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Immuno-Biochemical and Pathological Studies on Aspergillosis in Broiler Chicks with Trail of Prevention.

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Abstract

This study was aimed for isolation and identification of *Aspergillus* spp. from naturally infected broiler chicks with Aspergillosis and studying its effect on the growth performance, immune response, some hemato-biochemical parameters and pathological changes in addition to evaluating the effect of mycostatin in treatment of the disease in broiler chicks. Eighty swabs were collected from chickens showed respiratory signs (40 air sac and 40 lung) swabs for isolation and identification of *Aspergillus* spp. The examined swabs showed (41) were positive for *Aspergillus* spp. 21 air sac and 20 lung swabs were positive for *Aspergillus* spp. *A. fumigates* 20, *A. flavus* 9, *A. niger* 7 and *A. terreus* 5 were isolated from positive examined swabs. Also experimental study was carried out on 90 chicks-7 day old apparently healthy chicks, were randomly divided into three equal groups (30 chicks each). Group (1) healthy control group. Group (2) experimentally infected by intra-nasally inoculated with 1 drop of 10^7cfu spores of *A. fumigatus* per chick. Group (3) infected by the same dose, route as in (G2), and treated at 3 days post infection (PI) with 1 ml Mycostatin daily / liter drinking water for five consecutive days. Clinical signs of infected chicks with Aspergillosis appear within 48-72 h (PI). Three blood samples were taken at 1st, 14th and 21th days post treatment (PT) for immune-biochemical and hematological study. Specimens from lung, trachea, liver and heart were taken at 21th days (PT) for histopathological examination. Aspergillosis in chicks induces significant decrease in body weight, body weight gain, feed consumption, heterophile, lymphocyte, monocyte, phagocytic %, phagocytic index, total protein, albumin and globulin. Whereas associated with a significant increase in TLC, eosinophil, AST, ALT, ALP, T. cholesterol, uric acid, creatinine and poor feed conversion ratio all over the experimental periods when compared with control group. Microscopical examination of internal organs revealed adverse effects. Infected and treated chicks with Mycostatin showed improvement in all examined parameters and in pathological picture. It could be concluded that the Aspergillosis in broilers induce adverse effects on growth performance, immune response, hemato-biochemical parameters and on histological picture. But Mycostatin treatment induce improvement in these parameters and on the pathological changes.

Keywords: *Aspergillosis, A. fumigatus, broiler chicks, Mycostatin, immune-biochemical parameters, phagocytic activity and pathological lesions.*

Introduction

Mycotic diseases cause significant economic losses to poultry industry either due to their direct infectious nature or production of mycotoxins **(Dhama *et al*., 2013).** *Aspergillus* spp. can be isolated from environmental samples and are worldwide in distribution **(Marcel, 2011).** Aspergillus is the most common fungal disease of the avian respiratory system **(Beernaert *et al*., 2010).**

Aspergillosis is an infectious non-contagious fungal disease caused by *Aspergillus* spp. **(Ali *et al.*, 2018).** Aspergillosis is a major cause of high mortality in captained birds and less frequently in free-living birds **(Girma *et al.*, 2016)**. Aspergillus infection is a disease that impaired immunity **(Romani, 2004)**. Aspergillus members that may affect bird adversely are *A. fumigatus, A.terreus, A. glaucus, A.nidulans* and *A.niger* **(Dhama *et al*., 2012)**. *A. fumigates* infectious occurs more frequently in poultry, as the spores of this pathogen species are smaller than those of other *Aspergillus* spp.**(Arne *et al*., 2011)**.The disease develops in brooder stages in chicks as well as small birds especially below three days of age **(Chauhan and Roy, 2008)**. Aspergillosis occurs in two main forms acute and chronic **(Reese *et al*., 2006).**
Acute Aspergillosis (brooders pneumonia) occurs as a result of inhaling high number of spores causing high mortality (70-90%) can be seen within 24-48 h of infection **(Milos *et al.*, 2011)**. Chronic form occurs sporadically and is observed generally in adult breeder birds **(Femenia *et al*., 2007).** Diseased chicks showed gasping, fever, fetid diarrhea and rapid loss condition with convulsions **(Atasever and Gumussoy, 2004).** Infected chicks showed pathological lesions as uniform pinhead sized yellowish nodules in lungs **(Sultana *et al*., 2015a)**. Air sacs become thickened and cloudy hacking yellowish plaques **(Ahmet *et al*., 2009)**. Necrotic foci may occasionally be seen in the visceral organs **(Lotfy *et al.*, 1998).**
Using anti-fungal drugs, antiseptic compound help in reducing challenges with *A. fumigates* **(Sultana *et al*., 2015b).** Mycostatin and Nystatin that used in high value birds, where Nystatin is an antifungal antibiotic **(İcen *et al*., 2011 and Ziolkowska *et al.*, 2014).**

The aim of the present investigation is to isolate and identify *Aspergillus* spp. from naturally infected chickens with mycotic pneumonia as well as studies the growth performance, immune-biochemical, hematological parameters and pathological changes induced experimentally by *A. Fumigatus* in broiler chicks with trail of prevention.

Materials and Methods

1. Materials:

1.1. Collection of samples:

Eighty swab samples (40 air sac and 40 lung) were collected from a private chicken farm containing eight thousand chicks in Belbies, Sharkia Province from suspected diseased broiler chicks suffering from respiratory signs (respiratory distress, sneezing, nasal discharge, dyspnea, sinusitis, gasping (open mouth), emaciation, ruffled feather, greenish diarrhea).
Samples were collected aseptically (under hygienic condition) by scraping the lesion of lungs and air sacs using sterile scalpel blade and were put into sterile Petri dishes.

