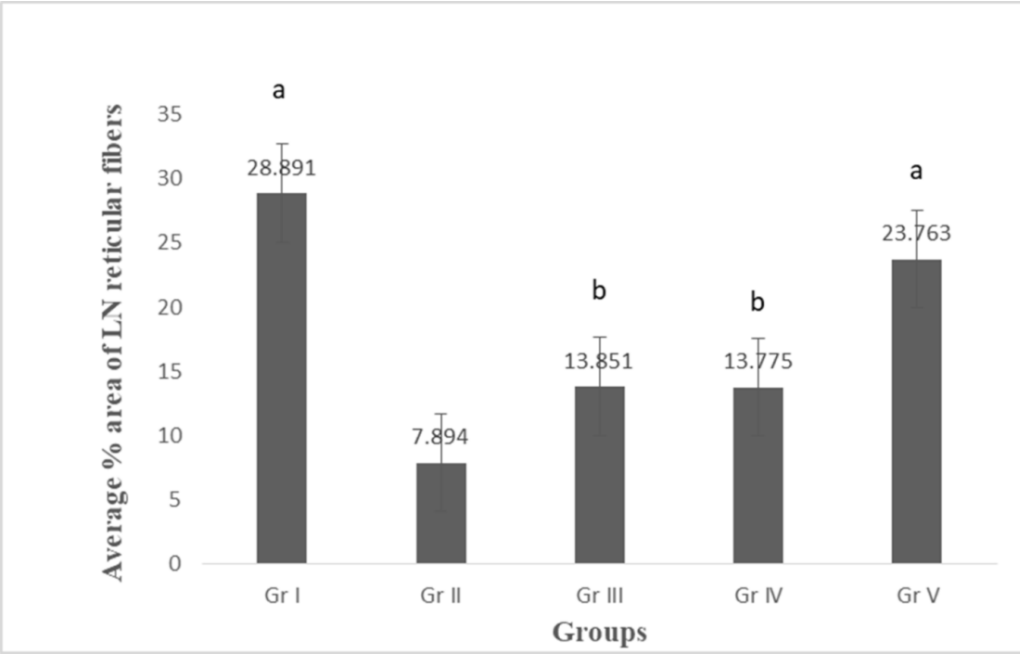

Figure 4. Histogram showing the area fraction of the reticular fibers in the LN of the different groups. The similar letters indicate there is no significance; the different letters indicate there is significance between groups.

