The Possible Effect of Ivermectin on Vasculitis Rat Model: Histopathological Study

Teeba Sarem Sadeq*, Nadia H Mohammed, Inam Sameh Arif*, Sayed Mahmood Alqallaf*****

Department of Pharmacology and Toxicology, College of Pharmacy, University of Mustansiriyah, Baghdad, Iraq

****Department of Microbiology and Immunology, College of Medicine, University of Mustansiriyah, Baghdad, Iraq***

*****Pharmacy Department, College of Health and Sport Sciences, University of Bahrain, Kingdom of Bahrain***

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Corresponding Author email:
teeba_sarim@uomustansiriyah.edu.iq
Orcid: https://orcid.org/0009-0003-9307-3005

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Abstract:

Vasculitis is an inflammation in the blood vessel wall, with variation in severity and the system
affected. Vasculitis pathogenesis is caused by three possible mechanisms, including autoantibodies anti-neutrophil cytoplasmic antibodies, activation of T lymphocyte cells, and immune complexes.

Various pathogens stimulate innate and adaptive immune systems, modulating the expression of pro-inflammatory cells, cytokines, and the complement system, that have a role in vasculitis progression. Ivermectin is an anti-parasitic drug, effective in treating parasitic infections. Thirty-three male albino rats were used for the experiment and divided into five groups. The disease was induced in all groups except group A (control), rats received the vehicles used in dissolving drug and chemicals. Group B (induction group) received Ovalbumin and Lipopolysaccharide. Group C, group D, and group E (Ivermectin groups) received 0.5mg/kg, 1 mg/kg and 1.5 mg/kg of Ivermectin, respectively, for seven days. All doses were injected by intraperitoneal route. Results of histopathological evaluation of lung sections of Ivermectin groups show a decrease in the inflammation severity, alveolar destruction, and vascular congestion and dilatation, with the absence of: hemorrhage, thrombi, and fibrinoid wall necrosis in comparison to the disease group. Therefore, the Ivermectin drug might have a protective effect against vasculitis.

Keywords: Vasculitis, Ivermectin, histopathological evaluation, alveolar destruction, vascular congestion and dilatation.

التأثير المحتمل للايفرمكتين على التهاب الاوعية الدموية في نموذج الجرذان: دراسة النسيج المرضي

طيبة صارم صديق*، نادية حميد محمد، انعام سامح عارف*، سيد محمود القلاف*****

فرع الأدوية والسموم، كلية الصيدلة، الجامعة المستنصرية، العراق

****فرع الأحياء المجهرية والمناعة، كلية الطب، الجامعة المستنصرية، العراق***

*****قسم الصيدلة، كلية العلوم الصحية والرياضية، جامعة البحرين، مملكة البحرين***

خلاصة

التهاب الأوعية الدموية هو مصطلح عام يشير إلى إلتهاب في جدار الأوعية الدموية والأنسجة المحيطة، مع إختلاف في شدة المرض والجهاز المصاب. يحدث إلتهاب الأوعية الدموية عن طريق ثلاث آليات محتملة والتي تشمل الأجسام المضادة الذاتية

وتنشيط الخلايا اللمفاوية والمجمعات المناعية. مسببات المرض المختلفة تعمل على تحفيز الأجهزة المناعية الفطرية والتكيفية، والتحكم بإظهار خلايا الإلتهاب ومساعدات الإلتهاب سيتوكين، والنظام التكميلي، التي لها دور في تطور إلتهاب الأوعية الدموية. الإيفرمكتين هو دواء مضاد للطفيليات، فعال في علاج الإلتهابات الطفيلية. تم إستخدام ثلاثة وثلاثين ذكور الجرذان في التجربة وتم تقسيمهم إلى خمس مجموعات. تم إحداث المرض في جميع المجموعات باستثناء المجموعة (أ) (السيطرة)، حيث تلقت الجرذان المركبات المستخدمة في إذابة الدواء والمواد الكيميائية. تلقت المجموعة (ب) (المجموعة المحفزة) الاوفالبومين وعديد السكريات الدهنية. المجموعة (ت) والمجموعة (ث) والمجموعة (ج) (مجاميع الايفرمكتين) تلقت 0.5 ملغم / كغم، 1 ملغم / كغم و1.5 ملغم / كغم من الإيفرمكتين على التوالي لمدة سبعة أيام. تم حقن جميع الجرع عن طريق غشاء الصفاق. أظهرت نتائج التقييم النسيجي المرضي لمقاطع الرئة في مجاميع الإيفرمكتين تثبيطاً في شدة الإلتهاب وتمزق الحويصلات الهوائية وإحتقان الأوعية الدموية وتوسعها، مع عدم وجود نزف وتخثر وتنخر في جدار الفبرينويد مقارنة بمجموعة المرض. لذلك ربما يملك دواء الإيفرمكتين تأثيرًا وقائيًا ضد إلتهاب الأوعية الدموية.

