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Article

Effect of *Solanum aculeastrum* on hematological parameters of Al-bino mice infected with *Aspergillus fumigatus*

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ABSTRACT

The goal of the current study was to research the changes in hematological parameters: WBC count, RBCs count, Hb, PCV, neutrophil, lymphocyte, and monocyte in albino mice infected with *Aspergillus fumigatus* by intraperitoneal injection after induced immunosuppression by intraperitoneal injection of cortisone. The current research also examined an attempt to reduce the infection load by treating with *Solanum aculeastrum*. The result shows higher decreased significance (P≤0. 05) in RBCs, Hb, and PCV after being infected with *A. fumigatus* 7. 1 ± 0. 8, 11. 3 ± 0. 5 and 41. 5 ± 2. 4, respectively, while the total WBC count, neutrophil, lymphocyte, and monocytes were increased significantly (P≤0. 05) after treatment with *S. aculeastrum* in groups infected with *A. fumigatus*, compared to other groups. According to these results, we conclude that the alcoholic extract of *S. Astrum* has significant therapeutic and antifungal characteristics that lead to an increase in the total WBC count and, therefore, is considered a necessary alternative therapy for increasing immunity.

Keywords: Cortisone, Hematology, Fungi, Iraq.

INTRODUCTION

Fungi are a major, diverse, ubiquitous group of heterotrophic organisms that live as saprophytes or parasites or are related to many other organisms as symbiotes [1]. According to estimates of global richness, it constitutes the second largest group of organisms, with about 3 million species expected. Also, it ranks third among the eukaryotic kingdoms regarding the wealth of known species [2, 3]. The most prevalent pathogenic species in the animal kingdom is *Aspergillus fumigatus*, a saprotrophic fungus that lives vegetatively on decaying organic matter in the soil and spreads by asexual sporulation [4, 5]. This fungus can survive high temperatures above (50°C) and may happen in piles of decomposing plant matter. The fungus releases a significant amount of asexual airborne spores [6]. These fungi have a broad clinical spectrum, ranging from allergy to chronic infections and acute invasive aspergillosis (I.A.) in humans and animals [7]. Aspergilli are known for their capacity to secrete different types of biologically active chemical compounds, including antibiotics, mycotoxins, immune-suppressant substances, and cholesterol-lowering factors [8]. According to the host's underlying immunological state, these fungi can cause overlapping chronic, noninvasive types of infection that range from the formation of a fungal ball (Aspergilloma) to a long-lasting inflammatory and fibrotic process that is presently categorized as chronic lung infection similar to invasive pulmonary aspergillosis (IPA) [4, 9].

Solanum aculeastrum, "goat bitter-apple" Hnzal (Arabic), is extensively dispersed in a native of Africa and South Africa. *S. aculeastrum* is a spiny perennial that grew to 3 m tall with white blooms and bears berries resembling lemons and turning yellow-green when ripe. For both people and domestic animals, the tart fruits of this plant are utilized medicinally in various techniques for the treatment of cancer, indigestion, and stomach disruption; the boiling extract of the fruits and leaves is administered orally route. Both fresh and cooked fruit are used as a therapy for acne, gonorrhea, and jigger wounds [10], according to the presence of bioavailable

phyto-constituents including steroidal saponins, steroidal alkaloids, terpenes, flavonoids, lignans, sterols, phenolic compounds, and coumarins. *Solanum* spp. is essential in the nutraceutical and pharmaceutical industries. Both steroidal alkaloids and glycoalkaloids serve as important chemical indicators of this genus. Both ancient and modern systems of medicine place special importance on steroid alkaloids and glycoalkaloids since they exhibit a variety of bioactivities, including antibacterial, analgesic, immunomodulatory, hepatoprotective, neurogenetic, anticancer, etc. [11]. Considering the above facts and minimal studies, this study was designed to study the effects of *S. aculeastrum* on hematological parameters in albino mice infected with *A. fumigatus*.

MATERIALS AND METHODS

Fungal isolation

The fungus was isolated from different veterinary clinics and stray cats in Baghdad province, identified on Sabouraud Dextrose Agar and malt extract agar, and diagnosed by traditional morphological examination laboratory methods.

Preparation of A. fumigatus fungal suspension

The suspension of A. fumigatus was prepared according to a previously carried out study [12].

Solanum aculeastrum fruit extraction

Solanum aculeastrum (Hanzal) was purchased from local Baghdad markets, and the extraction was performed previously [13].

Induction of immunosuppression

Immunosuppression was induced by treatment of the mice with cortisone intraperitoneal administration at a single dose one day before conidial administration. Each mouse receives 2mg of Dexamethasone per mouse (14).