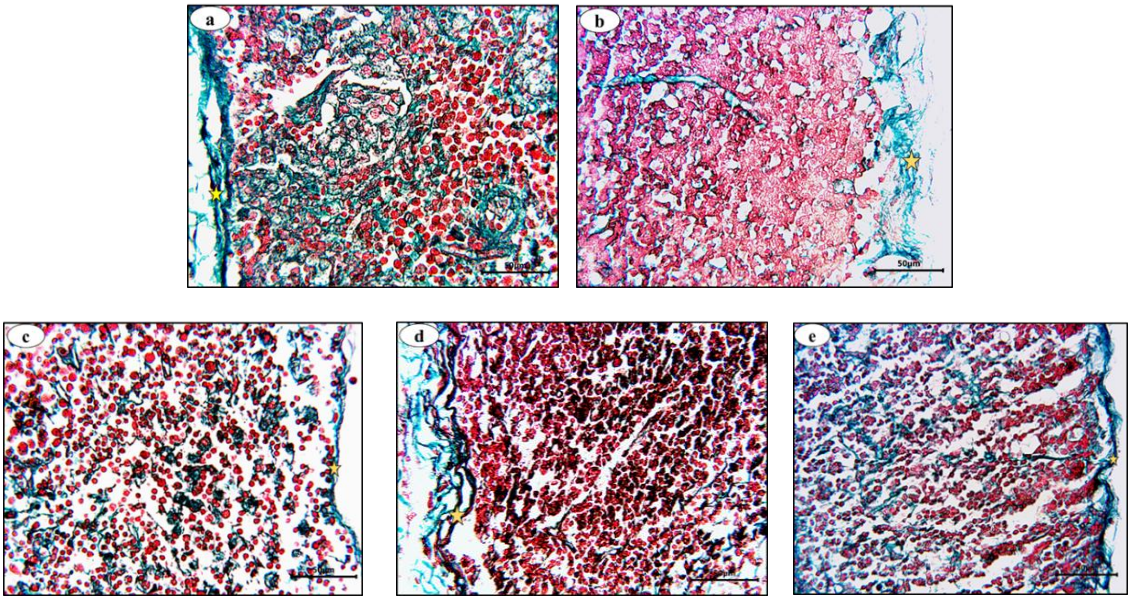

Figure 5. (a) Axillary lymph node section of the control group I showing normal area and distribution of collagen fibers, which take the blue color, in the capsule (star) and the ground substance of the cortex. (b) Group II showing a marked decrease in collagen fibers content and distribution in the cortex when compared with group I. (c and d) Groups III, and IV

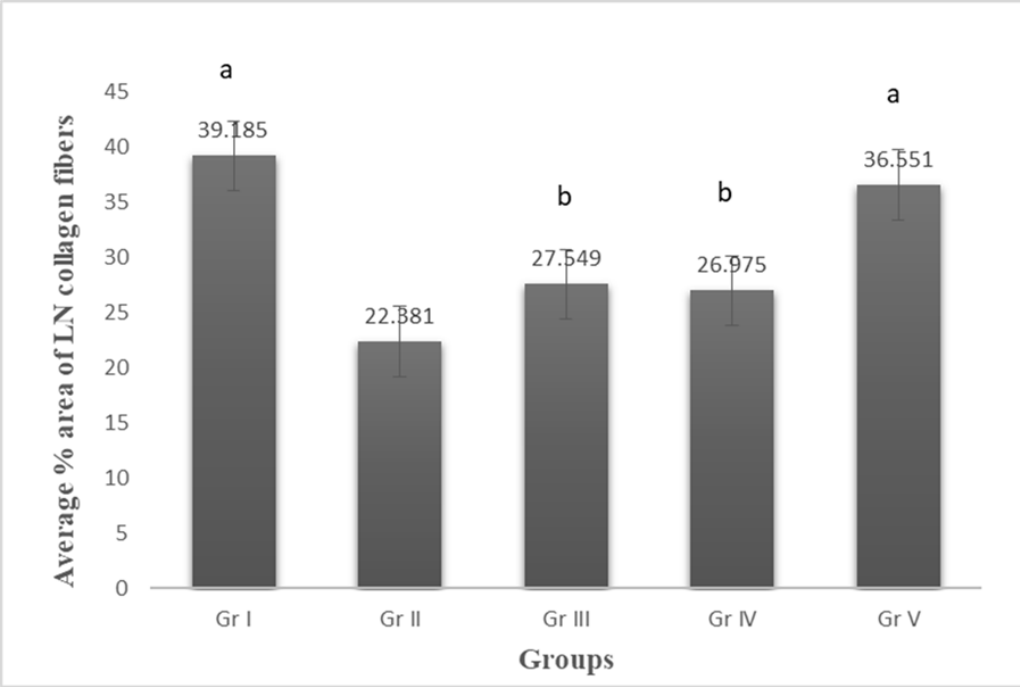

Figure 6. Histogram showing the area fraction of the collagen fibers in the LN of the different groups. The similar letters indicate there is no significance, the different letters indicate there is a significance between groups.