الكلمات المفتاحية: إلتهاب الأوعية الدموية ،ايفرمكتين ،تقييم النسيج المرضي ،تمزق الحويصلات ،احتقان وتوسع الاوعية.

Introduction

Vasculitis is a general term for inflammation in the blood vessel wall and surrounding tissue, with variation in severity and the system affected(1). From a pathological view, vasculitis is classified according to the target site, pathogenesis, etiology and the size of the blood vessels (2). Vasculitis pathogenesis is caused by three possible mechanisms, including autoantibodies of anti-neutrophil cytoplasmic antibodies, activation of T lymphocyte cells, and immune complexes (3,4).

Activation of innate and adaptive immune systems modulates the expression of pro-inflammatory cells and cytokines that have a role in the pathogenesis of vasculitis. Various microbes and allergens stimulate the expression of pattern recognition receptors such as toll-like receptors (TLR), CD4 co-receptors, and activation of macrophages, dendritic cells, and monocytes (5,6). Thus, leads to increase the secretion of pro-inflammatory cytokine, like interleukin-6 that links innate to adaptive immune responses by controlling the differentiation of T helper cell 17, B-cell, and plasma cell (7).

This sequence impairs vascular homeostasis and increases the risk of vascular congestion, dilatation of vessel walls, thrombi formation, and the incidence of vascular events (8). Pulmonary tissue may be involved in various types of vasculitis, clinical manifestation varies and includes rapture in the alveolar wall, interstitial oedema, fibrinoid wall necrosis and increased inflammatory cell infiltration (9).

Ivermectin is an anti-parasitic drug that acts by blocking synaptic transmission in invertebrates. It is a lipophilic drug that cannot cross the blood-brain barrier (10). It is considered a safe drug and highly effective for treating onchocerciasis (river blindness) and parasitic infections such as head lice and scabies in humans (11). Another possible action is that the drug may have a role in the attenuation of various viral replications(12,13).

Materials and Methods

Materials

Drug and chemicals used in the experiment are listed in table (1), along with their company and origin.

Table (1). Drugs and chemicals.

Drug or chemical	Company	Origin
Ivermectin crude powder	Meryer	China
Propylene glycol solution	BDH	British
Glycerol solution	Merck	Germany
Ovalbumin powder	Macklin	China
Lipopolysaccharide powder	Solarbio	Japan

Methods

Ivermectin preparation

Ivermectin powder (CAS number 70288-86-7, 98%) was purchased from Meryer/China. Ivermectin is a hydrophobic drug(14), therefore the powder was dissolved in a mixture of propylene glycol and glycerol (60:40% v/v) (15). A stock solution equal to 10 mg of Ivermectin was prepared using a magnetic stirrer device for 10 minutes to enhance powder and liquid dissolution. Ivermectin groups received the drug according to the rat's average weight, and injected by intraperitoneal route using a 1cc syringe.

Vasculitis induction

Vasculitis was induced in experimental rats using Ovalbumin (16) and Lipopolysaccharide(17). All the experiment groups (except the control group) received nine doses of Ovalbumin (7.5 mg/kg) from day 8 to day 32 every third day, followed by three doses of lipopolysaccharide (2 mg/kg) on the 27th, 30th, and 33rd days. Animal groups received doses modified according to the rat's average weight. Doses were injected by intraperitoneal route using a 1cc syringe(18).

Experimental design and animals grouping

Thirty-three male albino rats weighing between 120 g and 130 g, and aged almost six weeks were used. Rats were acquired from the animal house /Iraqi Center for Cancer and Medical Genetics Research/ Mustansiriyah University. The experimental animals were acclimatized in a well-ventilated, sterilized cage, 12/12 hours of light/dark cycle with free access to food and water in the animal house at the College of Pharmacy/ Mustansiriyiah University. The study was conducted after approval from the scientific and animal ethics committee at the College of Pharmacy/ Mustansiriyah University. Approval number: 22, date 10/7/2023.

