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## Effect of *Solanum aculeastrum* on hematological parameters of Albino mice infected with *Aspergillus fumigatus*

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**Abstract:** The goal of current study was designed to research about the changing in hematological parameters; WBCs count, RBCs count, Hb, PCV, neutrophil, lymphocyte and monocyte in albino mice that 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 load of infection by treated with *Solanum aculeastrum*. The result show higher decreased significant ( $P \leq 0.05$ ) in RBCs, Hb and PCV after being infected with *A. fumigatus*  $7.1 \pm 0.8$ ,  $11.3 \pm 0.5$  and  $41.5 \pm 2.4$ , respectively; while, the total WBCs count, neutrophil, lymphocyte and monocytes were increased significantly ( $P \leq 0.05$ ) 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* having a significant therapeutics and antifungal characteristics that lead to increasing the total WBCs count, and therefore, is considered as an important alternative therapy for increasing immunity.

**Keywords:** Cortisone; Hematology; Fungi; Iraq

### 1. Introduction

Fungi represent as a major, diverse, ubiquitous group of heterotrophic organisms that live as saprophytes or parasites, or it's related with other many organisms as symbiotes<sup>1</sup>. It is constitute the second largest group of organisms according to estimates of global richness, with about 3 million species expected. Also, it ranks third among the eukaryotic kingdoms in terms of the wealth of known species<sup>2,3</sup>. The most prevalent pathogenic species known to exist 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<sup>4,5</sup>. These fungus can survive high temperatures above (50°C) that may happen in piles of decomposing plant matter. The fungus releases a great amount of asexual airborne spores<sup>6</sup>. These fungi have a wide clinical spectrum, ranging from allergy to chronic infections and acute invasive aspergillosis (IA) in both humans and animals<sup>7</sup>. Aspergilli are known for their capacity to secrete a different types of biologically active chemical compounds included antibiotics, mycotoxins, immune-suppressants substances, and cholesterol lowering factors<sup>8</sup>. 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 to inflammatory and fibrotic process that is presently categorized as chronic lung infection similar to invasive pulmonary aspergillosis (IPA)<sup>4,9</sup>.

*Solanum aculeastrum*, referred as "goat bitter-apple" Hnzal (Arabic), is greatly dispersed in a native of Africa and South Africa. *S. aculeastrum* a spiny perennial that developed to 3 m in tall, with white blooms, and bears berries that resemble lemons and turn yellow-green when ripe. For both people and domestic animals, the tart fruits of this plantare utilized medicinally in a variety of technique for the treatment of cancer, indigestion, and stomach disruption, the boiling extract of the fruits and leaves is administered by orally route. The both fresh and cookedfruit is used as a therapy for acne, gonorrhea, and jigger wounds<sup>10</sup>. According to the presence of bioavailable phyto-constituents including steroidal saponins, steroidal alkaloids, terpenes, flavonoids, lignans, sterols, phenolic compounds, and coumarins. *Solanum* spp. are important in the nutraceutical and pharmaceutical industries. Both, steroidal alkaloids and glycoalkaloids serve as important chemical indicators of this genus. The both ancient and modern systems of medicine place a special importance on steroid alkaloids and glycoalkaloids since they exhibit a variety of bioactivities, in-

cluding antibacterial, analgesic, immunomodulatory, hepatoprotective, neurogenetic, anticancer, etc.<sup>11</sup>. Considering the above facts and very limited studies, so this study was designed to study the effects of *S. aculeastrum* on hematological parameters in albino mice infected with *A. fumigatus*.

## 2. Materials and Methods

### 2.1. Fungal isolation

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

### 2.2. Preparation of *A. fumigatus* fungal suspension

The suspension of *A. fumigatus* was prepared according to previously carried out study<sup>12</sup>.

### 2.3. *Solanum aculeastrum* fruit extraction

*Solanum aculeastrum* (Hanzal) were purchased from local Baghdad markets and the extraction was performed previously<sup>13</sup>.

### 2.4. Induction of immunosuppression

Immunosuppression was induced by treatment the mice with cortisone intraperitoneal administration at a single dose one day before conidial administration. Each mice receive 2mg per mice Dexamethasone<sup>14</sup>.