1.2. Drug: (Mycostatin):

Manufactured by Glaxo-*GSK* Smith Kline Beecham-Egypt. Each ml Mycostatin contains 100,000 I.U Nystatin / ml. Oral suspension-for oral use. The prophylactic, and therapeutic dosage is 1ml mycostatin / liter once daily in drinking water for 5 consecutive days **(Groll *et al.*, 1999 and Arne *et al*., 2011).**

1.3. Experimental birds:

Ninety, Hubbard broiler chicks 7-day old apparently healthy were used in this study. Chicks were fed on commercial un-medicated balanced ration and housed under hygienic conditions, managemental programmed, feed and water provided ad-libitum for 4 weeks.

1.4. Experimental inoculums (Standardization of infective dose):

On 7-day of age 60 chicks were Intra-nasally inoculated with 1 drop of 10^7cfu spores of *A. fumigatus* per chick by catheterization per nasal opening **(Lotfy *et al*. 1998)**.

2. Methods:

2.1. Mycological examination:

2.1.1. Isolation and identification of fungi from cases of diseased chickens:

The collected samples were cultured on Sabauroud's dextrose agar (SDA) media with

0.05 mg of Chloramphenicol / ml **(Larone, 1976).** The plates were incubated at 25-27 C˚for (5-7 days) and colonies identified **(Richard and Beneke, 1989).** The isolated fungi were sub-cultured and examined for macro-and micro-morphological characters and identified **(Pitt and Hocking, 2009).**

2.1.2. Preparation of *A. Fumigates* spore suspension:

The Spores were flushed with sterile physiological saline solution containing 0.05 tween 20-80 **(McClenny, 2005).** The spore suspension was shaken on a mechanical Shaker and the desired concentration of spores cfu 10^7 *A. fumigates* / 0.5 ml was determined, prepared and counted with hemocytometer **(Chaudhary and Sadana, 1988 and Lotfy *et al*., 1998).**

2.1.3. Experimental design:

Ninety, chicks 7-day old were kept for 4 weeks & then randomly divided into 3 equal groups (30 chicks each) in separated hygienic unit (cage). 1st group (G1) apparently healthy (control group). 2nd group (G2) and 3rd groups (G3) were infected by intranasal inoculated with 1 drop of 10^7cfu spores of *A. fumigates* / chick**.** As 2nd group infected non-treated (I non T) and 3rd groups infected and treated (I non T) with 1ml Mycostatine / liter drinking water once daily for 5 consecutive days. The treatment started at 3rd days post infection (PI) after appearance of clinical signs.

Table (l). Experimental Design: 90 chicks were divided into 3 equal group's (30 chicks each).

Chicks group	Number of chicks	Treatment	Dose	Time and duration of treatment
Gr1 Control gp.	30	Balanced diet.	--	4 weeks.
Gr2 Infected gp	30	Balanced diet + *A. fumigatus*.	-- 1 drop of 10^7cfu / chick intranasally.	4 weeks + then, infected.
Gr3 Infected and Treated gp.	30	Balanced diet + *A. fumigatus*, + Mycostatin.	-- 1 drop of 10^7cfu / chick intranasally, then 1ml / daily/ liter drinking water	4 weeks + then, infected for 3 days, Then treated for 5 consecutive days.

Clinical signs were recorded. The chicks in all groups were closely observed at least three times daily up to 4 weeks for the appearance of clinical signs, growth performance, morbidity and mortality with gross lesions in both recently dead and sacrificed chicks during the course of the experiment.

2.1.4. Re-isolation of *A. fumigatus* after the experiment:

Swabs from respiratory tract were collected for culture and microscopical examination of affected tissue. Isolation and identification of fungus under aseptic condition (by putting swabs or small pieces of affected tissue on sterilized Sabouraud's dextrose agar (SDA) or glucose agar and growth cultures kept and observed for 24- 48 h at 27°C, colonies are powdery gray, green / bluish in appearance and cottony colony shape, conidial heads (conidiophores) appeared as flask-shaped vesicle **(Girma *et al*., 2016).**

2.2. Body performance parameters:

All chicks of each groups were weighted at the start of the experiment and weekly for 4 weeks during the experiment. Feed consumption (FC)/gm/bird were recorded. Feed conversion ratio (FCR) equal consumed feed per gram on B.WT gain.

2.3. Blood samples:
Three blood samples were taken from wing vein of 5 chicks of each groups at the 1st, 14th and 21th days post treatment (PT).

First blood sample was taken on a tube contained EDTA for estimation. Total and Differential Leukocyte count **(Jain, 2000)**.

Second blood sample were collected on heparinized tube for phagocytic activity **:-**

a. Measurement of phagocytic activity of Peripheral Blood Monocyte **(**PBM**)** using *Candida albicans* was performed **(Anthony *et al*., 1985 and Chu and Dietert, 1989).**

b. Separation of Peripheral Blood Mononuclear Cells (PBMC) using ficoll-plaque density gradient was carried out **(Boyum, 1986 and Godeeris *et al.,* 1986).**

c. Phagocytic assay **(Wilikinson, 1976).**

d. Evaluation of phagocytic activity **(Wilikinson, 1976).**

Third blood sample were taken without anticoagulant in clean dry centrifuge tube to obtain clear serum and using spectrophotometer for estimation. T. protein **(Doumas *et al.,* 1981).** Albumin **(Drupt, 1974)** and Globulin (calculated as difference between T. proteins and albumin). AST-ALT **(Reitman and Frankel, 1957).** ALP **(John, 1982)**. T. cholesterol **(Allain *et al*., 1974)**. Uric acid **(Sanders *et al*., 1980)**. Creatinine **(Henry, 1974).**