Thirty-three male albino rats were divided into five groups, including:

1. Group A (n=6): Control group, rats received a propylene glycol and glycerol mixture (60:40% v/v) for seven days, followed by normal saline injection, doses were injected by intraperitoneal route as the exact timeline of the disease induction group.
2. Group B (n=6): disease induction group, rats received propylene glycol and glycerol mixture (60:40% v/v) for seven days, followed by nine doses of Ovalbumin (7.5 mg/kg) from day 8 to day 32 every third day. Then, three doses of lipopolysaccharide (2 mg/kg) on the

27th, 30th, and 33rd days, all doses were injected by intraperitoneal route.

3. Group C, group D, and group E (n=7 in each group): (Ivermectin groups) rats received Ivermectin dissolved in propylene glycol and glycerol mixture (60:40% v/v) for seven days. Group C rats were injected with 0.5 mg/kg, group D with 1 mg/kg, and finally, group E with 1.5 mg/kg, followed by nine doses of Ovalbumin (7.5 mg/kg) from day 8 to day 32 every third day, then, three doses of Lipopolysaccharide (2 mg/kg) were administered on the 27th, 30th, and 33rd days; all doses were injected using the intraperitoneal route.

Histopathology assay

Rats were received intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) (19). Lung tissue was isolated and kept in a 10% formalin buffer saline for histopathological evaluation of inflammatory indications in lung tissue(20). The process involved the following steps:

I. Fixation: All samples were fixed directly in 10% neutral buffered formalin at room temperature for 24 hours.

II. Dehydration: Water removal from samples was done manually by soaking them in ethanol serial concentrations.

III. Clearing and embedding: Xylol for 2 hours was used to ensure adequate dehydration of the lung tissue and for fat removal. A paraffin bath was used for lung tissue embedding at 57°C melting point.

IV. Sectioning: Microtome was used to prepare sliced sections with 4 µm thickness, then placed into a drying oven.

V. Staining: sections were stained with Hematoxylin and Eosin. Hematoxylin is added for 10 minutes and then washed with distilled water. Followed by Eosin stain for 5 minutes application and then washed with distilled water.

Histopthological evaluation of lung tissue

The lung tissue of each group was evaluated by the same pathologist to assign the degree of inflammation and the variation among experiment groups. The presence of dilated blood vessel walls, congested blood vessels, and fibrinoid necrosis were scored 0 to 1. The degree of ruptured alveolus, inflammatory cell infiltrate and hemorrhage were scored 1 to 3, as illustrated in table (2). Total lung tissue histology scores (maximum 12) were obtained from the sum of scores (21).

Table (2). Histopathology scoring for lung inflammation (21)

Inflammation findings	Presence/extent of findings	Score
Congested blood vessel	+ –	1 0
Dilated blood vessel wall	+ –	1 0
Fibrinoid necrosis	+ –	1 0
Ruptured alveoli	1-33 % 34-66% 67-100 %	1 2 3
Inflammatory cell infiltrate	1-33 % 34-66% 67-100 %	1 2 3
Hemorrhage	1-33 % 34-66% 67-100 %	1 2 3

Result and discussion

Result

Effect of Ivermectin on histopathological changes of lung tissue in male rats.

Lung tissue sections were evaluated to differentiate inflammatory and morphological changes among the main study groups. The stained lung sections with Hematoxylin and Eosin (H&E) were observed under the microscope.

A section of **group A** rat lung tissue is shown in figure (1). It shows normal lung architecture with expanded alveoli lined by normal alveolar walls with macrophages, pneumocytes, and alveolar capillaries.

