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CLINICAL AND PATHOLOGICAL INVESTIGATIONS AND THERAPEUTIC TRIALS ON EXPERIMENTAL INFECTION BY *ASPERIGILLUS FUMIGATUS* IN MONGREL DOGS

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ABSTRACT

This work was carried out to investigate the findings of clinical, haemotological, biochemical, histopathological and mycological analyses and to evaluate the efficiency of itraconazole treatment in mongrel dogs experimentally infected with *Aspergillus fumigatus.* Twenty healthy dogs were used, the animals were divided into 4groups: group I (control), group II (non-immunosuppressed infected), group III (immune-suppressed infected) and group IV (immunosuppressed infected & treated), each group consisting of 5 dogs. Clinical findings of aspergillosis developed after day15thfollowing *A. fumigates* inoculation. Hematological analysis revealed leukocytosis, lymphocytosis, monocytosis and granulocytosis in groups II and III. In the same groups The red blood cell, haemoglobin haematocrit values and glucose concentrations decreased while serum alkaline phosphatase activity, urea nitrogen, total protein, globulin, magnesium and phosphorus concentrations increased. *Aspergillus fumigatus* was re isolated from lungs, kidneys, liver and spleen of the dogs, granulomatous inflammation was observed in the lungs, kidneys, liver and spleen by histopathological examination. The treatment of the animals by Itraconazole started on day 35 PI and lasted for 6 weeks. At the end of the experiment, all clinical signs in the treated

dogs had improved except for crepitation rales of the lung and dyspnea. Haematological, biochemical and urine analysis findings returned to close to normal values.

In conclusion, *A. fumigatus* should be considered among the diseases of the lungs and kidneys. Immuno suppression has a great role in occurrence of canine asperigellosis and severity of its clinical manifestations and pathological findings. The success rate which obtained with itraconazole in the present study may indicate that a large-scale and detailed study would be of practical value.

Keywords: *Aspergillus fumigatus*, Clinical, histopathological, itraconazole

INTRODUCTION

Aspergillus is a hyphomycete which is widely spread in the environment and is commonly found in soil, water and organic matter [1,2&3]. Aspergillos is has been recorded in different species as dogs, cats, sheep, cattle, birds and human beings causing localized and disseminated infection in animals and humans [4&2]. Canine Aspergillosis mostly involve the upper respiratory tract in form of sinonasal (SNA) infection while pulmonary and disseminated infection are less commonly described [4,5&6].Although canine aspergillosis has been recorded in every breed and age of dogs , it commonly affects young to middle aged dogs [1,7 & 8] immune suppression and spore count play an important role in development of infection [7 & 9]. Experimental infection via intratracheal and intravenous routes have been recorded [10]. Common clinical signs in dogs with disseminated aspergillosis are weight loss, pyrexia, inflammatory ocular disease and muscular pain [2, 3 & 6].

Various antifungal drugs have been used for treatment of aspergillosis [11]**.** Itraconazole is synthetic antifungal drug has been widely used in human medicine and was recently used in veterinary medicine in treatment of aspergillosis [12].Administration of itraconazole has results in 60 to 70 % success rate in treatment of aspergillosis [8]. It used in dose of 5mg /kg .b.w orally for 6 weeks in treatment of experimental aspergillus infection in stray dogs [4] with good success rate. There have been limited studies on treatment of canine asperigillosis with itraconazole [11]

So, this study was carried out to investigate the clinical, haematological, biochemical, histopathological and mycological findings and to evaluate the efficiency of itraconazole treatment in immune-suppressed and non-immuno-suppressed

mongrel dogs experimentally infected with *A. fumigates*.

MATERIALS AND METHODS

Experimental animals:

Twenty apparently healthy mongrel dogs of different sex, 1-3years old with average body weight 20-25 kg were housed in dog kennels of clinical hospital of department of medicine and infectious disease, faculty of veterinary medicine, Cairo University. All animals were fed fresh rice, bread and meat residues twice daily and were given ad-libtiumwater throughout the experimental period (from April till July, 2013).