2.4. Histopathological Studies:
Specimens were taken at 21th days post treatment (PT) from lung, trachea, liver and heart from all groups and fixed in10 % neutral buffered formalin solution then dehydrated, cleared and embedded in paraffin wax, then specimens were sectioned to 4-5 micron thickness and stained with Hematoxyline and Eosin stain (H&E) and examined microscopically **(Survarna *et al.,* 2013).**

2.5. Statistical Analysis: The obtained data were statistically analyzed by the students, t-test **(Tamhane and Dunlop, 2000).**

Results and Discussion
Aspergillosis is one of the most common mycotic disease causing significant economic loss in poultry industry and animals, also cause direct harm to human health either due to their direct infectious nature or due to production of mycotoxins **(Kamble, 2017).** Acute Aspergillosis that affects young chicks called (brooder pneumonia) with high morbidity and mortality than chronic form which affect adult birds **(Ali *et al*., 2018).**

Forty one positive sample for *Aspergillus* spp. as total (21 air sac and 20 lung) identified into *A. fumigatus* 20, *A. flavus* 9, *A. niger* 7, and *A. terreus* 5 respectively (Table. 2). *A. fumigatus* is the most common fungus isolates. This may be attributed to environmental condition of poultry rearing and intensive use of antibiotics, which occur in broiler than layer flock **(Debey *et al.,* 1995 and Girma *et al.,* 2016).**

Our findings agreed with the results obtained by **Lotfy *et al*. (1998)** they isolated high incidence of *A. fumigatus* from naturally infected fattening than layers chickens as identified *Aspergillus* spp. into (*A. fumigatus, A. flavus, A. niger* and *A. glaucus*). **Sajid *et al.* (2006)** described similar *A. fumigates* in commercial poultry flock**.** Also, **Kamble (2017)** reported that the 2 major species of *Aspergillus* spp. causing Aspergillosis in poultry are *A. fumigates* and *A. flavus*, in addition to others species include *A. terreus, A. glaucus* and *A. niger.* **Richared and Debey (1995)** who reported that the conidia of *A. fumigatus* small enough (2-3 µm) in diameter to bypass initial physical barriers and disseminate deeply in respiratory system. However, large diameter of *A. flavus* conidia (3.5-4.5 µm) may explain lower pathogenicity when compared to *A. fumigatus* in experimental infection, but not in other conidia of *A. nidulans, A. niger* and *A. oryza*. Also the same findings confirmed by **Femenia**

***et al*. (2009).** While lower incidence of *Aspergillus* spp. in lungs was recorded by **Lobna and Abd El-Fatah (2014)** they reported that *Aspergillus* spp. was isolated by 24% from lung tissues of chickens.

In our study the infected chicks with *A. fumigatus* showed clinical signs within (2-3 days) post infection as nasal discharge, conjunctivitis, off feed, ruffled feather, depression, greenish diarrhea, accelerated respiration and respiratory distress, dyspnea and gasping (open mouth) (Fig.1&2), while others showed sudden death in some chicks, when compared with control group all over the experimental period, this may be due to the gradual obstruction of the air passage and the fatal effects of toxins that produced by *A. fumigatus* which impair mucocillary action of upper respiratory system resulted in prolonged residence of the fungus at surface of epithelium **(Madadi *et al*., 2014 and Sultana *et al*., 2015a)**. Others showed typical signs by **Milos *et al*. (2011) and Ali *et al*. (2018)** in broiler this may be due to aspergillosis in young chicks is highly fatal in the first 10 days of life resulted in major respiratory distress. Also these findings agreed with **(Ahmet *et al*., 2009 and Abdulrahman *et al*., 2014)** in pullets, ostrich and broiler farms. In addition **(Ariese *et al*., 2013)** reported that the clinical manifestations depend on the infective dose, spores distribution, pre-existing diseases and immune response of the host.

In this study the chicks infected with Aspergillosis showed significant reduction in body weight, F.C and body weight gain beside poor FCR throughout the experimental period when compared with control group as shown in (Table.3).This observation may be due to malabsorption from inflamed gut, malnutrition and anorexia caused by Aspergillosis. Similar findings were previously recorded by **Sajid *et al*. (2006) and Jacquie (2015)** who mentioned that Aspergillosis in poultry induce significant decrease of body weight and body weight gain. Also the same results were coincided with **Girma *et al*., (2016) and Ali *et al*., (2018)** who showed that hatchery contamination by Aspergillosis led to decreased feed intake, lower growth rate and enteritis of chicks.

The present work showed significant increase in WBC, eosinophil which associated with significant decrease heterophile, lymphocyte, and monocyte in chicks challenged with *A. fumigatus* when compared with control group throughout the experimental periods (Table.4). Increase leukocytosis may be indicated to acute inflammation and stress factor of Aspergillosis in birds as a result of inhalation over number of spores by respiratory passage to lungs and response of leukograme as body defense mechanism against enteritis **(Coles, 1986 and Peter *et al*., 2013) .**

Our results similar to that obtained by **Bolu *et al.* (2011)** who mentioned that broiler challenged with *A. flavus* showed significant increase in leukocyte count and eosinophil which associated with significant decrease in heterophil and monocyte.