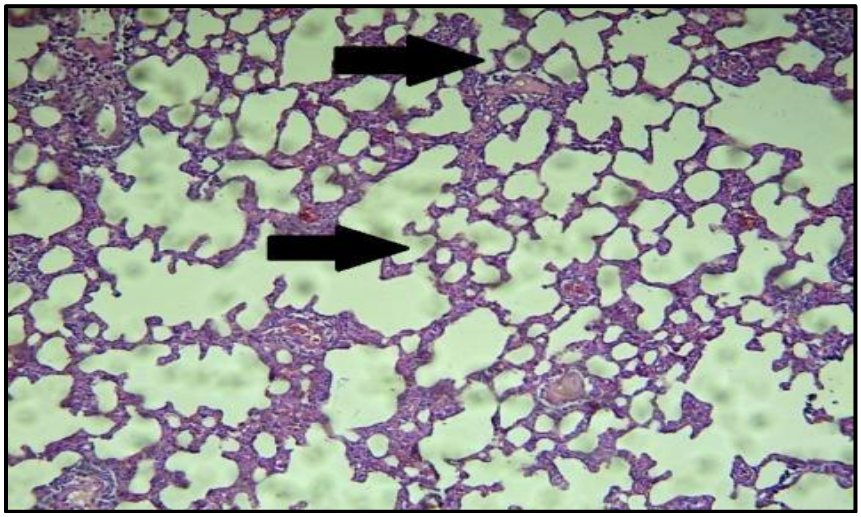

Figure (1). Histopathological section of rat lung tissue (group A) (100x magnification, Hematoxylin-Eosin). Showing normal expanded alveolus (black arrow).

The histopathological section of **group B** rat lung tissue displays moderate to severe interstitial acute pulmonary inflammation in figure (2). Multifocal areas with inflammatory cells, primarily macrophages, neutrophils, lymphocytes, and eosinophils. Vascular congestion with thickness in the vessel wall, thrombosis and fibrinoid wall necrosis (fibrinoid vasculitis) are all present.

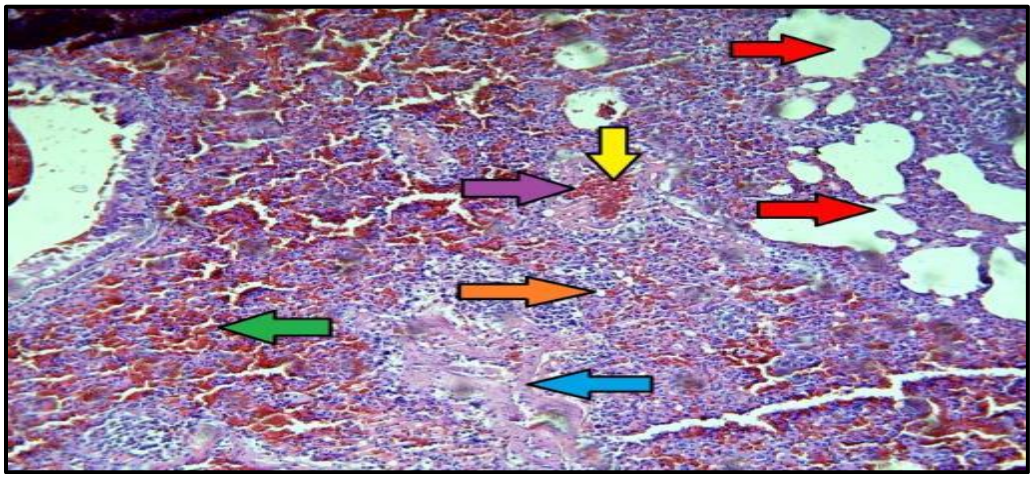

Figure (2). Histopathological section of rat lung tissue (group B) (100x magnification, Hematoxylin-Eosin). Inflammatory cells infiltrate (orange arrow), vascular congestion (purple arrow), thicken blood vessel wall (yellow arrow), thrombosis (green arrow), fibrinoid wall necrosis (blue arrow) and ruptured alveolus (red arrow).

The histopathological section of rat lung tissue from **group C** (Ivermectin group, received 0.5 mg/kg) with mild to moderate inflammation is displayed in figure (3). Ruptured alveoli, vascular dilatation, congestion, thrombosis and inflammatory cell infiltration were found upon examination. Other areas display normal alveoli. There is no evidence of interstitial oedema or fibrinoid wall necrosis.