Before the start of experimental work all dogs were kept one week for adaptation and were given anthelmintic, albendazole (Bendax®,Sigma company , 10ml PO q24hr) for 3 successive days and repeated after 14 days for another 3 days. Also all dogs were vaccinated with rabies vaccine (Defensor®, Pfizer Company).

The animals were randomly divided into 4 groups: group I (control), group II (non-immunosuppressed infected), group III (immunosuppressed infected) and group IV(treated) each group was consisting of 5 dogs.

All procedures of using laboratory animals in this study met the regulations of Ethics of Research Committee at Faculty of Veterinary Medicine, Cairo University and received the approval number: Cu-Vet. F. Med-1-2015.

Preparation of inoculum:

A. fumigatus was supplied by Department of Medical Microbiology, Faculty of Veterinary Medicine, Cairo University. Sabaroud Dextrose Agar (SDA with 2IU penicillin G/ml + 40 µg streptomycin/ml were used for the growth of *A. fumigatus*. Macroscopical and Microscopical examinations of colonies were carried out. The concentration of 2.7 x 10^6 spores/ml was obtained by measuring the optic density of the spore suspension.

Induction of experimental infection and treatment:

Animals in group II were injected with *Aspergillus fumigates* inoculum with dose 2.5 ml I/V/dog. Animals in group III and IV were injected with dexamethasone (Dexmethasone®, Al- Amrya company, 1ml I/M for 3 successive days for each dog)then they were injected with *Aspergillus fumigates* inoculum with dose 2.5 ml I/V. On day 35 PI animals in group IV were treated with itraconazole (Itranox®, AdwiaCompany, 5mg/ kg b.wdissolved in 2 ml distal water and given PO q 24hr) dailyfor 6weeks.

Clinical examination and samples collection:

Clinical examination including rectal temperature, respiratory rate and heart rate,

were observed and recorded at the beginning of studyand on day 34 PI(post infection)in all groups.Nasal and lacrimal swabs and 2 blood samples for hematological and biochemical analysis were taken at the beginning of study and on day 34 PI(post infection)from all groups. Animals in group IV were exposed to samples collection after 6 weeks of treatment with recording of clinical examination criteria.

Hematological and biochemical analysis:

The white blood cell (WBC), red blood corpuscles (RBC's), lymphocyte, monocyte, Haemoglobin (Hgb) and haematocrit (PCV) were determined according to[13] and blood indices were measured as described by [14]

Serum alkaline phosphates (ALP), aspartate aminotransferase (AST) activities (Spinreact company ,Spain), creatinine, glucose, totalprotein (Bio-Diagnostic, Giza, Egypt), albumin, calcium,magnesium and phosphorus concentrations (Spectrum company ,Egypt) and globulin were measured with commercial kitsby a spectrophotometer (Apple 302, USA).

Mycological analysis:

Samples of nasal swabs (live dogs) and organs at postmortem examination (lungs, kidneys, liver and spleen) from all groups before and after treatment were inoculated on SDA. The isolates were identified on the basis of morphology and cultural characteristics.

Postmortem and histopathological examination:

At the begging of experiment 2 dogs from group I were euthanized, on day 34 PI5 dogs from group II and 5 dogs from group III were euthanized, at the end of experiment (after 6 weeks from treatment)5 dogs from group IV were euthanized. All dogs were subjected to postmortem examination to detect any abnormal gross changes. Tissue specimens from lungs, kidneys, liver and spleen of these dogs were collected, fixed in 10 % neutral buffered formalin, processed and embedded in Paraffin wax, sectioned at 4 µm and stained with Hematoxylin and Eosin and PAS [15] and examined under an Olympus microscope (Olympus, Japan). Euthanasia was performed by intravenous injection of overdose of barbiturates.

Statistical analysis:

The data were subjected to variance analysis (one-way ANOVA) and student T-test in treated group before and after treatment was done using SPSS program Version 16 All data were expressed as means ± SEM.

