Research Article

Study of Anti-Inflammatory Effect of Dipyridamole by Evaluation Inflammatory Cells and Histopathology in Rat: Airway Models

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Abstract | Pneumonitis, another name for lung inflammation, can be caused by respiratory infections, exposure to pollutants or allergens from the air, and lung conditions such as chronic bronchitis or asthma. An inhibitor of phosphodiesterase enzymes 3 and 5, dipyridamole is a conventional antiplatelet drug that increases adenosine. The purpose of this study was to investigate whether dipyridamole plays a role as an anti-inflammatory to improve airway inflammation. A total of 24 healthy rats (albino, male), were weighted (150-300 g), were divided into four groups, each group consisting of six rats; Group A rats were given oral distal water and was considered a negative control group. Group B rats received distal water orally with ovalbumin (ova) sensitization, which was considered a positive control group. Group C rats were given dipyridamole (26.4 mg / kg) orally with sensitization to the ova. Group D rats received prednisolone (4.12mg/kg) orally with sensitization to the ova. Subsequently, the right lung was lavage, by insertion of a tracheal cannula, three times with 10 ml of normal saline (NS 0.9%) at 37 ˚C and the broncho alveolar lavage fluid (BALF) was sent for laboratory evaluations and the left lung was incised for histopathological evaluation. Data analysis indicated that a substantial decrease in total white blood cell counts (WBC) was observed (p-value <0.05) and cells including (neutrophils, mononuclear) in BALF of rats treated with dipyridamole (group C) and prednisolone (group D) compared to positive control (group B), but reduction in eosinophils cells in dipyridamole treated group C was non significant (p-value>0.05) there was also an improvement in histological characteristics regarding bronchial dilatation and improvement in the alveolar sac and modification of inflammatory cells in lung tissues in dipyridamole treated rats (group C) and prednisolone treated (group D) was observed in contrast to ovalbumin (ova) sensitized rats (group B). Collectively, dipyridamole has the ability to decrease inflammation to a lower limit in the airways in rats through its ability to decrease total and differential WBCs in BALF.

Keywords | Rat lung model, Dipyridamole, Inflammatory cells, Ovalbumin.

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INTRODUCTION

Pneumonitis, characterised by the lung inflammation, can be caused by respiratory infections, exposure to airborne pollutants or irritants, and lung conditions such as chronic bronchitis or asthma (Chen et al., 2018). Chronic lung inflammation can alter airway content, thickness, or volume over time, resulting in bronchiectasis. (Hill et al., 2019). These changes can also result in hypercapnia (Defnet et al., 2020).

Regarding eosinophils, which are significant inflammatory cells that play a pathogenic role in the pathobiology of airway disorders; in fact, their recruitment, maturation, activation, and survival within the airway lumen and bronchial wall are important pathogenic events (Lambrecht and Hammad, 2015; Brusselle et al., 2013). Inhaled allergens can activate several recognizing receptors once they pene-

trate the airway epithelium. This triggers a series of events that culminate in the production of Th2 cytokines (Larché et al., 2003).

Prolonged infiltration and degranulation of eosinophils leads to the continuous release of cytotoxic products, such as eosinophil cationic protein, main basic protein, neurotoxin derived from eosinophils, and eosinophil peroxidase. These factors play an important role in airway epithelium damage, goblet cell mucus overproduction, bronchial hyper responsiveness, and impaired ciliary beating (Bandeira-Melo et al., 2002; Peters-Golden and Henderson Jr., 2007; Hallstrand and Henderson Jr, 2010; Hall and Agrawal, 2014). Furthermore, growth factors, including transforming growth factor alpha (TGF-a) are produced by eosinophils (Humbles et al., 2004). In response to neutrophilic airway inflammation, T-helper1(Th1) cells, and particularly Th17 lymphocytes, are the initiators. (Aujla and Alcorn, 2011; Trejo Bittar et al., 2015). Specifically, the unique airway environment, which is composed of specialized costimulatory molecules and cytokines that drive the various Th cells, is vital for the commitment to the multiple Th lineages. (Vroman et al., 2015). Neutrophil-induced airway inflammation is frequently associated with the most severe asthmatic phenotypes (Newcomb and Peebles, 2013).