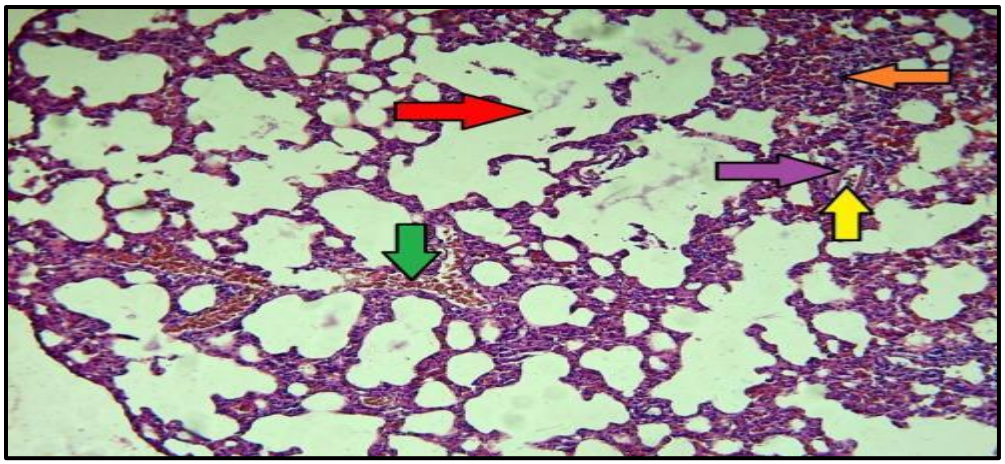

Figure (3). Histopathological section of rat lung tissue (group C) (100x magnification, Hematoxylin-Eosin). Inflammatory cells infiltrate (orange arrow), vascular congestion

(purple arrow), thicken blood vessel wall (yellow arrow), thrombosis (green arrow), and ruptured alveolus (red arrow).

The second Ivermectin group section (**group D**, received 1 mg/kg) in figure (4) shows lung tissue with mild inflammation; minor distortion of alveoli, blood vessel congestion and dilatation. Inflammatory cells reduced in number with no evidence of interstitial oedema, fibrinoid wall necrosis, and thrombosis upon microscopic examination.

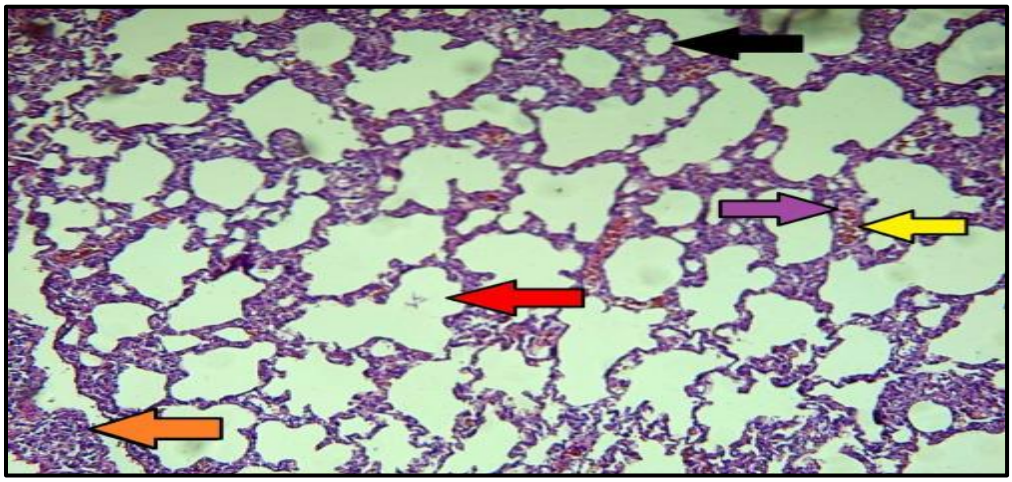

Figure (4). Histopathological section of rat lung tissue (group D) (100x magnification, Hematoxylin-Eosin). Normal expanded alveoli (black arrow), inflammatory cells infiltrate (orange arrow), vascular congestion (purple arrow), thicken blood vessel wall (yellow arrow) and ruptured alveolus (red arrow).

Finally, a section of rat lung tissue from **group E** (Ivermectin group, received 1 mg/kg) in figure (5) shows multi-focal areas with mild inflammatory reactions. Few vascular dilatation and congestion were observed. Absence of thrombosis, interstitial oedema, and fibrinoid wall necrosis under microscopic examination. Emphysema was observed in some regions of the tissue.