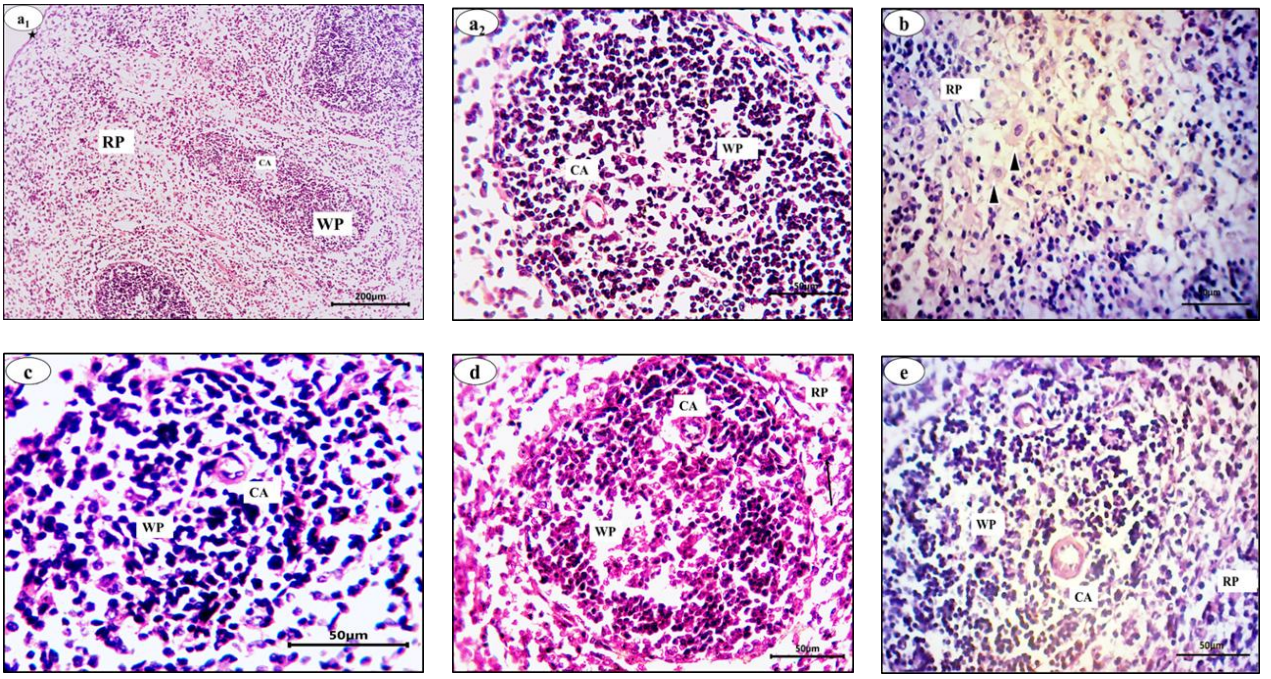

Figure 7. (a_1) Spleen section of control group I showing normal spleen structure of red (RP) and white pulp (WP) with central artery (CA), the capsule (star) surrounds the organ, H&E, X10. (a_2) High magnification of a_1 showing normal cellular components of red and splenic follicle of white pulp with central artery. (b) Group II showing marked edema with appearance of histiocytes (triangle). (c) Group III showing normal cellular components of splenic structures white and red pulps. (d) Group IV showing few