Interlukin-23 (IL-23) cytokine is crucial to preserve Th17 cell capacity to operate (McGeachy et al., 2009). Bronchial neutrophilia has been associated with cigarette smoking. (Thomson et al., 2004; Polosa and Thomson, 2013), Airborne pollutants could trigger severe asthma (Vroman et al., 2015). The innate immune response to inflammation, on the other hand, is highly dependent on monocytes, which circulate in the blood (Geissmann et al., 2003). Monocytes migrate to areas of inflammation, so it is essential for host defense (Ley et al., 2007). The release of monocytes from the bone marrow into the circulation depends on the chemokine receptor 2 and monocyte chemoattractant protein-1/monocyte chemoattractant protein-3. (Tsou et al., 2007; Serbina and Pamer, 2006)

Macrophages function as sentinels in tissues, detecting harm and infections. Macrophages have the ability to self-renew, examples of tissue-resident are alveolar macrophages in the lung and Kupffer cells in the liver (Yona et al., 2013; Hashimoto et al., 2013). Alveolar macrophages and interstitial macrophages that exist in the lung seem to be two different populations with different ancestry in the lung (Bharat et al., 2016). Alveolar macrophages in the lung act as "first responders" to tissue damage, particles, and infections. Together with alveolar epithelial cells, they make up the mucosal barrier of the lung. Therefore, alveolar macrophages have been associated with inflammatory and fibrotic lung conditions. Host defense is the important function macrophages play in, this is demonstrated by the fact that reduction of lung damage is associated with depletion of alveolar macrophages while simultaneously being linked to poor pathogen clearance (Ghoneim et al., 2013).

Adenosine receptors are found in T lymphocytes (Bours et al., 2006). Furthermore, T cells produce large amounts of chemokines and cytokines (Säfholm et al., 2015), which leads to increased proliferation of T cells and smooth muscle contraction in the airways in asthma (Ayakannu et al., 2019).

Dipyridamole is a long-standing antiplatelet and coronary vasodilator drug; By blocking platelet phosphodiesterase, it reduces platelet aggregation and causes accumulation of cyclic adenosine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP) (Kim and Liao, 2008).

The vasculature, particularly the endothelium, can benefit from dipyridamole's direct and indirect actions due to its anti-inflammatory, antioxidant, and proliferation-inhibiting qualities (Chakrabarti and Freedman, 2008).

MATERIALS AND METHODS

The drugs that were used in this study include dipyridamole tablets 75 mg (Medocheme Ltd- Cyprus, prednisolone tablets 5 mg (The State company for the pharmaceutical industry and medical appliances- Iraq), phenobarbital ampule 200 mg ml (IBN HAYYAN Pharmaceutical co.- Syria), 0.9% sodium chloride solution (Pharmaceutical solution industry- Saudi Arabia), whereas other substances were Ovalbumin powder (OVA) (RIEDEL-DE HAEN AG,SEELZE- HANNOVER-Germany), aluminum hydroxide powder (MERK Darmstadt, Germany), Formaldehyde 37% (Aqua medical- Turkey).

Animals

Twenty-four albino male rats were obtained from Nahrain Collage, Biotechnology Research Center, Animal facilities of Baghdad University and Faculty of Pharmacy, Basra University; they were kept under normal conditions of temperature (21 ± 4 ° C), humidity, and light-dark cycle (12 l/12 d) and received pelleted feed and reverse osmosis water. The Research Ethics Committee of the Faculty of Medicine of the University of Basra approved the research protocol.

Experimental design

The animals were divided into four groups, each group containing six male rats:
Group (A) represents the negative control group, rats were administered distal water without sensitization for 14 days.
Group (B) represents the positive control group; rats were

administered distal water with ova sensitization. Group (C) treated group, rats were administered orally dipyridamole (26.4 mg kg - day) (Shin JW et al., 2010) with ova sensitization. Group (D) treated group, rats were administered orally prednisolone (4.12 mg kg - day) (Ahmed et al., 2021) with ova sensitization.