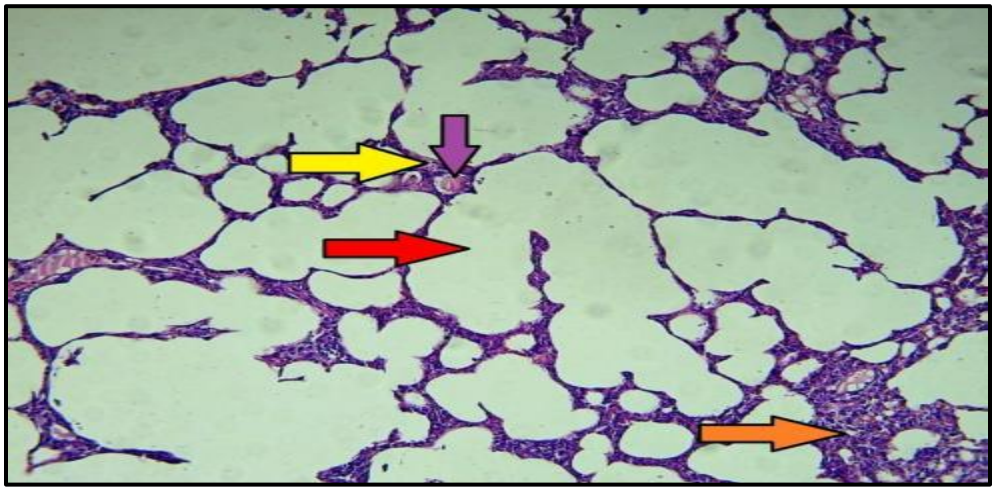

Figure (5). Histopathological section of rat lung tissue (group E) (100x magnification, Hematoxylin-Eosin). Inflammatory cells infiltrate (orange arrow), vascular congestion (purple arrow), thicken blood vessel wall (yellow arrow) and ruptured alveolus (red arrow).

Histopathology scoring for lung tissue has benefits in obtaining semi-quantitative data and experimental groups comparison. The pathologist examined tissues at different levels. Followed by using scoring criteria "ordered'' to identify group-specific differences in tissue morphology as mentioned in table (3). The presence and degree of fibrinoid necrosis, congested blood vessels, dilated blood vessel wall, inflammatory cell infiltrate, hemorrhage, distortion and rupture of the alveoli were evaluated.

Table (3). Total score for pathological changes in lung tissue.

Group	Group A (control group)					Group B (disease group)					Group C (IVM group) 0.5 mg/kg					Group D (IVM group) 1 mg/kg					Group E (IVM group) 1.5 mg/kg				
Rat number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Dilated blood vessel wall	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1
Congested blood vessel	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Fibrinoid necrosis	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	1
Ruptured alveolus	1	1	1	0	1	2	3	3	2	3	2	2	1	1	2	1	1	1	1	1	1	2	1	2	1
Inflammatory cell infiltrate	1	1	1	1	1	2	3	3	2	3	2	2	1	2	2	1	1	1	1	1	1	1	1	1	1
Hemorrhage	0	1	0	1	0	2	3	3	2	2	1	2	1	1	1	1	1	0	1	1	1	1	2	1	1
Final score	5	5	3	4	3	9	12	12	9	11	8	9	6	7	8	5	6	4	6	5	5	7	5	7	6

Discussion

Histopathological sections of Ivermectin groups show agreeable results, and the drug may have a protective effect against vasculitis. The drug inhibits the disease severity in lung tissue and preserves endothelial and vascular integrity by reducing the stimulation of innate immune cells and the production of pro-inflammatory cytokines and chemokines. Thus, decreasing the initiation of adaptive immune cells, Th-cell differentiation and activation of B cell producing antibodies (4,22). Consequently, inhibiting further stimulation of complement pathways and the release of endothelin-1 and adhesion molecules. These sequences are responsible for causing vascular damage, endothelial cell rapture and thrombus formation by losing of endothelial defense mechanism. Ivermectin reduces fibrosis mediated by transforming growth factor-β and signal transducers and activators of transcription (STAT) activation (23). In addition, ivermectin reduces pulmonary oedema by activated and primed neutrophils that are responsible for damaging endothelial cells, alveolar epithelial cells and capillaries by producing reactive oxygen sepsis, proteolytic enzymes, and arachidonic acid metabolites (23,24).

The rats section in the second Ivermectin group (received 1 mg/kg) shows a similar tissue appearance for the control group. While third Ivermectin group sections (received 1.5 mg/kg) displayed reduce in inflammation with emphysema signs, resulted from activation and accumulation of inflammatory cells, release of neutrophil chemotactic factors, and proteinases. This sequence leads to reactive oxygen sepsis

production, excessive mucus secretion, alveoli damage and the development of emphysema (25).

Conclusion

Ivermectin can modulate the innate immune response by affecting pro-inflammatory cytokine production, such as Interleukin-6 and Toll-like receptor 4 expression and may have a protective role against pathogens that enhance vasculitis. Also, Ivermectin decreases Anti-neutrophil cytoplasmic antibody levels with a subsequent reduction in neutrophil priming, complement activation, and reactive oxygen species formation. This may protect the tissue from the destructive effect of vasculitis.

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