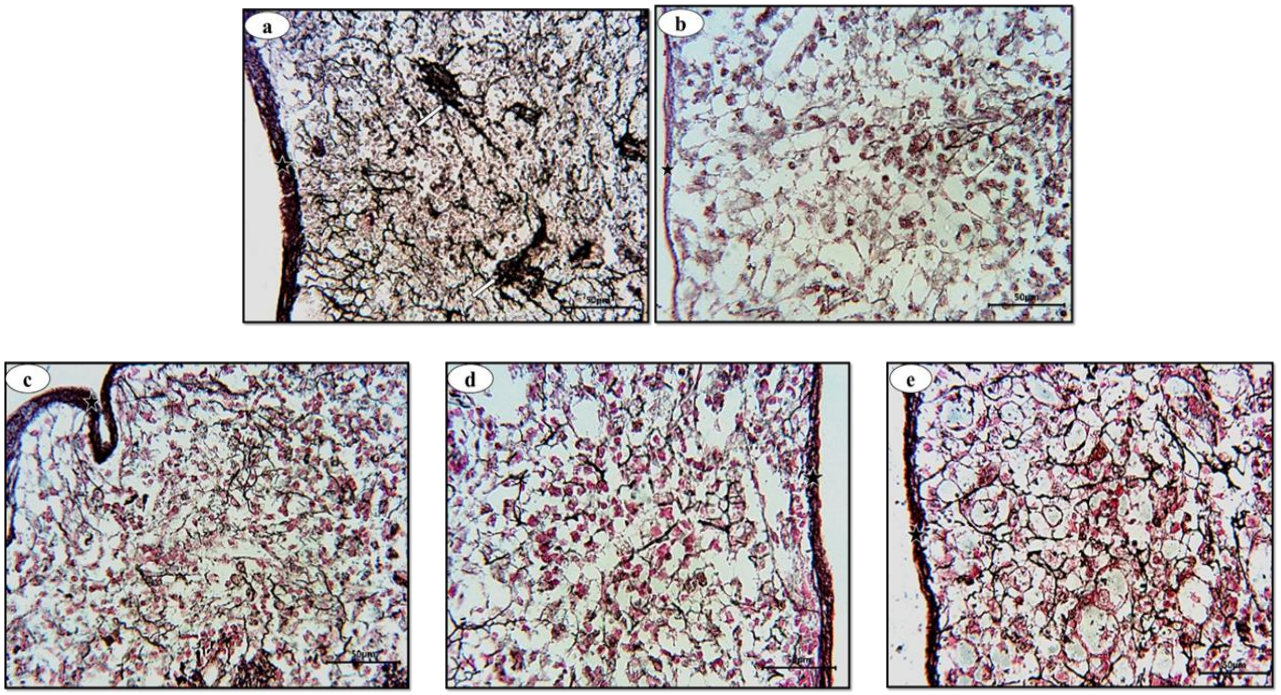

Figure 8. (a) Spleen section of control group I showing deeply stained dark grey to black reticular meshwork in the dense connective tissue capsule (star) surrounding the spleen, in trabeculae (white arrows), and in the stroma. (b) Group II showing an obvious decrease in the reticular fibers content of the capsule and the stroma when compared with control group I. (c, d, & e) Groups III, IV, & V showing a slight increase in the content and recurrence of the reticular fibers than group II (the capsule is thicker), marked increase in the content and recurrence in the last group (V) than the previous two groups. Reticulin stain, X40

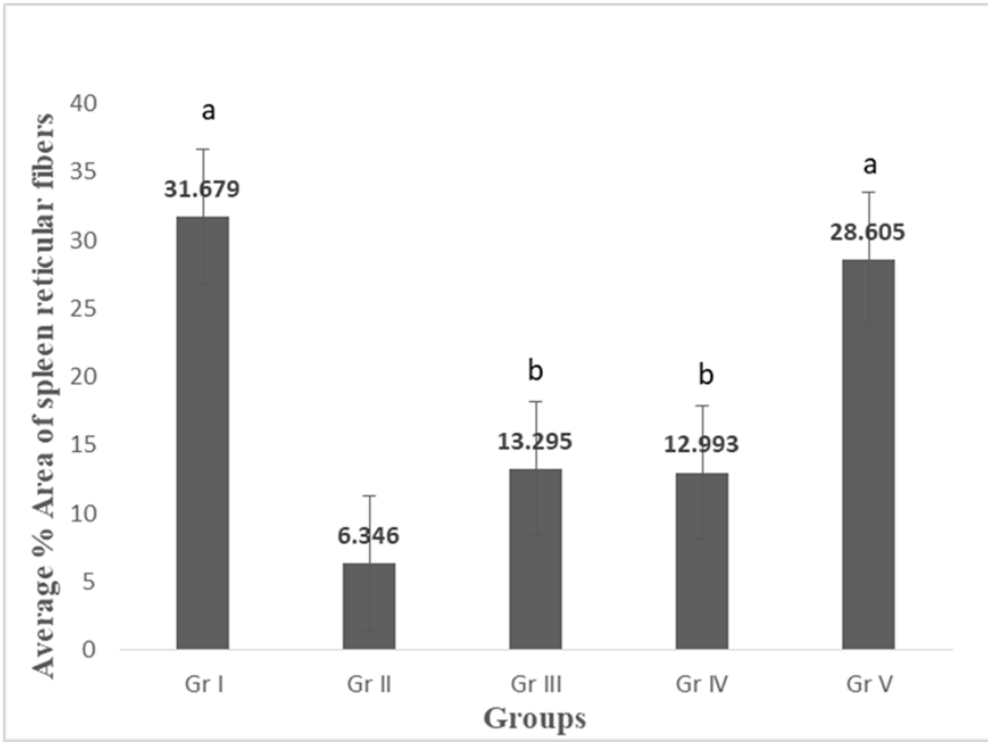

Figure 9. Histogram showing the area fraction of the reticular fibers in the spleen of the different groups. The similar letters indicate there is no significance; the different letters indicate there is a significance between groups.