RESULTS AND DISCUSSION

Clinical findings:

On days 15-20 PI(post inoculation) , dullness, anorexia, weight loss, dehydration, sneezing, coughing, dyspnoea, hardness of respiratory sounds , crepitation rales on auscultation of the lungs , and lacrimation with conjugativitis were observed in all dogs in groups, II, III and IV. The same observation were recorded by [2, 3, 4, 6, 16 & 17]

The severity of the clinical findings in group III, IV was more than in group II which confirm the role of immunosuppression in intense and spread of clinical sings in canine asperigillosis. Respiratory rate, heart rate and rectal temperature were increased in all dogs in groups II,III as in (Table 1).

Animals in group IV that exposed to antifungal treatment with itraconazole for 6 weeks were recovered from clinical findings except for crepitation rales of the lungs and dyspnea with improvement in its physical parameters.

Concerning the results of statistical analysis of clinical examination parameters of group IV .Itrevealed that the use of itraconzole as antifungal agent had a significant improvement effect ($p \leq 0.05$) on respiratory and heart rates of infected dogs (Table 2).

Hematological findings

Concerning to the results of statistical analysis of hematological parameters, it revealed reduction in RBCs, Hb and PCV values in group II and III (Table 3) as the results recorded by[2,3,4 &18] this reduction may be due to sever infection . Leucocytosis due to increased numbers of monocytes, lymphocytes and granulocytes WBCs was determined (Table 3) as mentioned previously by [3,4,6,18 &19]. This finding was supported by the histopathological finding.

Concerning to the results of statistical analysis of hematological parameters in group IV, it revealed slightly improved hematological picture with treatment by itraconazole which confirmed by disappearance of most clinical signs (Table 4).

Biochemical findings:

The results of statistical analysis of biochemical parameters revealed increase in serum ALP as reported by [2,4,18 &20], BUN as reported by [3,4,6 &18], total protein and globulin as recorded by [2,3,4,6 &20]. Hypoalbuminemia was observed in infected dogs, similar result was mentioned by [3,4&20].

Hypoglycemia in affected dogs may be due to lack of appetite and anorexia. On the other hand hyperphosphatemia and hypermagnesaemia were recorded as the same findings by [1&4] which suggested that kidneys were involved in infection and that was confirmed by histopathological

and mycological findings. Although the nasal cavity were reported as the most sensitive organ to naturally acquired *A. fumigatus* infection as mentioned by[4,5,6 &11].Concerning the results of statistical analysis of biochemical parameters.in group IV, it revealed great improvement of biochemical panel with treatment by itraconazole which confirmed by disappearance of mostclinical signs.Levels of hematological and biochemical parameters in treated dogs with itraconazole returned close to normal values which confirm the success of it in treatment of canine asperigillosis.

Several antifungal drugs have been used for treatment of aspergillosis [11], and these measures succeeded by 43-60%. Itraconazole is synthetic antifungal drug which has been widely used in human medicine and recently used in veterinary medicine in treatment of aspergillosis and is 5-100 times more effective against aspergillus spp. than ketoconazole, and it has fewer side effects and a much longer half-life [12].[21]have reported that a dog with disseminated aspergillosis responded to itraconazole treatment but died due to a hemorrhage in the kidney. However, [1 &22] found that itraconazole was effective in treating aspergillosis in 1 out of 4 infected dogs, while the remaining dogs died for different reasons after a long time of treatment. Therefore, the authors suggested that the treatment of aspergillosis with itraconazole had a positive effect on life span. [8] reported a 60- 70% success rate in the treatment of aspergillosis with itraconazole. In the present study treatment of 3 dogs from group I/Vwith itraconazole for 6 weeks have agreat effect on restoration of all clinical signs except crepitation rales of the lung and dyspnea which confirmed by histopathological findings .

Mycological findings:

Allnasal swabs collected fromall dogs at zero day of experiment were free from *A. fumigatus*. Nasal swabs (at 7 day & at the end of the experiment) and organs collected at the end of experiment showed variable degree of *A. fumigatus* isolation as concluded in (Table 7). The findings of mycotic cultures indicate that the lungs and kidneys were more sensitive than the otherorgans.