### 2.5. Animal and design experiments

A fifty six of albino mice were used in the study, at the age of 10-12 weeks and weight  $25.3 \pm 0.9$  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 single dose of *A. fumigatus* at  $2 \times 10^7$  cells / ml per mouse intraperitoneally (I/P).
- G3: Treated mice with cortisone at a dose of 2mg / mice I/P.
- G4: Treated mice with a single dose of *S. aculeastrum* at 10 mg/kg B. W, orally.
- 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*  $2 \times 10^7$  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 *A. fumigates*  $2 \times 10^7$  cells / ml per mouse I/P.
- G7: In which, mice were treated with a single dose of *S. aculestrum* at 10 mg/kg B. W orally, at the same time of fungal infection.

### 2.6. Blood samples collection

According to<sup>15,16</sup> the blood samples were taken at the end of experiment from the all groups

### 2.7. Statistical analysis

Data was calculated by SPSS for windows TM version 23. 0 by using one way ANOVA. All experimental data are presented as Mean  $\pm$  SE and significant differences at  $P \leq 0.05$ <sup>17</sup>.

## 3. Results

### 3.1. Hematological study

Hematological parameters; total WBCs counts, neutrophil, lymphocytes, monocytes, RBCs count, Hb level and PCV. The blood samples were taken from all the groups to analyze in laboratory by employment tubes containing an anticoagulant agent. In currently study the results showed that control positive group of *A. fumigatus* infection, cortisone and group infected with *A. fumigatus* plus cortisone treated cases significant ( $p \leq 0.05$ ) decrease in RBCs count, Hb and PCV, whereas, no significant ( $p \leq 0.05$ ) differences were notice 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).

**Table 1.** Effects of *A. fumigatus* infection, cortisone and *S. aculeastrum* treated after two weeks on parameters of RBCs count, Hb and PCV (mean  $\pm$  SE).

Parameter	G1	G2	G3	G4	G5	G6	G7
RBCs $10^6/\text{mm}^3$	9.9 $\pm$ 0.4 a	7.1 $\pm$ 0.8 b	7.8 $\pm$ 0.3 b	9.7 $\pm$ 1.3 a	7.9 $\pm$ 0.1 b	9.9 $\pm$ 0.3 a	9.7 $\pm$ 0.5 a
Hb mg/dl	14.2 $\pm$ 0.1a	11.3 $\pm$ 0.5 b	11.4 $\pm$ 0.3 b	14.4 $\pm$ 1.5 a	12.4 $\pm$ 0.6 b	13.7 $\pm$ 0.9 a	13.4 $\pm$ 0.3 a
PCV %	46.8 $\pm$ 0.2a	41.5 $\pm$ 2.4 b	39.9 $\pm$ 0.4 b	44.7 $\pm$ 6.3 a	43.6 $\pm$ 1.5 b	43.5 $\pm$ 1.8 a	47.4 $\pm$ 2.4 a

Variation in horizontal small letters refer to significant differences ( $P < 0.05$ )

Total WBCs, neutrophils, lymphocytes and monocytes count were found to be decreased significantly ( $p \leq 0.05$ ) in both groups infected with *A. fumigatus* and *S. aculeastrum* alone. Also, mice that represents control positive of cortisone showed decreased significantly ( $p \leq 0.05$ ) in neutrophils and monocytes while total WBCs and lymphocytes were not affected significantly ( $p \leq 0.05$ ). The results also showed no significant differences ( $p \leq 0.05$ ) in group of mice treated with cortisone plus infection of *A. fumigatus*. Total WBCs and lymphocytes were increased significantly ( $p \leq 0.05$ ), neutrophils and monocytes were not affected significantly ( $p \leq 0.05$ ) in G6 that treated with *S. aculeastrum* after one week of infection. Total WBCs, neutrophils and lymphocytes were increased significantly ( $p \leq 0.05$ ), monocytes was not affected significantly ( $p \leq 0.05$ ) in group treated with *S. aculeastrum* in same time of infection comparable with the controls regarding the WBCs (Table 2).

**Table 2.** Effects of *Aspergillus fumigatus* infection, cortisone and *Solanum aculeastrum* treated after two weeks on parameters of (WBCs, Neutrophil, Lymphocyte and monocyte) (mean  $\pm$  SE).