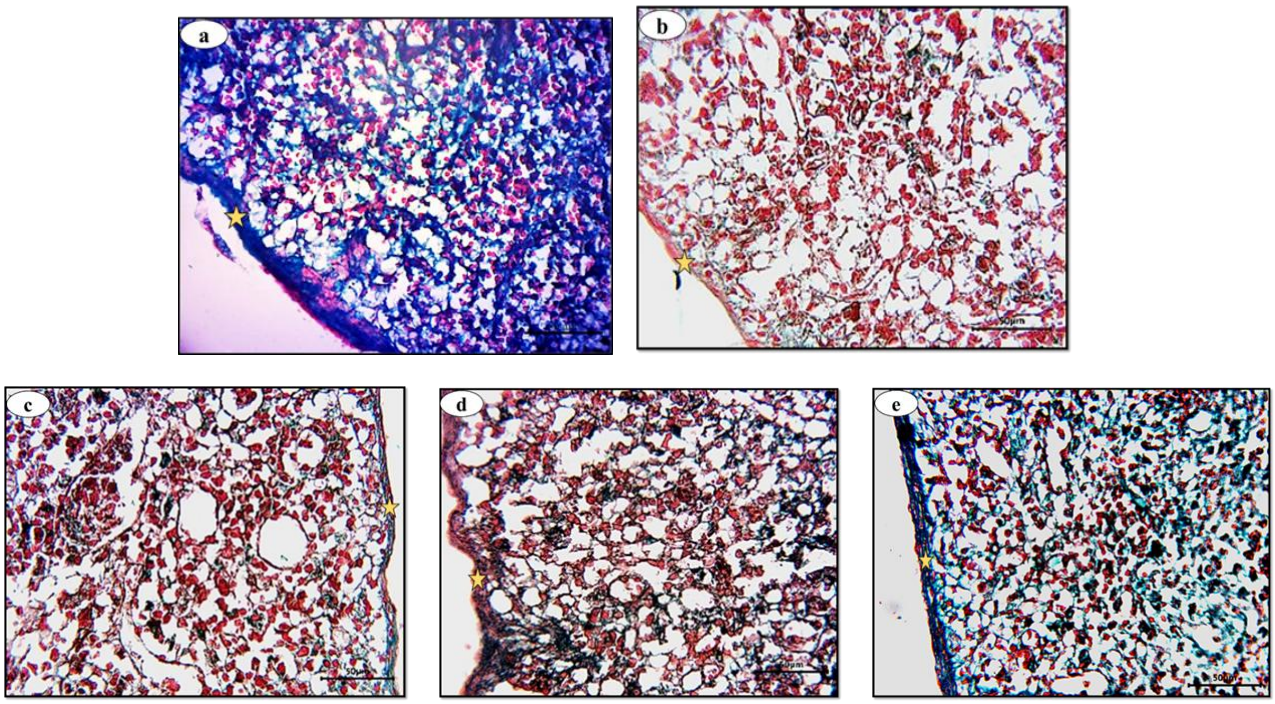

Figure 10. (a) Spleen section of control group I showing the normal content and distribution of the blue collagen fibers in capsule (star) and the stroma. (b) Group II showing an obvious decrease and faint staining collagen fibers. (c & d) Groups III and IV showing a slight increase in the collagen fibers content than group II. (e) Group V showing a sharp increase in collagen fibers content than groups III and IV. Masson trichrome, X40