Groups B, C, and D rats were exposed to ova sensitization (Algaem et al., 2013; Wang et al., 2008) as follows: Intraperitoneal (IP) injection of 1 milligrams of ova and 100 mg of Al $(OH)_3$ dissolved in 1 ml of 0.9% sodium chloride solution (N/S) is used to sensitize rats. This procedure is repeated from day one until the third day. On the sixth day, rats receive an IP injection of 100 mg of ova and 100 mg of AL (OH) 3 dissolved in 1 ml of 0.9%N/S. On day nine, rats in groups B, C and D received 1% ova (1g ova in 100 ml 0.9% N - S) by nebulization every day for six days, lasting 30 minutes each. The animal is placed in a glass chamber 20 cm by 30 cm by 40 cm, and the mixture is nebulized using an electric nebulizer through a hole in the glass chamber. After that, all animals in groups A, B, C and D received an IP injection of phenobarbital at a dose of 800mg/kg to induce anesthesia after 24 hours of the previous dose (Zatroch et al., 2017).

A computerized auto-analysis equipment was used to perform a differential WBC analysis in the BALF of the right lung after it had been lavaged, by insertion of a tracheal cannula, three times using 10 ml N - S 0.9% at 37 ° C, then it was collected and stored in simple tubes within the laboratory for evaluation.

A histological examination of the left lung was performed after making an incision to the left lung preserved in formaldehyde 37% with dilution, embedded in paraffin, and a series of micro sections (5 um) were cut on a microtome, stained with Hematoxylin and eosin (H&E), then preparing glass slides, these glass slides were then examined under a microscope developed by GENEX Laboratories, which could capture a picture of the area under examination on the glass slides at a magnification of 40x. The microscope examined the alveoli, bronchus, bronchioles, and inflammatory cells.

Statistical Analysis

The study used the standard error of the mean (SEM) ± mean to express the data. Analysis of was done to compare different groups. Variance (ANOVA) and the unpaired student t-test were used to determine the significance between the two groups. P-values less than 0.05 (P-value<0.05) were considered significant for this work; otherwise, they were considered nonsignificant.

RESULTS

Impact of dipyridamole on the total WBC level in BALF rats

The total count of WBCs (mean ± SEM) in BALF of rats in the positive group sensitized by ova (group B) was substantially higher (P-value<0.05) than that of the normal control group (group A), with values of 52.08±18.22 and 1.46±0.25 *10^9cells\l, respectively. These findings are presented in Figure 1 and Table 1. Furthermore, total WBC counts in the dipyridamole (group C) and prednisolone (group D) treated groups were significantly lower than those of the positive control group (group B), at 2.86±1.43 and 1.02±0.162 *10^9cells\l, respectively.

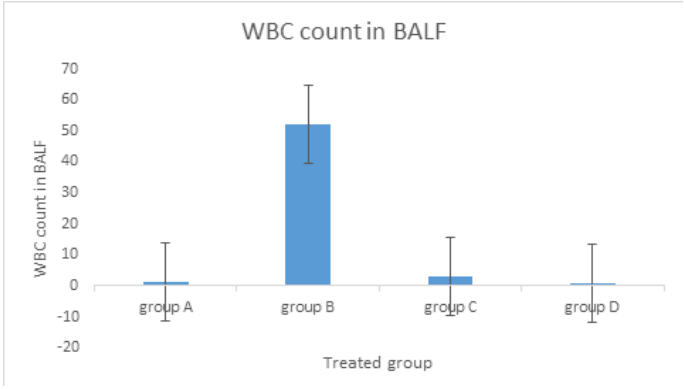

Figure 1: Influence of dipyridamole on BALF WBC count. Rats in Group A received distilled water as a control for a period of 14 days. Group B: rats treated solely with airway ova sensitization served as a positive control group. Group C: oral dipyridamole (26.4 mg / kg / d) in conjunction with airway sensitization by ova. Group D: received oral prednisolone (4.12 mg/kg/d) along with airway ova sensitization.