Postmortem and histopathological findings:

Concerning group I (Control group), no abnormal pathological changes were detected, while in dogs of groupII, gross examination of lungs showed areas of congestion and hepatization with focal areas of hemorrhages and also showed presence of small white nodule embedded in lung tissue and this result was

compatible with that recorded by [4]. Kidneys revealed presence of severe congestion and pinpoint white foci in both cortex and medulla and this finding agreed with [2&6], also there was subcapsular hemorrhage. Liver revealed presence of severe congestion and this result was similar with that recorded by [4]. Spleen showed congestion and focal areas of hemorrhages and this was in agreement with [23]. Microscopically, lungs of groupII revealed interstitial pneumonia in which there was thickening of alveolar wall that infiltrated with neutrophils and mononuclear cells with severe congestion of interstitial blood vessels and interstitial hemorrhages; there were also focal interstitial granulomes consisted of focal aggregation of neutrophils, macrophages, plasma cells and lymphocytes with presence of areas of caseuos necrosis, these granulomes contained spores and branched hyphae of the fungus (Fig. 1)and this result was smilar with that recorded by [4].Some lung alveoli showed emphysema while others showed alveolar collapse. There was also hyperplasia of lining epithelium of bronchi and bronchiole with presence of inflammatory exudate and inflammatory cells inside bronchial and bronchiolar lumen, peribronchial and peribrochiolar fibrosis also present with dilatation of peribronchial and peribronchiolar blood vessels. Kidneys of infected dogs revealed microscopically degeneration and coagulative necrosis of lining epithelium of renal tubule in both cortex and medulla with severe congestion of interstitial blood vessels and this finding was smilar with that mentioned by [4]. There were focal granulomes formed of focal aggregation of macrophages, plasma cells and lymphocytes, these granulomes contains spores and hyphae of the fungus (Fig. 2) and this result was in agreement with [23]. There were also focal areas of fibrosis in both cortex and medulla and subcapsular hemorrhage. Histopathological examination of liver showed vacuolar degeneration of hepatocytes with hepatocellular coagulative necrosis, severe congestion of central veins and blood sinusoids with presence of fibrosis in-between hepatocytes. Portal areas showed fibroplasia with severe congestion of portal blood vessels. There were small foci of hepatocellular necrosis that infiltrated with mononuclear cells (Fig. 3) and this picture was previously recorded by [23]. Spleen showed lymphoid depletion, hemorrhage, hemosiderosis and severe congestion with presence of focal granulomes formed of focal aggregation of macrophages, giant cells, plasma cells and lymphocytes (Fig. 4), these granulomes contains spores and

hyphae of the fungus, and this finding agreed with [6].

Concerning group III, gross examination of lungs revealed presence of congestion and lung hepatization, bronchi and bronchiole contained exudate in their lumen, there were also pinhead sized pale nodules embedded in lung tissue. Kidneys were small and pale with thickening of renal capsule; there were small white foci in both cortex and medulla these results agreed with [23]. Liver was pale with focal areas of congestion. Spleen showed congestion and small focal areas of paleness. Histopathological picture of lungs of experimentally infected dogs revealed diffuse alveolar edema in which there was homogenous esinophilic proteinacious transudate inside alveolar lumen, other alveoli showed alveolar emphysema, hyperplasia of lining epithelium of bronchi and bronchiole and peribrochial and peribronchiolar fibrosis also detected. Inflammatory exudate and inflammatory cells were detected inside bronchial and bronchiolar lumen. There were focal granulomes consisted of central areas of caseuos necrosis surrounded by neutrophils, macrophages, plasma cells and lymphocytes and encapsulated with fibrous connective tissue capsule, these granulomes contains spores and branched hyphae of the fungus (Fig. 5) this picture was seen by [4]. There was severe congestion of interstitial blood vessels.