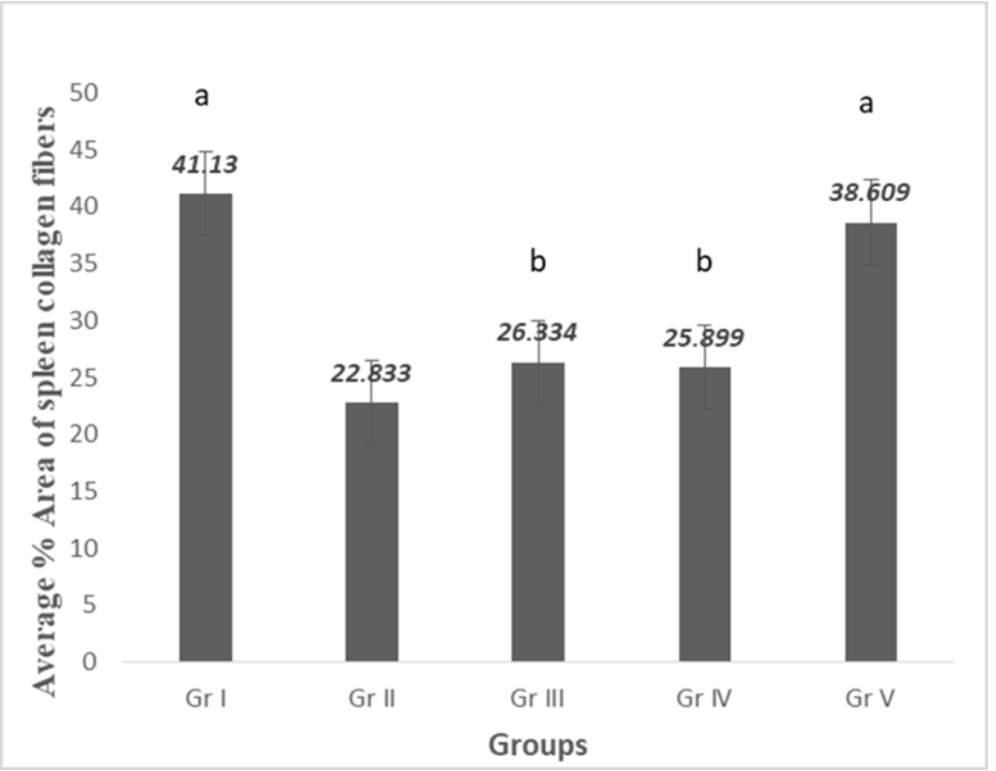

Figure 11. Histogram showing the area fraction of the collagen fibers in the spleen of the different groups. The similar letters indicate there is no significance; the different letters indicate there is a significance between groups.

4. DISCUSSION

RA affects joints, as well, extra-articular manifestations have been reported in human patients [27, 28]. The purpose of the current study is to investigate the oral administration effect of ginger and curcuma, separately and in combination, on LNs and spleen of CIA rat model. Several reports concordant us and suggested the anti-arthritic efficacy of ginger and curcuma, either separately [29-43] or in a mix [44, 45].

Scientists direct their efforts towards understanding the drainage LNs in RA patients to understand the arthritic behavior in the body [16]. The

juxta-articular LNs (located nearby the joints) play an important role in regulating the inflammatory actions [16]; hence**, t**he axillary lymph nodes which are close to the injection site (forelimbs) were selected in our study.

Our results showed an increase in the LN size of arthritic rats than controls. This is in compliance with a previous report that showed increase sizes of the drainage LNs in RA rat model [19]**.** Lymphadenopathy (increase in LN size or LN enlargement) was reported in the RA patients [46-49]. Macrophages (histiocytes in tissue), that were showed in control sections, are normal immune cells, derived from blood monocytes, and found in many parts of the body especially in bone marrow, bloodstream, skin, liver, lungs, lymph nodes, and spleen. They cling to the reticular meshwork like spiders on a web and snare bacteria, cell debris, red blood cells, carbon, and other particulates suspended in the lymph as it flows through the meshes of this biological filter. Marked sub-capsular distentions with monocytoid–like cells were detected in the axillary LN sections of arthritic animals. Monocytoid cells are unusual subsets of LN B-cells indicating LN reactivity. Morphologically, they are characterized by more abundant cytoplasm and round to oval nuclei similar to monocyte-derived cells, hence their name [50]. One more study reported polyclonal plasma cell infiltration in the interfollicular area and in the increased germinal centers (GCs) as well as a vascular proliferation to a moderate degree [51]. Further literature in 2013 revealed LN atrophy with medullary hyperplasia and granulomas in the LN sections of arthritic rats [19]. Many studies reported that histiocytes accumulation could occur in LNs in response to diverse stimuli, including infections, foreign materials, tumors, and autoimmune diseases such as RA [52-54].