Impact of dipyridamole on the level of eosinophils in BALF rats

The total eosinophil count (mean ± SEM) in the BALF of rats in the positive group sensitized to ova (group B) was substantially higher (P-value<0.05) than that of the normal control (group A), with values of 6.89±1.99 and 0 *10^9 cells\ l, correspondingly. These findings are shown in Figure 2 and Table 1. Gleichzeitig, there was a notable reduction in overall Eosinophil counts in groups C and D treated with dipyridamole and prednisolone, respectively, compared to group B, the positive control. These values were 5.95±2.11 and 0 *10^9 cells\l respectively but the reduction in eosinophils cells was non significant in dipyridamole treated group C (p-value> 0.05).

Impact of dipyridamole on neutrophils in BALF rats

Table 1 and Figure 3 demonstrate that the total neutrophil count (mean ± SEM) in BALF of rats in the positive group

Table 1: Shows how well dipyridamole works on WBC, Eosinophils, Neutrophils, and mononuclear cells counts (monocytes and lymphocytes) in BALF rat following models of airway inflammation.

Treatment group	Type of treatment	WBC count in BALF mean± SE	Eosinophil count in BALF mean± SE	Neutrophil count in BALF mean± SE	Mononuclear (monocyte +lymphocyte) count in BALF mean± SE
Group A	Negative control/DW	1.46±0.25	0	0	0
Group B	Positive control/OVA sensitization	52.08±18.22*	6.89±1.99*	28.10±10.35*	22.87±6.18*
Group C	Dipyridamole 26.4mg\kg\day	2.86±1.43[a]	5.95±2.11	1.86±1.21[a]	1.10±0.35[a]
Group D	Prednisolone (4.12mg/kg/day)	1.02±0.162[a]	0[a]	0.468±0.064[a]	0.485±0.106[a]

Standard error of means (SEM) is used to represent values as means ±. *= Significantly different from the negative control group (P-value< 0.05). In relation to group B, the values denoted by the superscript (a) are substantially different (P-value<0.05). D/W stands for distilled water; OVA stands for ovalbumin.

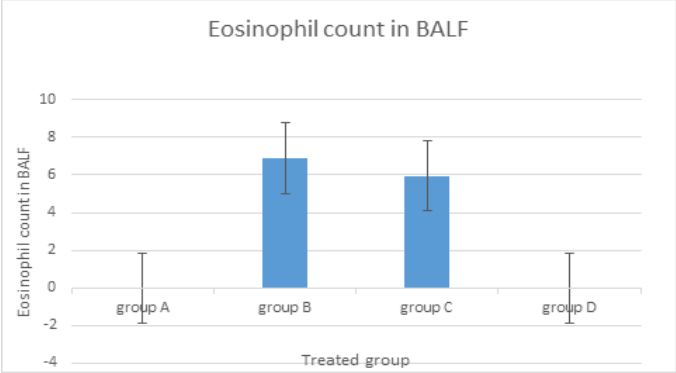

Figure 2: Effect of dipyridamole on BALF eosinophil count. Rats in Group A received distilled water as a control for a period of 14 days. Group B: rats treated solely with airway ova sensitization served as a positive control group. Group C: oral dipyridamole (26.4 mg / kg / d) in conjunction with airway sensitization by ova. Group D: received oral prednisolone (4.12 mg/kg/d) along with airway ova sensitization.