Kidneys of infected dogs revealed cell degeneration and coagulative necrosis of lining epithelium of renal tubule in both cortex and medulla, with congestion of interstitial blood vessels. There were focal granulomes consisted of areas of caseuos necrosis with mononuclear cells infiltration and fibrous connective tissue proliferation with presence of hyphae and spores in the granulome(Fig. 6). There was renal capsular fibrosis and thickening. Histopathological examination of liver of experimentally infected dogs showed portal fibrosis with congestion of portal blood vessels. There was vacuolar degeneration of hepatocytes and hepatocellular necrosis, severe congestion of central veins and blood sinusoids with presence of fibrosis between hepatocytes. There was focal area of hepatocellular necrosis, that area was surrounded by fibrous connective tissue capsule. Spleen showed congestion and focal infiltration with mononuclear cells. Lesions of kidneys, liver and spleen were reported previously by [6].

Large amount of mononuclear cell infiltration and few numbers of neutrophil in areas of granulomatous inflammation may indicate that cellular immunity is responsible for the defense against *A. fumigatus* infections as reported [19&24].

These previous results showed that lungs and kidneys were more sensitive to this infection than other body organs and this agreed with [4].Also the pathological lesions were more severe in immunosuppressed group than other groups. In this experiment, no primary lesion was detected at site of inoculation.

Presence of *Asperigillus Fumigatus* in different organs means that the fungus is able to invade the blood vessels. In man, natural infection occurs via inhalation of spores the fungus proliferates in lungs of infected person and disseminated from primary lesion. Disseminated mycosis occurs mostly in immunosuppressed animals [23].

In group IV (treated group), no lesions or fungal spores or hyphae were detected in organs after termination of the treatment period (Fig. 7) except for mild alveolar edema andmild thickening in alveolar wall in lungs, few tubular epithelium necrosis in kidneys and few hepatocellular degeneration in liver.

By PAS, thin walled septated branched hyphae were detected in lungs, kidneys, liver and spleen of groups II and III. While were not detected in control and treated groups

Table (1): Clinical examination of dogs in group I at the beginning of the experiment, group II, group III on 34 day post inoculation of *A.fumigates*and group IV at the end of treatment.

Parameters	Group I	Group II	Group III	Group IV
Respiratory rate (/ min)	19.94 ± 0.22^{A}	22.22 ± 0.27^{B}	23.18 ± 0.13^{C}	21.14 ± 0.28^{A}
Heart rate (/ min)	110.06 ± 0.53^{A}	120.90 ± 0.55^{B}	127.64 ± 0.39^{C}	116.59 ± 1.37^{A}
Rectal temperature (C°)	38.58 ± 0.12^{A}	38.94 ± 0.10^{A}	39.52 ± 0.10^{B}	38.84 ± 0.08^{A}

Means with different superscripts are significantly different at p≤0.05

Table (2): Clinical examination of dogs in group IV before and after treatment with itraconazole for 6 weeks

Parameters	Before treatment	After treatment
Respiratory rate (/ min)	21.76 ± 0.31	$21.14 \pm 0.28^{*}$
Heart rate (/ min)	119.11 ± 1.42	$116.59 \pm 1.37^{*}$
Rectal temperature(C°)	39.04 ± 0.10	38.84± 0.08

*** P≤0.05 ** P≤0.01 ***P≤ 0.001**

Table (3): Hematological parameters of dogs in group Iat the beginning of the experiment , group II , group III on 34 day post inoculation of *A.fumigates*and group IV at the end of treatment