In this study the significant increase in TLC may be due to eosinophilia to compensate the decrease in heterophile and lymphocyte, monocyte. These finding agreed with the results obtained by **Aravind *et al*. (2003) and Adeymo and Sani (2013)** who reported leukocytosis due to peripheral blood eosinophilia as *A. fumigatus* is an opportunistic funguss caused Eosinophilic Allergic Broncho Pulmonary Aspergillosis (EABPA). Monocytopenia in our study discussed by **Arne *et al.* (2011)** due to toxic effect of gliotoxin as a secondary metabolic product by *A. fumigatus* which suppress cellular immune response via gliotoxin-mediated apoptosis of monocyt . In addition to **(Marcel, 2011)** who found that Aspergillosis in bird resulted in significant decrease in heterophil due to fatal toxic effects of gliotoxin that capable of altering host defenses through interrupt function of leukocytes by inhibiting migration of heterophil which is the principle target cell of this toxin, as mentioned by **Latage (2001).**

Avian lung associated with immune system combining lymphocytes, macrophages and phagocyte system **(Toth, 2000 and Reese *et al.,* 2006)**. During acute phase response when birds inhaled numerous spores by respiratory passage to lungs migration of heterophils in large numbers from tissues to lumen of lungs, which responsible for destruction of hyphae of *A. fumigatus* and able to kill conidia that escaped and destroyed by macrophage (**Mills and Ley, 2014**). In addition to, the phagocytes cells have essential role against disease that is the direct defense cells when bird inhaled conidia as the macrophages prevent germination of it, depend on anatomic location, where avian macrophages properties include chemotaxis, phagocytosis, pathogen elimination and cytokine production **(Marcel 2011).** Also, **(Redig, 2005)** mentioned that the primary polymorph nuclear leukocytes are the vital cellular components of innate immunity and function it killing pathogen, beside chemotaxis and cytokine induction antigen presentation following by phagocytosis and pathogen elimination**.** So the ability of birds to respond to fungal antigens depend on organizing good cell-mediated response and strong activated phagocytes that is one of key events in establishment of Aspergillosis **(Beernaert *et al.,* 2010).**

In the present study the infected broiler with Aspergillosis revealed significant decrease phagocytic % and phagocytic index (Table.5) when compared with control group throughout the experimental period. The similar finding agreed with the results obtained by **Qureshi and Miller (1991)** as they mentioned that Aspergillosis in broilers compromised immune system (impaired immunity) and led to overcome natural defines by decreasing phagocytic activity.

Our results showed significant decrease in phagocytic % and phagocytic index in chicks suffering from Aspergillosis, this may be due to toxic effects of gliotoxin and other several mycotoxins produced by *A. fumigatus* which suppress cellular immune response via-mediated apoptosis of monocyte, in addition to enhance cellular apoptosis of macrophages so retared phagocytosis and impaired chemotaxis **(Corrier, 1991 and Arne *et al*., 2011).** In addition to delay leukocyte migration and intra cellular killing potency of heterophils and monocytes as well as cell-mediated responses, where the toxin is highly immunosuppression mycotoxin produced by various isolate *A. fumigates* so resulting in significant decreased in phagocytic activity (phagocytosis) and CMI, as mentioned by **Femenia *et al*. (2018).**

Our results (Table.6) revealed that Aspergillosis in broiler induce significant decrease in T. protein, albumin, globulin and A/G ratio when compared with control group throughout experimental period, these may be due to hepatocellular damage and renal involvement.

In present study the reduction in protein picture may be due to anorexia (loss appetite) caused by Aspergillosis, malnutrition and malabsorption due to inflamed gut, or may be the loss of protein in urine due to nephritis as a result of systemic Aspergillosis, that led to lower serum protein level and albumin with loss of globulin (gamma) so decrease resistance to infection. Also the decreased albumin may be due to hepatocellular damage as present necrosis in liver cells where liver is main site of albumin syntheses and partially globulin. Our results similar to that obtained by **Marcel (2011) and Peter *et al*. (2013).**

Our data (Table.7) showed that broilers affected with Aspergillosis caused significant increase in liver enzymes (AST- ALT- ALP) when compared with control group during the experimental period, these may be due to hepatic damage. Similar results were reported by **Basmacioglu *et al*. (2005)** who mentioned that liver enzymes increased significantly reflect liver damage which induced by Aspergillus toxin as led to leakage of enzymes outside necrotic hepatic cells into blood stream. And may be due to increase cellular permeability of damaged liver cells. Also the same results

agreed with the finding of **Reidarson and McBain (1995).**

Significant increase of total cholesterol (Table.7) in our study may be due to stenosis of blood stream with present blood vessels thrombosis due to vegetative hyphae where conidia disseminated through visceral organs to blood capillary, as mentioned by **lofty *et al.* (1998) and Beytut *et al*. (2004).** In addition to **Merkley *et al*., (1987)** reported that the increase in total liver lipids (cholesterol) due to hepatic and kidney damages in chickens exposed to aflatoxin.

In our study the significant increase in uric acid and creatinine (Table.7) in broiler suffering from Aspergillosis when compared with control group during the experimental period, may be due to kidneys damage. And may be attributed to disturbance and increase in protein catabolism due to toxic effect of mycotoxins as (proteases enzymes, gliotoxin and galactommanan) that produced by *A. fumigatus* which degrade (lysis) protein into nutrients for further assimilation of fungus and led to renal failure. The same results were in harmony with **(Basmacioglu *et al*., 2005 and Marcel, 2011).** Also our data agreed with previous reports of **Bolu *et al.,* (2011)** in broiler infected with Aspergillosis as increase in uric acid and creatinine may be due to kidneys damage. **Adeymo and Sani (2013)** stated that uric acid is primary catabolic product of protein and non-protein nitrogenous compounds in birds and increase uric acid in case of starvation beside massive tissue destruction and renal failure. As increase protein catabolism and decrease renal blood flow led to increase uric acid in birds when disseminated conidia via blood capillary to major organs such as (kidneys, spleen, heart, brain, liver, and intestine) caused renal dysfunction, that uric acid is the major end product of protein metabolism **(Le Loch *et al.,* 2006).**