Animal and design experiments

Fifty-six albino mice were used in the study at 10-12 weeks and weighing 25. 3 ± 0. 9 g, maintained on a standard laboratory diet, water and temperature-controlled at the animal house laboratory at the College of Veterinary Medicine (University of Baghdad, Baghdad, Iraq), were randomly assigned to different groups: 8 mice per group as following:

- G1: Negative control mice (untreated).
- G2: Infected mice with a single dose *of A. fumigatus* at 2×10^7 cells/ml per mouse intraperitoneally (I/P).
- G3: Treated mice with cortisone at 2 mg/mice I/P.
- G4: Treated mice orally with a single dose of *S. aculeastrum* at 10 mg/kg B. W.
- G5: In which mice were treated with a single dose of cortisone at 2mg/mice I/P for one day prior to fungal spore infection and then infected with a single dose of *A. fumigates* 2×10^7 cells/ml per mouse I/P.
- G6: In which mice were treated with a single dose of *S. aculestrum* at 10 mg/kg B. W orally, one week after infection with a single dose *of A. fumigates* 2×10^7 cells/ml per mouse I/P.
- G7: Mice were treated orally with a single dose of *S. aculestrum* at 10 mg/kg B. W simultaneously with the fungal infection.

Blood samples collection

According to (16), the blood samples from all groups were taken at the end of the experiment.

Statistical analysis

SPSS calculated data for Windows TM version 23. 0 by using one-way ANOVA. All experimental data are presented as Mean ± S.E. and significant differences at P≤ 0. 05 [17].

RESULTS

Hematological study

Hematological parameters: total WBC counts, neutrophils, lymphocytes, monocytes, RBC count, Hb level, and PCV. The blood samples were taken from all the groups to analyze by employment tubes containing an anticoagulant agent in the laboratory. In the current study, the results showed that the control positive group of *A. fumigatus* infection, cortisone and group infected with *A. fumigatus* plus cortisone treated caused significant (p≤ 0. 05) decrease in RBCs count, Hb and PCV, whereas, no significant (p≤ 0. 05) differences were noticed in groups of mice treated with Solanumaculeastrum alone or plus cortisone and *A. fumigatus* in both G6 and G7 when compare with G1 (Table 1).

Parameter	G1	G2	G3	G4	G5	G6	G7
RBCs 10^6/mm^3	9. 9 ± 0. 4 a	7. 1 ± 0. 8 b	7. 8 ± 0. 3 b	9. 7 ± 1. 3 a	7. 9 ± 0. 1 b	9. 9 ± 0. 3 a	9. 7 ± 0. 5 a
Hb mg/dl	14. 2 ± 0. 1a	11. 3 ± 0. 5 b	11. 4 ± 0. 3 b	14. 4 ± 1. 5 a	12. 4 ± 0. 6 b	13. 7 ± 0. 9 a	13. 4 ± 0. 3 a
PCV %	46. 8 ± 0. 2a	41. 5 ± 2. 4 b	39. 9 ± 0. 4 b	44. 7 ± 6. 3 a	43. 6 ± 1. 5 b	43. 5 ± 1. 8 a	47. 4 ± 2. 4 a

Variation in horizontal small letters refers to significant differences (P<0. 05).

Table 1: Effects of *A. fumigatus* infection, cortisone, and *S. aculeastrum* treated after two weeks on parameters of RBC count, Hb, and PCV (mean ± S.E.).

Total WBCs, neutrophils, lymphocytes, and monocyte count were found to be decreased significantly (p≤ 0. 05) in both groups infected with *A. fumigates* and *S. aculeastrum* alone. Also, mice representing control-positive cortisone decreased significantly (p≤ 0. 05) in neutrophils and monocytes. At the same time, total WBCs and lymphocytes were not affected significantly (p≤0. 05). The results also showed no significant differences (p≤0. 05) in a group of mice treated with cortisone plus infection of *A. fumigatus*. Total WBCs and lymphocytes were increased significantly (p≤0. 05), and neutrophils and monocytes were not affected significantly (p≤0. 05) in G6 treated with *S. aculeastrum* after one week of infection. Total WBCs, neutrophils, and lymphocytes were increased significantly (p≤0. 05), and monocytes were not affected significantly (p≤0. 05) in the group treated with *S. aculeastrum* at the same time of infection comparable with the controls regarding the WBCs (Table 2).

Parameter	G1	G2	G3	G4	G5	G6	G7
WBCs 10^9/L	5. 3 ± 0. 5 a	3. 7 ± 0. 8 b	5. 0 ± 0. 5 a	2. 5 ± 0. 4 b	5. 8 ± 1. 9 a	6. 6 ± 0. 9 a	7. 6 ± 3. 0 b
Neutrophil %	3. 2 ± 0. 8 a	1. 8 ± 0. 7 b	1. 8 ± 0. 1 b	1. 6 ± 0. 5 b	2. 4 ± 0. 9 a	3. 4 ± 1. 1 a	4. 2 ± 1. 0 b
Lymphocyte %	2. 1 ± 0. 4 a	1. 4 ± 0. 2 b	2. 6 ± 0. 4 a	1. 1 ± 0. 5b	2. 5 ± 0. 8 a	1. 5 ± 0. 2 b	4. 0 ± 0. 3 b
Monocyte %	1. 2 ± 0. 1 a	0. 1 ± 0 b	0. 1 ± 0 b	0. 1 ± 0. 5 b	0. 2 ± 0 a	0. 6 ± 0. 1 a	0. 7 ± 0. 04 a

Variation in horizontal small letters refers to significant differences (P<0. 05).