Parameters	Group I	Group II	Group III	Group IV
RBCs ($\times 10^6$/µl)	6.37 ± 0.30^{A}	6.23 ± 0.26^{A}	6.11 ± 0.25^{A}	6.22 ± 0.11^{A}
Hb (g/dl)	14.99 ± 0.09^{A}	13.97 ± 0.03^{B}	13.51 ± 0.23^{BC}	14.10 ± 0.13^{BD}
PCV (%)	42.00 ± 0.55^{A}	38.70 ± 0.39^{B}	37.15 ± 0.41^{C}	39.42 ± 0.36^{BD}
MCV (fl)	66.67 ± 3.93^{A}	62.46 ± 2.14^{A}	61.28 ± 3.24^{A}	63.84 ± 1.37^{A}
MCH (pg)	23.73 ± 1.08^{A}	22.54 ± 0.85^{A}	22.22 ± 0.83^{A}	22.79 ± 0.40^{A}
MCHC(g/dl)	35.72 ± 0.65^{A}	36.10 ± 0.76^{A}	36.38 ± 0.81^{A}	35.79 ± 0.32^{A}
WBCs($\times 10^3$/µl)	10.16 ± 0.12^{A}	30.09 ± 0.13^{B}	32.11 ± 0.58^{C}	20.23 ± 1.85^{D}
Lymphoctes($\times 10^3$/µl)	3.40 ± 0.01^{A}	7.95 ± 0.03^{B}	8.89 ± 0.10^{C}	5.77 ± 0.45^{D}
Monocytes($\times 10^3$/µl)	0.91 ± 0.10^{A}	4.01 ± 0.04^{B}	4.42 ± 0.10^{C}	2.27 ± 0.32^{D}
Granulocytes WBC($\times 10^3$/µl)	5.30 ± 0.07^{A}	18.25 ± 0.24^{B}	19.38 ± 0.13^{C}	11.27 ± 1.26^{D}

Means with different superscripts are significantly different at p≤0.05

Table (4): Hematological parameters of dogs in group IV before and after treatment with itraconazole for 6 weeks

Parameters	Before treatment	After treatment
RBCs ($\times 10^6$/µl)	6.20 ± 0.11	6.22 ± 0.11
Hb (g/dl)	13.99 ± 0.14	14.10 ± 0.13
PCV (%)	38.81 ± 0.42	39.42 ± 0.36
MCV (fl)	63.08 ± 1.36	63.84 ± 1.37
MCH (pg)	22.71 ± 0.39	22.79 ± 0.40
MCHC(g/dl)	36.10 ± 0.34	35.79 ± 0.32
WBCs($\times 10^3$/µl)	23.47 ± 1.89	20.23 ± 1.85
Lymphoctes($\times 10^3$/µl)	6.65 ± 0.47	5.77 ± 0.45
Monocytes($\times 10^3$/µl)	2.94 ± 0.33	2.27 ± 0.32
Granulocytes WBC($\times 10^3$/µl)	13.71 ± 1.29	11.27 ± 1.26

*** P≤0.05 ** P≤0.01 ***P≤ 0.001**

Table (5): Biochemical parameters of dogs in group I (~~control~~) at the beginning of the experiment, group II and III on 34 day post inoculation of *A.fumigates*and group IV at the end of treatment

Parameters	Group I	Group II	Group III	Group IV
ALP (IU/L)	139.98 ± 1.09^{A}	308.26 ± 4.58^{B}	331.34 ± 2.14^{C}	262.81 ± 6.54^{D}
ALT (IU/L)	58.92 ± 4.22^{A}	52.34 ± 2.77^{A}	57.65 ± 4.52^{A}	56.5 ± 3.28^{A}
BUN(mg/dl)	35.45 ± 1.41^{A}	40.57 ± 1.14^{B}	60.35 ± 1.61^{C}	44.52 ± 1.53^{AB}
Creatinine(mg/dl)	0.93 ± 0.02^{A}	0.88 ± 0.01^{A}	0.90 ± 0.01^{A}	0.91 ± 0.01^{A}
Glucose(mg/dl)	57.76 ± 2.31^{D}	52.18 ± 2.63^{AD}	30.90 ± 1.12^{B}	49.18 ± 2.04^{AC}
Total protein (g/dl)	6.15 ± 0.09^{A}	6.25 ± 0.10^{A}	6.84 ± 0.08^{B}	6.15 ± 0.09^{A}
Albumin(g/dl)	3.05 ± 0.05^{A}	2.98 ± 0.05^{A}	2.95 ± 0.04^{A}	3.01 ± 0.06^{A}
Globulin(g/dl)	3.09 ± 0.10^{A}	3.26 ± 0.12^{A}	3.89 ± 0.11^{B}	3.14 ± 0.13^{A}
Ca (mg/dl)	10.67 ± 0.29^{A}	10.42 ± 0.21^{A}	10.35 ± 0.23^{A}	10.63 ± 0.26^{A}
Mg(mg/dl)	2.20 ± 0.06^{A}	2.33 ± 0.02^{AB}	3.27 ± 0.02^{C}	2.22 ± 0.01^{A}
P(mg/dl)	4.91 ± 0.30^{A}	5.54 ± 0.24^{A}	10.30 ± 0.09^{B}	7.62 ± 0.16^{C}