In the present study the infected and treated groups by Mycostatin showed improvement in clinical signs, body performance, all immune-biochemical parmeters and pathological changes post Mycostatin treatment. As these levels decline indicating regeneration processes. Similar findings were coincided with previous study by **(Dyar *et al*., 1984 and Arne *et al* ., 2011)** as they stated that mycostatin is an antimycotic drug indicated for prevention and treatment of fungal infection as it reduce challenge with *A. fumigatus* in chicks, also these may be due to Nystatin is an antifungal, antibiotic that interferes with permeability of the cell membrane of sensitive fungi by binding to sterols, chiefly ergosterol, where its main action against fungal infection and *Candida* spp. (candidiasis), so it used for prophylaxis and reduced fungus–mediated tissue injury that reduce pulmonary fungal tissue, as mentioned by **Groll *et al.* (1999) and McMullin (2004).**

In the present study the infected chicks with Aspergillosis showed congestion of all internal organs (liver, kidney, lung, heart and intestine) in addition to numerous yellowish white nodules with thickening in wall of affected intestine.

The postmortem examination (PM) of broiler suffering from Aspergillosis showed white to yellowish spherical caseous nodules of various sizes in lungs & liver (fig.3). Similar observations were demonstrated by **Sajid *et al*., (2006**) who described congestion in lungs with grayish white nodules and frothy exudates due to *A. fumigatus* affected poultry and ostrich. Also these results agreed with **(Medani *et al*., 2004 and Francoise *et al.,* 2007)** in turkeys. **Ahmet *et al.* (2009**) mentioned that *A. fumigates* infection in ostrich lead to fatal pulmonary Aspergillosis beside prominent enlargement with amyloid accumulation in liver, but necropsy revealed several grayish to white nodules scattered throughout lungs and thoracic air sac. In addition, our results agreed with **(Madadi *et al*., 2014 and Ibrahiem *et al.,* 2016**) they showed yellow, green or white and numerous military granulomatous foci in lung parenchyma in broiler and poultry affected with As-

pergillosis. In addition to multi systemic Aspergillosis with granulomas in layer chickens, as recorded by **Kim and Kim (2011).**

The lung of infected bird after 21th days (PT) showed recent granuloma contain macrophages, histocytes and lymphocytes cells infiltration (fig.4) in addition to granuloma with caseated center (fig.5). The lung showed hyphae of Aspergillus with histocytes, and lymphocytes infiltration (fig.6).These results are discussed by **Kunkle and Rimiler (1996) and Zafra *et al.* (2008)** who mentioned that infection occur by inhalation of over number of small hydrophobic spores (conidia) into respiratory tract that invade trachea, nasal, bronchial epithelia, penetrate respiratory tissue, reproduce by single division of tubular hyphae to form mycelia, initiation into granulomas then disseminate to other tissue like brain, heart, pericardium, bone marrow, kidney, other tissue. As inflammatory response with hetrophile, lymphocyte, monocyte and some giant cell infiltration produce lesion in turkeys**.** In addition, these results agreed with **Abdulrahman *et al*. (2014) and Madadi *et al*. (2014).**

The lung of infected and treated chickens of the third group showed some improvement of lung tissue with congested and thickening in wall of blood vessels were noticed (fig.7). These results agreed with **Lotfy *et al.* (1998) and Sultana *et al.* (2015a)** who found congestion and perivascular edema in infected birds.

Liver of infected chickens (group 2) after 21th days (PT) showed granuloma with central caseation surrounded by heterophils, lymphocytes and macrophage cells infiltration (fig.8), hyphae of Aspergillus with fibrin threads and histocytic cells infiltration (fig.9). The same findings coincide with **(Lotfy *et al.,* 1998 and Ahmet *et al.,* 2009)** who said that the broilers were highly infected with Aspergillosis than layers, they detected multifocal areas of coagulated necrosis replacement of hepatic parenchyma with lymphocytes and few macrophages, beside mild degenerative changes.

Liver of infected and treated chickens (group 3) showed some improvement where it showed congestion of blood vessels and hydrobic degeneration of hepatic cells (fig. 10).

Trachea of infected chickens after 21th days (PT) showed inflammatory cells infiltration and discontinuity of tracheal muscles beside inflammatory edema between muscles layers (fig.11)**.**

Heart of infected chickens after 21th days (PT) showed hyaline degeneration and perivascular edema with mild congestion, macrophage and lymphocyte among cardiac muscles (fig.12).This observation agreed with **Julian and Goryo (1990).**

These results agreed with **Girma *et al*. (2016) and Ali *et al.* (2018)** they mentioned that visceral organs were involved in Aspergillosis with formation of nodular granulomatous lesions. Also **Beernaert *et al* (2010)** said that all organs can be involved, leading to a variety of manifestations ranging from acute to chronic infections.

In contrast, the infected and treated chick organs showed marked improvement in visceral organs as lungs (fig.7). Also liver, trachea and heart are showed mild congestion, as these demonstrated by **Sultana *et al.,* (2015b)**.

Conclusion

It could be concluded that Aspergillosis in broiler chicks induce severe adverse effects on growth performance, immune-biochemical, hematological parameters and histological appearance. Mycostatin is effective in treatment and prevention of Aspergillosis in broiler chicks.

Table (2). Identification of Isolated *Aspergillus* species in the examined samples.

No. of Swabs	Total number	+ ve swabs	- ve swabs	Frequency of *Aspergillus* spp.				
				Type	Air sac	Lung		
		No.	No.		No.	No.	Total No.	%
Air sac	40	21	19	*A. fumigatus*	11	9	20	48,78
				A. flavus	5	4	9	21,95
Lung	40	20	20	*A. niger*	3	4	7	17,07
				A. terreus	2	3	5	12,19
Total	80	41	39		21	20	41	

Table (3). Effect of Aspergillosis and Mycostatin on body performance of broiler chickens at 1st, 2nd, 3rd and 4th week post treatment (N=5) (mean± S.E).