Parameter	G1	G2	G3	G4	G5	G6	G7
WBCs $10^9/\text{L}$	5.3 $\pm$ 0.5 a	3.7 $\pm$ 0.8 b	5.0 $\pm$ 0.5 a	2.5 $\pm$ 0.4 b	5.8 $\pm$ 1.9 a	6.6 $\pm$ 0.9 a	7.6 $\pm$ 3.0 b
Neutrophil %	3.2 $\pm$ 0.8 a	1.8 $\pm$ 0.7 b	1.8 $\pm$ 0.1 b	1.6 $\pm$ 0.5 b	2.4 $\pm$ 0.9 a	3.4 $\pm$ 1.1 a	4.2 $\pm$ 1.0 b
Lymphocyte %	2.1 $\pm$ 0.4 a	1.4 $\pm$ 0.2 b	2.6 $\pm$ 0.4 a	1.1 $\pm$ 0.5 b	2.5 $\pm$ 0.8 a	1.5 $\pm$ 0.2 b	4.0 $\pm$ 0.3 b
Monocyte %	1.2 $\pm$ 0.1 a	0.1 $\pm$ 0 b	0.1 $\pm$ 0 b	0.1 $\pm$ 0.5 b	0.2 $\pm$ 0 a	0.6 $\pm$ 0.1 a	0.7 $\pm$ 0.04 a

Variation in horizontal small letters refer to significant differences ( $P < 0.05$ )

#### 4. Discussion

The present results indicated that hematological picture of *A. fumigatus* infected mice similar to those reported by other study<sup>18</sup>. The mycotoxin from pathogenic fungi caused anemia observed as decreases PCV and Hb% and RBCs count also the phospholipase enzyme that present in *Aspergillus* spp. as the most important virulence factor that can cause destabilization, penetration and breaks down membrane phospholipids that surrounds the red blood cell and generates arachidonic acid<sup>19</sup>. 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 swelling before exploding<sup>20</sup>.

Previous study also found decrease of the hematological parameter because of cortisone treated<sup>21,22</sup>, the exposure to dexamethasone demonstrate a significant change in red blood cell such as anemia, destruction of cells or inhibition of hematopoiesis all these reasons effect on RBC, Hb and PCV level and these changing leading to observed the result comparing with control group and explain the result that dexamethasone may cause suppression of the bone marrow. Such decreases could also be attributed to hemodilution, but they could also be the result of 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 to 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 a decrease in cell size, sphering, and increased susceptibility to osmotic stress, which altered the cell's functional properties and caused cell lysis<sup>23</sup>. Another reasons represented that phospholipase enzyme and *Aspergillus* spp. infection effect on hormones responsible for production of blood cell, represented by the erythropoietin hormone from the kidney, which is the main catalyst for production of blood cell, this finding agree with other researchers<sup>24</sup>.

The reduction in neutrophil and monocytes counts after cortisone treated mice was indicative of immunosuppression this finding agree with other study<sup>25</sup>. Actually, 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<sup>26</sup>.

*Solanum aculeastrum* have an effect on hematological parameters, the results indicated that plant cause decrease in total WBC count and differential cells, WBCs count is an indicator of organism capacity to eradicate infection, a reduction in WBCs count in the group of mice treated with

Solanumaculeastrum, which is agree with other study<sup>27</sup>. The animals 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 serious affect. Also the WBCs count decrease as observed by other results<sup>28</sup>. 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, proliferation, differentiation, and maturation by granulocyte-macrophage colony stimulating factor, interleukins IL-2, IL-4, and IL-5. Therefore, the extract might have interfered with the sensitivity of the committed stem cells in responsible for generating these white blood cells and differential cells, or it might have decreased the synthesis of these regulatory factors<sup>29,30,31,32</sup>.

Mice in both G6 and G7 showed an increasing in total WBCs count and its differential cells compared with other treated groups, best results obtained when mice treated same time of infection compared with mice received the plant extract after one week of fungal spores infected, the stress factor of infection on 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<sup>33</sup>.

Previous researches proved that *S. aculeastrum* having a chemical and pharmaceutical component that act as antifungal against number of fungal species<sup>34,35</sup>. Other researchers have investigated the effect of ethanolic extract using diffusion method, and the findings showed the strongest antifungal activity was by *A. fumigatus*<sup>13,36,37</sup>. While, other authors found that alcohol extracts from fruit completely impeded the growth of *A. flavus* (100%)<sup>28,35,36,37</sup>. The results of present study agree with all researches above, the hematological findings showed improved in white blood cell, neutrophil, lymphocyte and monocyte when given the ethanolic extract orally at the same time of infected with *A. fumigatus* that indicated the treatment power of 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.

## 5. Conclusions

This study concluded that the using alcoholic extract of *S. aculeastrum* could provide an effective therapeutic tool in treatment of fungal infections as well as in 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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**Conflicts of Interest:** The authors declare no conflict of interest.

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