Means with different superscripts are significantly different at p≤0.05

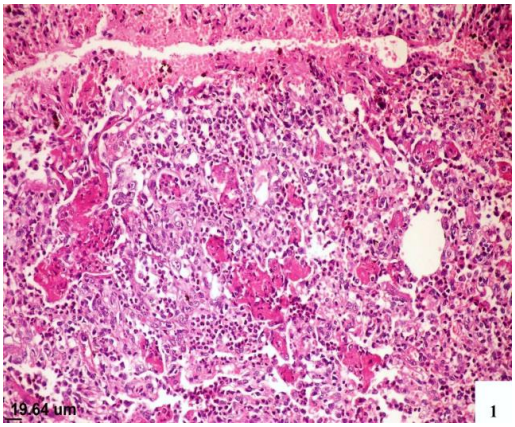
Table (6): Biochemical parameters of dogs in group IV before and after treatment with itraconazole for 6 weeks

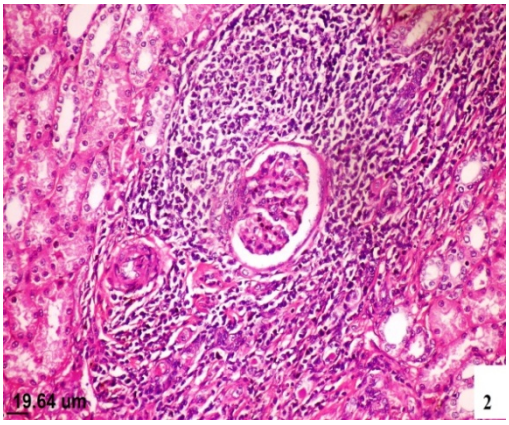
Parameters	Before treatment	After treatment
ALP (IU/L)	318.71± 3.17	$262.81\pm 6.54^{***}$
ALT (IU/L)	58.42± 2.96	56.5± 3.28
BUN(mg/dl)	57.80± 1.10	$44.52 \pm 1.53^{***}$
Creatinine(mg/dl)	0.89 ±0.19	0.91± 0.01
Glucose(mg/dl)	29.86 ± 0.55	$49.18\pm 2.04^{***}$
Total protein (g/dl)	6.79 ± 0.06	$6.15\pm 0.09^{**}$
Albumin(g/dl)	2.96 ± 0.48	3.01± 0.06
Globulin(g/dl)	3.83 ± 0.09	$3.14\pm 0.13^{***}$
Ca (mg/dl)	10.33 ± 0.23	10.63± 0.26
Mg(mg/dl)	3.26 ± 0.02	$2.22\pm 0.01^{***}$
P(mg/dl)	9.92 ± 0.09	$7.62\pm 0.16^{***}$