Groups	1st week					2nd week				3rd week				4th week			
	I BW	FBW	WG	FC	FCR	FBW	WG	FC	FCR	FBW	WG	FC	FCR	FBW	WG	FC	FCR
C G1	48.24 ± 0.67	228.06± 3.44	179.94± 2.54	270.54	1.51	514.23 ± 4.55	286.17± 2.85	489.84	1.71	1010.6± 5.07	496.37± 2.85	1008.5	2.03	1966.21 ± 6.09	955.40± 2.85	1970.76	2.06
I non T G2	48.16 ± 0.35	198.15± 7.99**	149.99± 5.64**	230.73	1.54	474.17 ± 6.93**	276.02± 5.64**	474.17	1.76	954.1 ± 3.87**	479.93± 5.64**	997.7	2.07	1837.73 ± 7.93***	883.63± 5.64**	1860.81	2.11
I and T G3	49.06 ± 0.38	228.89± 1.86	179.83± 1.99	265.56	1.52	484.18 ± 9.67*	255.29± 1.99	445.09	1.74	978.95± 4.94**	491.77± 1.99	1004.8	2.04	1894.35± 8.96***	915.4 ± 1.99	1921.76	2.09

C= Control G1 I non T = Infected Non Treated G2 I and T = Infected and Treated G3 FC=Feed Consumption. FCR= Feed Conversion Rate G= Group IBW= initial body weight FBW=final body weight WG= weight gain BW= body weight *Significant at $P < 0.05$. ** Significant at $P < 0.001$.

Table (4). Effect of Aspergillosis and Mycostatin on Leukogram of broilers (mean± SE).

Groups		Leukocytic count					
		TLC (10^3/mm^3)	Differential count(10^3/mm^3)				
			Heterophile	Lymphocyte	Monocyte	Eosinophile	Basophile
1st Day	Control G1	20.07± 0.21	7.26± 0.22	4.44± 0.25	3.56± 0.26	2.44± 0.50	2.37± 0.44
	I non T G2	23.19± 0.33**	5.85± 0.13*	3.68± 0.17*	2.75± 0.16*	5.95± 0.45*	4.96± 0.31*
	I and T G3	22.09± 0.18*	6.82± 0.17	4.07± 0.19	3.26± 0.21	3.98± 0.79	3.96± 0.63
14th Day	Control G1	20.95± 0.31	7.50± 0.41	4.44± 0.24	3.81± 0.20	2.59± 0.33	2.61± 0.60
	I non T G2	23.18± 0.22**	5.95± 0.15*	3.63± 0.19*	2.69± 0.13*	5.92± 0.53*	4.99± 0.40*
	I and T G3	20.99± 0.65	6.90± 0.26	4.18± 0.31	3.30± 0.17	3.08± 0.43	3.53± 0.35
21th Day	Control G1	20.52± 0.28	7.41± 0.36	4.50± 0.22	3.61± 0.17	2.60± 0.16	2.40± 0.21
	I non T G2	23.09± 0.28**	6.07± 0.10*	3.55± 0.21*	2.60± 0.14*	5.98± 0.61*	4.89± 0.45*
	I and T G3	20.74± 0.56	7.10± 0.34	4.22± 0.41	3.42± 0.33	2.95± 0.38	3.05± 0.43

C= Control G1 I non T = Infected Non Treated G2 I and T = Infected and Treated G3
* Significant at $P < 0.05$. * * Significant at $P < 0.001$

Table (5). Effect of Aspergillosis and Mycostatin on Phagocytic% and Phagocytic Index of broiler (mean± S.E).

Groups		Control G1	I non T G2	I and T G3
1st day (PT)	Phagocytosis %	63.20±1.35	43.12±1.31**	53.61±1.36**
	Phagocytic Index	4.3±0.15	3.91±0.06*	3.86±0.12*
14th day (PT)	Phagocytosis %	65.36±1.23	42.16±1.15**	58.72±1.26
	Phagocytic Index	4.53±0.13	3.10±0.30*	4.39±0.19
21th day (PT)	Phagocytosis %	67.38±1.41	39.42±1.36**	64.21±1.36
	Phagocytic Index	5.66±0.13	2.93±0.32*	4.23±0.82

C= Control G1 I non T = Infected Non Treated G2 I and T = Infected and Treated G3 (PT)=post treatment
* Significant at P < 0.05. * * Significant at P < 0.001.

Table (6). Effect of Aspergillosis and Mycostatin on protein profile of broiler (mean± S.E).

Groups	1st day			14th day			21th day		
	C	I non T	I and T	C	I non T	I and T	C	I non T	I and T
T. Protein (gm/dl)	5.20± 0.31	3.50± 0.18**	4.10± 0.16*	5.91± 0.28	3.60± 0.15**	4.86± 0.36	5.50 ± 0.29	3.15± 0.16**	4.33± 0.13
Albumin (gm/dl)	2.91± 0.21	2.10± 0.18*	2.20± 0.13*	3.61± 0.26	2.20± 0.11*	3.00± 0.43	3.42 ± 0.15	2.06± 0.20*	2.66± 0.32
Globulin (gm/dl)	2.29± 0.19	1.40± 0.21*	1.90± 0.12	2.30± 0.22	1.40± 0.32*	1.86± 0.21	2.08 ± 0.41	1.09± 0.23*	1.67± 0.32
A/G Ratio	1.27± 0.19	1.50± 0.25	1.16± 0.25	1.57± 0.34	1.57± 0.31	1.61± 0.33	1.64 ± 0.34	1.89± 0.32	1.59± 0.29

C= Control G1 I non T = Infected Non Treated G2 I and T = Infected and Treated G3
*Significant at P < 0.05. * * Significant at P < 0.001.