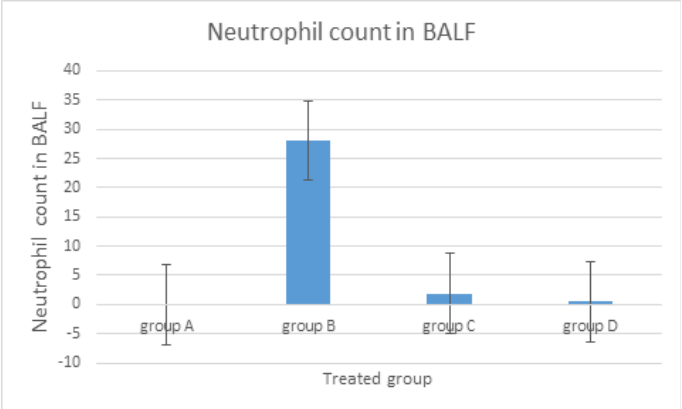

Figure 3: The influence of dipyridamole on the BALF neutrophil count. Rats in Group A received distilled water as a control for a period of 14 days. Group B: rats treated solely with airway ova sensitization served as a positive control group. Group C: oral dipyridamole (26.4 mg / kg / d) in conjunction with airway sensitization by ova. Group D: received oral prednisolone (4.12 mg/kg/d) along with airway ova sensitization.

sensitized to ova (group B) was substantially higher (P-value<0.05) than that of the normal control (group A), at 28.10±10.35 and 0 *10^9 cells\l, respectively. Similarly, total neutrophil counts in groups C and D treated with dipyridamole and prednisolone, respectively, showed a substantial drop compared to group B, the positive control. These values were 1.86±1.21 and 0.468±0.064*10^9 cells\l, respectively.

Impact of dipyridamole on mononuclear cells (monocyte +lymphocyte) in BALF rats

The total number of mononuclear cells in BALF (mean ± SEM) of rats in the positive ova sensitized group (group B) was substantially higher (P-value<0.05) than in the normal control group (group A), with values of 22.87±6.18 and 0 *10 9 cells l, respectively. These findings are shown in Figure 4 and Table 1. At the same time, the total counts of mononuclear cells in groups C and D treated with dipyridamole and prednisolone showed a substantial decrease compared to group B, the positive control. The respective values were 1.10±0.35 and 0.485±0.106 *10 9 cells l, respectively.

Effect of dipyridamole on lung tissue histopathology in rats

Dissected tissues from the left lung were soaked in 37% with dilution formaldehyde solution (v / v), rinsed with normal saline, and then embedded in paraffin. H and E dyes were used to stain lung specimens after they had been sectioned. Images of particular slices were taken with a microscope equipped with a digital camera (GENEX Laboratories) at 40X magnifications.

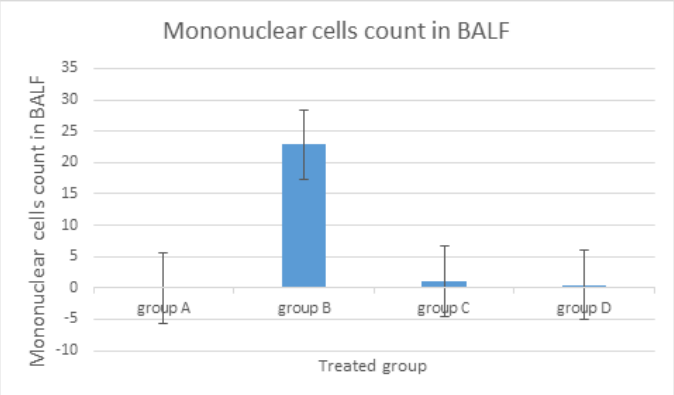

Figure 4: Dipyridamole influence on the count of mononuclear cells (lymphocytes + monocytes) in BALF. Rats in Group A received distilled water as a control for a period of 14 days. Group B: rats treated solely with airway ova sensitization served as a positive control group. Group C: oral dipyridamole (26.4 mg / kg / d) in conjunction with airway sensitization by ova. Group D: received oral prednisolone (4.12 mg/kg/d) along with airway ova sensitization.

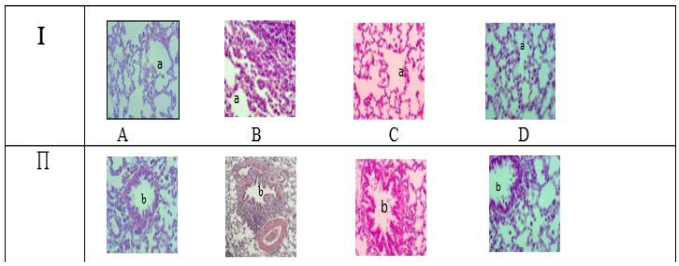

Figure 5: Histopathology of rat lung tissue in response to dipyridamole. Images of lung tissue taken using an X-40 light microscope. Alveolar Sac (a) in Section I. The bronchi in Section (II) (b). Group A is the negative control; Group B is the positive control; Group C is the Dipyridamole-treated group; Group D is the group treated with prednisolone.