The presence of monocytoid cells under the LN capsule of arthritic rats indicates the inflammatory action, due to immunization, that happened in animals' bodies. In both ginger and curcuma treated groups, the histiocytes replaced the monocytoid cells in arthritic rats, indicating the ameliorating action of both herbs. Fibroblasts, in ginger treated sections,

are an indication of pathological healing conditions in the tissue, we could not observe these cells in curcuma administered rats. This suggests that the role of ginger in ameliorating inflammation may be better than curcuma in the used doses. This is reverse as a previous study that used Adjuvant Induced Arthritis arthritic rat model and concluded that the curcuma was more effective in alleviating the inflammation than ginger [44]. In ginger/curcuma treated rats, no aggregation of monocytoid cells or histiocytes was noticed, this suggests that the ameliorating action of the combined two herbs is better than any of them separately. This is in parallel to a report that concluded that the two herbal mixtures had beneficial effects on the articular and extra-articular complications [45].

Reticular fibers are abundant in LN and spleen to form framework supports the cellular components, they are secreted by the sponge-like, stellate, elongated, spindle shaped fibroblastic reticular cells [55], they are composed of thin and delicately woven strands of collagen type III which was found to be dense in the interfollicular cortex, and peripheral paracortex while scarce in the follicles and central paracortex [56]. They are not visible with H&E stain. So, they are stained by silver (reticulin stain) which renders them black.

In 1970, It has been proved that, in the enlarged LNs sections stained with reticulin, the separation between the reticular fibers was increased than that of the normal [57]. This is compatible with our results where an obvious decrease in reticular fibers content of the arthritic LNs sections in compared with controls has been detected. We could explain that the LN enlargement wasn't accompanied by a proliferation of its supporting structures (reticular fibers). Administration of ginger, curcuma, and their mixture caused reduction in the LN enlargement, consequently, reduction in the separation between the reticular fibers and the reticular fibers area fractions in our image analysis results versus those of arthritic rats.

The reticular fibers consist of collagen fibrils core enveloped in a basement membrane layer [55]. Consequently, the collagen fibers, stained with Masson trichrome, behaved the same as reticular fibers, stained with reticulin in our groups sections.

In most RA cases, as be observed in this study, there are no detective changes in the spleen morphology, accordingly, spleen studies in RA patients and autoimmune diseases are scarce [58, 59]. Splenomegaly (spleen enlargment) has been reported in only 20 % of chronic autoimmune diseases [60]. On the other hand, reports included a necrotizing process, with haematoxyphilic bodies in both spleen and lymph nodes [61]. Furthermore, The spleen showed focal and extensive histiocytic necrotizing lesions and white pulp follicular hyperplasia in some reports [58]. In our study, edema and histiocytes were observed in the spleen of arthritic group. The arthritic treated groups showed normal spleen cellularity, except histiocytes were observed in the spleen of curcuma-treated rats. This suggests that the ameliorating effect of ginger separately or in combination with curcuma is better than treating with curcuma alone in the used doses.

Image analysis data of the reticular and collagen fibers stained sections in LN and spleen support the microscopic findings where arthritic animals showed a significant decrease in their contents than controls due to arthritis. The significant increase in those fibers contents in all herbs treated groups compared to arthritic untreated one indicates the ameliorating action of both herbs separately or in combination. The significant difference between both ginger group and curcuma group in comparison to the combined group indicates that the ameliorating effect of the mixture of two herbs is better than any of them alone.