Table (7). Effect of Aspergillosis and Mycostatin on Liver enzymes, Kidney function and Cholesterol of broiler chickens (mean± S.E).

Groups		Liver enzymes			Kidney function		Cholesterol (mg/dl)
		AST (U/L)	ALT (U/L)	ALP (U/L)	Uric acid (mg/dl)	Creatinine (mg/dl)	
1st day	Control G1	39.22± 0.65	23.31± 0.21	44.37± 0.88	5.03± 0.16	0.79± 0.13	115.49± 1.04
	I non T G2	60.65± 0.98**	29.21± 0.43 **	57.32± 0.87**	9.16± 0.32**	1.43± 0.11**	121.04± 1.22*
	I and T G3	45.04± 0.93*	26.44± 0.36*	48.54± 0.90	8.42`± 0.15*	1.14`± 0.10*	118.32± 1.85
14th day	Control G1	39.53± 0.61	22.27± 0.26	44.95± 0.79	5.14± 0.14	0.80± 0.11	115.38± 1.17
	I non T G2	65.23± 0.89**	30.85± 0.32**	55.98± 0.77**	9.84± 0.41**	1.60± 0.13**	120.89± 1.42*
	I and T G3	41.53± 0.99	24.21± 0.53	46.85± 0.89	7.31`± 0.28	1.06`± 0.17	116.49± 1.94
21th day	Control G1	40.43± 0.63	22.31± 0.19	44.97± 0.68	5.56± 0.24	0.77± 0.21	115.84± 1.21
	I non T G2	59.05± 0.64**	30.93± 0.42**	56.87± 0.84**	9.85± 0.27**	1.76± 0.18**	121.75± 1.73*
	I and T G3	43.43± 0.57	23.48± 0.83	47.05± 0.78	6.64± 0.28	1.09`±| 0.19	116.04± 1.98

. C= Control G1 I non T = Infected Non Treated G2 I and T = Infected and Treated G3
*Significant at P < 0.05. * * Significant at P < 0.001.

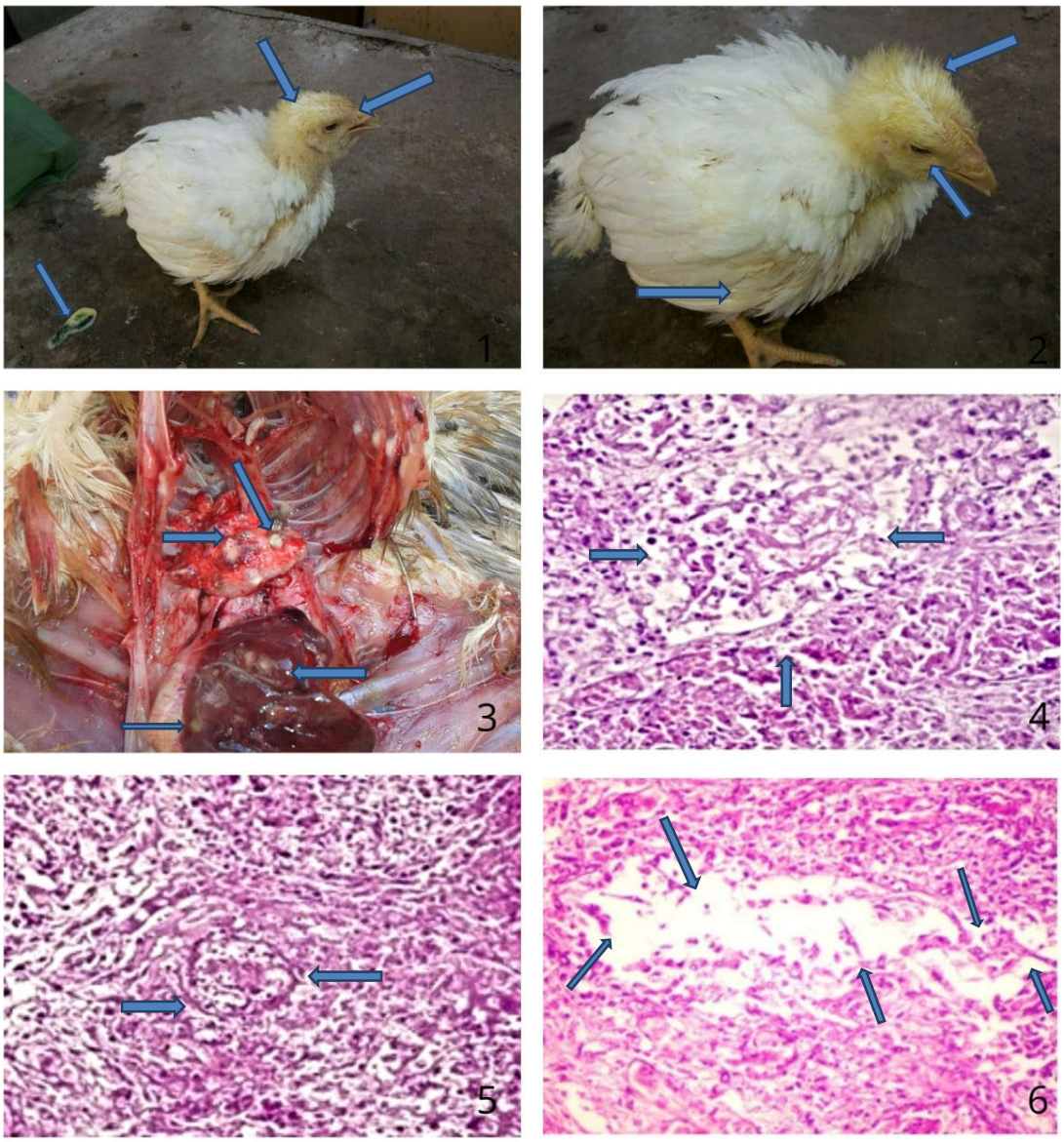

Fig. (1): Infected chicken suffering from difficult breathing (gasping) and green diarrhea.