Histopathological investigation revealed that the bronchioles and alveolar sacs in the negative control (group A) had a normal epithelium. This is depicted in Figure 5 (I, II). Sensitized rats of Group B showed an increased accumulation of inflammatory cells around the alveolar sac and bronchioles. Group C, who received dipyridamole treatment, did not show excaudate in their bronchioles, decreased paraplegia with dilatation, and minimal infiltration of inflammatory cells surrounding the bronchus. The alveolar sac was normal and the lumen contained few macrophages. Compared to the positive control (group B), prednisolone-treated (group D) showed an unquestionably improved histological appearance, less infiltration of inflammatory cells, and cleaner bronchial and alveolar sacs.

DISCUSSION

Various lung conditions, such as asthma and chronic obstructive pulmonary disease (COPD), can be clinically diagnosed using lung function, which is a reflection of respiratory health (Vogelmeier et al., 2017). White blood cell (WBC) counts in the circulation are commonly recognized indicators of systemic inflammation and may have a harmful function throughout the pulmonary damage process (Rovina et al., 2013; Doherty et al., 2019).

According to Table 1 figure 1, rats sensitized by ova (group B) had higher total WBC counts in their BALF ($P < 0.05$) as compared to normal control (group A); this is consistent with the study conducted by (Thakur et al., 2019).

According to a study by (Yu and Chen, 2018), airway inflammation was one of the distinctive characteristics of ovalbumin-induced eosinophilic asthma. Consistent with the findings of this study, Table 1 Figure 2 indicates that, compared to normal control (group A), ova sensitized (group B) rats had more eosinophils ($P < 0.05$) in their BALF.

It is important to say (Kim et al., 2019) reported that the ova-sensitized group had a higher number of neutrophil cells than the normal control group. The results of this study are consistent with those reported in this research, as seen in Table 1 Figure 3, there is a significant increase in neutrophils ($P < 0.05$) in BALF ova sensitized (group B) compared to normal control (group A).

Another researcher studied, in rats sensitized by ova, found that the number of lymphocytes in these rats had increased significantly (Khaldi et al., 2018). Furthermore, monocytes were found to be higher in a study by (Abreu et al., 2014).

In this study, when comparing the ova sensitized (group B) with the normal control (group A), Table 1 Figure 4 reports an increase ($P < 0.05$) in monocyte and lymphocyte counts in rat BALF, which is consistent with the studies previously discussed.

Dipyridamole has anti-inflammatory effects that act synergistically with glucocorticoids, making it a potential candidate drug to be added to the SLE treatment regime (Kyttaris et al., 2011).

In this study Table 1 Figure 1 and 2 showed that total WBC cells in BALF of dipyridamole treated rats (group C) were markedly decreased ($P < 0.05$) as compared to the positive control (group B). Additionally, eosinophil cells in BALF of dipyridamole-treated rats (group C) slightly decreased but this reduction was non significant (P-value>

0.05) as compared to the positive control (group B).

Dipyridamole has also been demonstrated to prevent neutrophil adherence to the vascular endothelium in patients with ischemic stroke (Hallevi et al., 2007). In total insight to Table 1, Figure 3, the neutrophil cells in BALF of dipyridamole treated rats (group C) showed a remarkable reduction ($P < 0.05$) compared to ova-sensitized rats (group B).

Furthermore, the recruitment, activation, and secretion of pro-inflammatory mediators by lymphocytes is inhibited by dipyridamole (Dong et al., 2006). Also in a study done by (Weyrich et al., 2005), he approved that dipyridamole has anti-inflammatory effects on human monocytes. When looking at Table 1 Figure 4 the level of mononuclear cells (lymphocytes and monocytes) in BALF of dipyridamole-treated rats (group C) decreased significantly ($P < 0.05$) in contrast to positive ova sensitized (group B).