Table 2: Effects of *Aspergillus fumigatus* infection, cortisone, and *Solanumaculeastrum* treated after two weeks on parameters of (WBCs, neutrophils, lymphocytes, and monocytes) (mean ± S.E.).

DISCUSSION

The present results indicated that the hematological picture of *A. fumigatus*-infected mice is similar to that of another study [18]. The mycotoxin from pathogenic fungi caused anemia, observed as decreases in PCV Hb% and RBC count. Also, the phospholipase enzyme in *Aspergillu*s spp. is the essential virulence factor that can cause destabilization and penetration, break down membrane phospholipids surrounding the red blood cell, and generate arachidonic acid [19]. Additionally, phospholipase hydrolyzes RBCs to release phosphatidylserine and produce lysophosphatidic acid (LPA), the latter of which results in the flow of substances through a blood cell membrane and causes swilling before exploding [20].

Previous studies also found decreased hematological parameters because of cortisone treatment [21, 22]. Exposure to Dexamethasone demonstrates a significant change in red blood cells, such as anemia, destruction of cells, or inhibition of hematopoiesis. All these reasons affect RBC, Hb, and PCV levels, and these changes led to observing the result compared with a control group and explaining the result that Dexamethasone may cause suppression of the bone marrow. Such decreases could also be attributed to hemodilution. However, they could also result from RBC reaction or inhibition of RBC synthesis, which limits the capacity of these cells to absorb oxygen in conjunction with increased hemolysis brought on by excessive physical stress and results in severe anemia. The WBC decrease results in the lysis of neutrophils affected by phospholipase enzymes, the elimination of lipid phosphorus, and hydrolysis of membrane phospholipids occurred after exposure to this enzyme. This led to decreased cell size, sphering, and increased susceptibility to osmotic stress, which altered the cell's functional properties and caused cell lysis [23]. Another reason represented that phospholipase enzyme and *Aspergillus* spp. The infection effect on hormones responsible for the production of blood cells is represented by the erythropoietin hormone from the kidney, which is the primary catalyst for the production of blood cells; this finding agrees with other researchers [24].

The reduction in neutrophil and monocyte counts after cortisone-treated mice indicated immunosuppression. This finding agrees with another study [25]. Steroids impair alveolar macrophage activity, lowering the primary defense against lung infection. Additionally, they affect T and B cell lymphocytes and reduce cytokine production, which impairs the adaptive immune response to invasive aspergillosis [26].

Solanum aculeastrum affects hematological parameters; the results indicated that plants cause a decrease in total WBC count and differential cells. WBC count is an indicator of an organism's capacity to eradicate infection. A reduction in WBC count in the group of mice treated with Solanumaculeastrum agrees with another study [27]. The animal's capacity to fight infection, attack, and destroy infectious agents in the blood may be adversely impacted by the decline in WBC levels. Additionally, the immune system's effector cells may have a severe effect. Also, the WBC count decreases, as observed by other results [28]. The decrease in WBC count suggests that the extract ingredients may have damaged or prevented the maturation of these blood cells. The committed stem cells that produce these blood cells are regulated by proliferation, differentiation, and maturation by granulocyte-macrophage colony-stimulating factors, interleukins IL-2, IL-4, and IL-5. Therefore, the extract might have interfered with the sensitivity of the committed stem cells responsible for generating these white blood cells and differential cells, or it might have decreased the synthesis of these regulatory factors [29-32].

Mice in both G6 and G7 showed an increase in total WBC count and its differential cells compared with other treated groups; best results were obtained when mice treated at the same time of infection compared with mice received the plant extract after one week of fungal spores infected, the stress factor of infection on the immune system and weakness of mice body can interfere with immune responses. Also, lymphocyte counts may be decreased with handling or other stressors and with age. Increased neutrophil counts (neutrophilia) are commonly seen in conditions of infection and acute inflammation and are related to immune system reactions to stress or excitement and infectious diseases [33].

Previous research proved that *S. aculeastrum* has a chemical and pharmaceutical component that acts as an antifungal against a number of fungal species [34, 35.]. Other researchers have investigated the effect of ethanolic extract using the diffusion method, and the findings showed that the strongest antifungal activity was by *A. fumigates* [13,36, 37]. At the same time, other authors found that alcohol extracts from fruit ultimately impeded the growth of *A. flavus* (100%) [28, 35-37]. The results of the present study agree with all the research above; the hematological findings showed improvements in white blood cells, neutrophils, lymphocytes and monocytes when given the ethanolic extract orally at the same time as infected with *A. fumigatus*, indicating the treatment power of the plant at the applied dose of 10 mg/kg B. W was elevated the immune status by increasing of immune cells that fight infection comparing with other groups.

CONCLUSIONS

This study concluded that using the alcoholic extract of *S. aculeasttrum* could provide an effective therapeutic tool in the treatment of fungal infections as well as in the correction of abnormalities in hematological and immunological markers due to exposure to different infections. Furthermore, studies are of great importance to detect the efficacy of this extract on body organs and other infections.

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