Fig. (2): Infected chicken suffering from ruffled feather and depression.

Fig. (3): Lung of infected chicken showing creamy color nodules in plural surface and yellowish white nodules in liver.

Fig. (4): Lung of infected chicken showing recent granuloma contain macrophages, histocytes, lymphocytes and fibroblast infiltration (H&E X400).

Fig. (5): Lung of infected chicken showing granuloma with caseated center (H& E X400).

Fig. (6): Lung of infected chicken showing hyphae of aspergillus with histocytes and lymphocytes infiltration (H& E X 400).

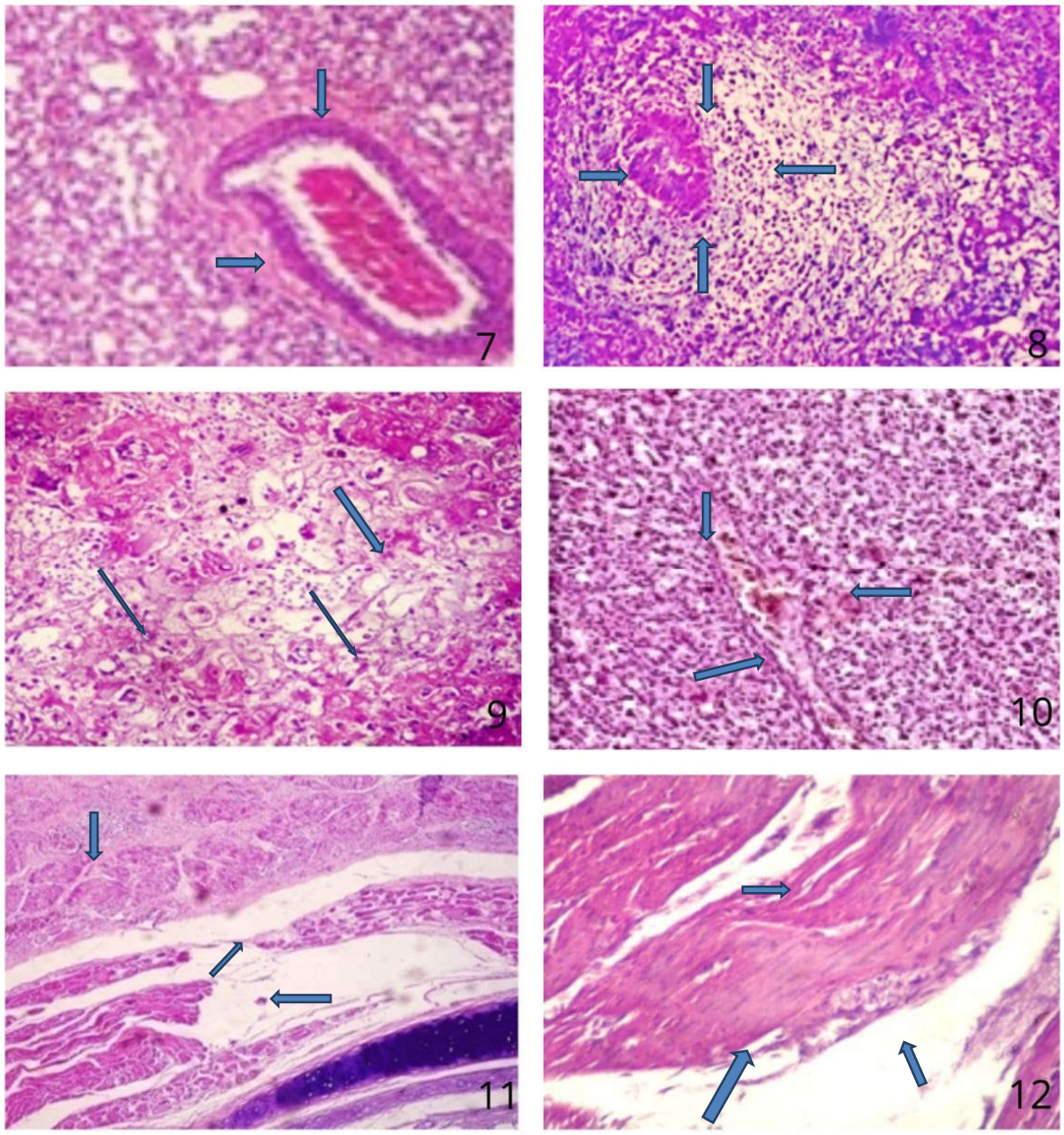

Fig. (7): Lung of infected and treated chicken showing some improvement of pulmonary tissues with congested and thickening in wall of blood vessels (H& E X400).

Fig. (8): Liver of infected chicken showing granuloma with central caseation surrounded by heterophil, lymphocytes and macrophage (H & E X400).

Fig. (9): Liver of infected chicken showing hyphae of aspergillus with fibrin threads and histocytic cells infiltration (H & E X400).

Fig. (10): Liver of infected and treated chicken showing mild congested blood vessels and hydrobic degeneration of hepatic cells (H & E X 200).

Fig. (11): Trachea of infected chicken showing inflammatory cells infiltration and discontinuity of tracheal muscles with inflammatory edema between muscle layer (H& E X 200).

Fig. (12): Heart of infected chicken showing hyaline degeneration and perivascular edema with mild congested blood vessels. (H & EX 400).

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