Many literatures explained the efficacy of ginger and curcuma or their constituents as anti-inflammatory agents. Moreover, gingerols could inhibit the synthesis of some inflammatory mediators as prostaglandins (PG) and leukotrienes *in vitro* [40]. Ginger extracts inhibited the expression of chemokine in the cell line of human monocytes and beta-amyloid peptide-induced cytokine [62]. In addition, to prevention more progression of arthritis via suppression of both Monocytes Chemoattractant Proteins (MPC-1) and IFN-γ activated protein (IP-10) in

human synoviocytes [63]. It has been proved that the ginger anti-inflammatory effect is similar to Betamethasone is occurred by inhibiting the IL-1 and IL-6 cytokines production [38]. Additionally, ginger extracts can make strong inhibition of Cyclooxygenases COX-1 and COX-2 enzymes, the pro-inflammatory cytokines (IL-12, TNF- α, and IL-1 β), leukotrienes, and pro-inflammatory chemokines [37]. Curcumin reduced the angiogenesis-linked genes expression, vascular endothelial growth factor (VEGF), and matrix metalloproteinase-9 (MMP-9) that responsible for the formation of new blood vessels [64]. In addition, curcumin inhibits the human synovial fibroblasts growth through apoptosis induction and causes PGE2 release as well as COX-2 expression [65]. It also lowers the expression of adhesion molecules on the surface of monocyte [42]. Cyclo-curcumin treatment can inhibit the release of TNF- α from LPS-stimulated macrophages in human [66].

CONCLUSION

We explained the lymph node and spleen histopathological appraisals in the CIA rat model and showed the ameliorated efficacy of ginger, curcuma, and their mixture. Our results showed that ginger and curcuma, alone or in combination could ameliorate the histopathological appraisals in the spleen and LN. We observed that the ginger/curcuma mixture could retrieve the normal spleen and lymph node histology better than any of them alone.

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الملخص العربى

التهاب المفاصل الروماتويدي (RA) هو مرض مناعي ذاتي مزمن والتهابي وتقدمي يتميز بالعديد من التأثيرات على المفصل وخارجه بالاضافه الى التاثيرات الجهازية الداخليه. يبحث العلماء عن علاج بالأعشاب (مثل الزنجبيل والكركم) التي لها خصائص مضادة للالتهابات وتسبب آثارًا جانبية أقل من الأدوية العادية. في الدراسة الحالية ، تم استخدام أحد أمراض المناعة الذاتية التجريبية (نموذج الفئران الناجم عن التهاب المفاصل الناتج عن الكولاجين) (CIA) الذي يشترك في العديد من صفات التهاب المفاصل لفحص التقييمات المرضية للنسيج في العقدة الليمفاوية والطحال لدى الفئران بالإضافة إلى التحسن الناتج من تناول الزنجبيل والكركم بالفم بعد ٢٠ يومًا متتاليًا. تم تقسيم خمسين ذكر من الجرذان البيضاء إلى ٥ مجموعات متساوية. المجموعة الأولى كانت الضابطه، المجموعة الثانية والثالثة والرابعة والخامسة كانت مصابة بالتهاب المفاصل ، المجموعة الثالثة تلقت الزنجبيل والرابعة تلقت الكركم والخامسة تلقت خليط من كليهما عن طريق الفم. تم جمع الغدد الليمفاوية والطحال ومعالجتها من أجل الفحص وتم تقطيعها وصباغتها بكل من (الهيماتوكسيلين والايوسين) ، الريتيكيولين و ماسونترايكروم. أظهرت نتائجنا أن قطاعات العقد الليمفاويه من المجموعة الثالثة تسللًا للنسيج يحل محل الخلايا الشبيهة بالخلية الأحادية في المجموعة الثانية ، وأظهرت المجموعة الخامسة استعادة بنية العقد الليمفاويه الطبيعية. أظهرت أقسام الطحال مكونات خلوية طبيعية في المجموعات المعالجة بدلاً من زيادة عدد المنسجات في المجموعة الثانية. أظهرت محتويات ألياف الريتيكولين والكولاجين في الطحال و العقد الليمفاويه زيادة معنوية في المجموعة الضابطة مقارنة بالمجموعة الثانية ، وأظهرت المعالجة بكلتا الأعشاب بشكل فردي أو مجتمعي زيادة معنويه في محتويات شبكية الكولاجين ($P < 0.05$).