In terms of corticosteroids, they have the ability to prevent leukocytes from migrating from post-capillary venules into the extravascular matrix (Chanez et al., 2004).

Table 1, Figure 1 shows a significant reduction in total leukocytes in BALF of prednisolone-treated rats (group D) compared to ova sensitized rats (group B), which is the same as the study by (Roumestan et al., 2007).

(Sakae et al., 2014) declare that a significant mean decrease in sputum eosinophils was found by pooling prednisone versus control and another drug. The eosinophil count in the BALF of prednisolone-treated rats (group D) decreased significantly in this investigation and increased in ova sensitized (group B), as indicated in Table 1 Figure 2, which is consistent with the study mentioned.

(Pauluse et al., 2013) conducted a study in which neutrophil lung protein levels were considerably reduced. In this investigation, the BALF of the prednisolone-treated rat (group D) showed a significantly lower neutrophil count than the sensitized ova (group B). Table 1 Figure 3 shows this.

In a study conducted by (Fleishaker et al., 2016), the results showed that the largest reductions in lymphocyte counts relative to placebo were observed with most doses of prednisolone.

It is interesting to note that (Buters et al., 2022) evaluated that the number of dendritic cells, T cells, natural killer cells, and monocyte were dramatically decreased by clobetasol and Prednisolone.

Compared to ova-sensitized cells (group B), mononuclear cells (lymphocytes and monocytes) in BALF decreased in this study in prednisolone treated cells (group D). This is consistent with the research mentioned (Fleishaker et al., 2016; Buters et al., 2022) and is clearly observed in Table 1 Figure 4.

The transcription factor NF-κB, which is dimeric, widely expressed and has evolved to be conserved, is essential for immunological and inflammatory responses (Soni, 2016). Strong anti-inflammatory chemical adenosine is essential for innate immunity (Haskó and Cronstein, 2004).

Dipyridamole raises plasma adenosine levels by preventing erythrocytes, endothelial cells, and platelets from reabsorbing adenosine (Chakrabarti and Freedman, 2008). Tissue perfusion is enhanced by dipyridamole's vasodilator action (Chakrabarti et al., 2005). Thus, NF-KB will be inhibited as a result.

According to histological evaluation, the bronchioles and alveolar sac in the negative control (group A) did not show any alterations, but the positive control (group B) showed an accumulation of inflammatory cells around the bronchioles and alveolar sac.

Group C, who received dipyridamole treatment, did not show excaudate in their bronchioles, decreased paraplegia with dilatation, and minimal infiltration of inflammatory cells surrounding the bronchus. The alveolar sac was normal, and the lumen contained few macrophages. Furthermore, in (group D), prednisolone produced a similar effect, with less inflammatory cell infiltration and more clean alveolar and bronchial sacs. This aligns with a research conducted by (Mina et al., 2023).

CONCLUSIONS

In BALF animal, dipyridamole exhibited a good anti-inflammatory effect by reducing total WBC, eosinophil, neutrophil, and mononuclear cells. Nevertheless, prednisolone is still more effective than dipyridamole in reducing these cells. Additionally, dipyridamole and prednisolone exhibit a marked improvement in inflammatory cells and the histological properties of the bronchial and alveolar sacs. It is recommended to do more research to see whether other steroids have more or less effects than dipyridamole.

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CONFLICT OF INTEREST

No conflict has been exists.

NOVELTY STATEMENT

The research is a novel and there was no any previous study in Iraq dealing with the anti-inflammatory effect of Dipyridamole.

AUTHORS CONTRIBUTION

-**Ali D. Nashmi**: is responsible for writing the manuscript and all the experimental methods regarding the dealing with and administration of required dose of drugs for animal.
2-**Jawad K. Hasan:** is responsible for statistical analysis and add of the suitable words for the manuscript.
3-**Manal A. Ibrahim:** is responsible for study design and checks the correct references for manuscript.

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