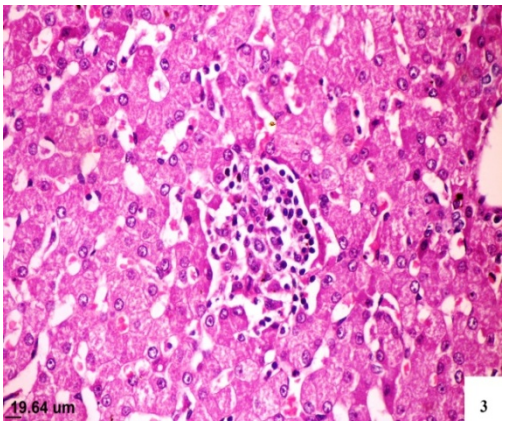
*** P≤0.05 ** P≤0.01 ***P≤ 0.001**

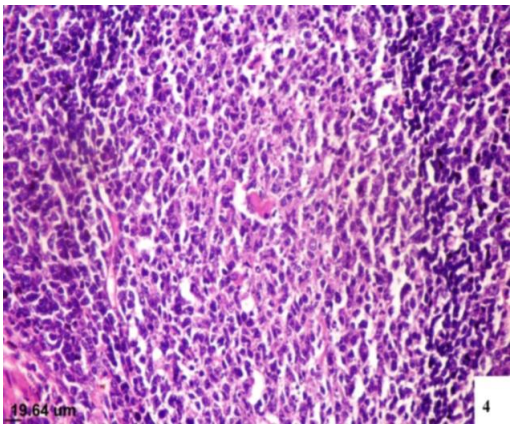
Table (7): prevalence of *A. fumigatus* from collected samples:

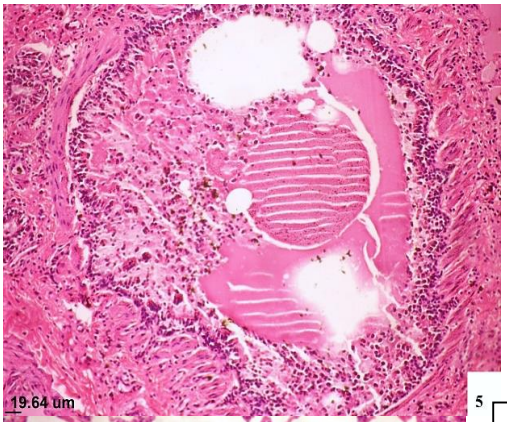
Parameters	Group I (n= 5)	Group II (n= 5)	Group III (n= 5)	Group IV (n= 5) After treatment
Nasal swabs at 7 day of experiment	-	2/5 (40%)	3/5 (60%)	2/5 (40%)
Nasal swabs at the end of experiment	-	3/5 (60%)	4/5 (80%)	1/5 (20%)
Lungs	-	5/5 (100%)	5/5 (100%)	1/5 (20%)
Kidneys	-	3/5 (60%)	4/5 (80%)	0/5 (0%)
Spleen	-	1/5 (20%)	1/5 (20%)	0/5 (0%)

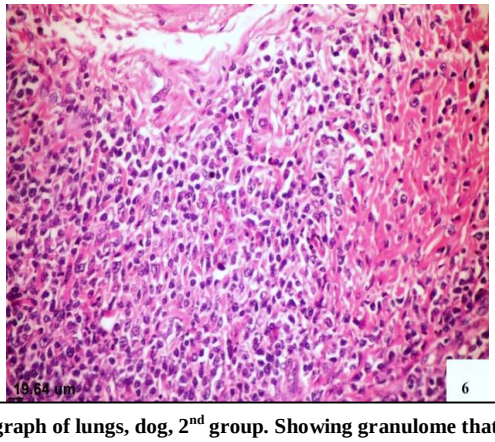












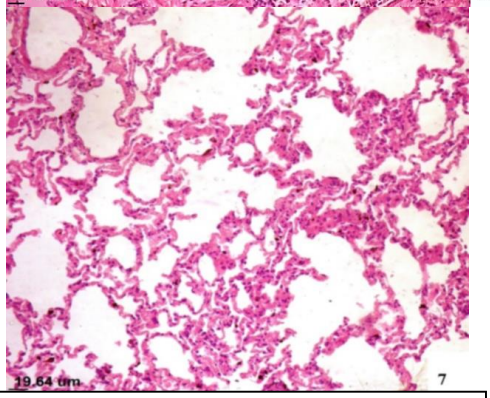


Fig. 1: Micrograph of lungs, dog, 2nd group. Showing granulome that consists of focal aggregation of neutrophils, macrophages, plasma cells and lymphocytes with areas of caseuos necrosis, this granulomes contains spores and hyphae of the fungus (H&E bar 19.64 µm).

Fig. 2: Micrograph of kidneys, dog, 2nd group. Note focal infiltration of cortex by macrophages, plasma cells and lymphocytes (H&E bar 19.64 µm).

Fig. 3: Micrograph of liver, dog, 2nd group. Notice small foci of hepatocellular necrosis that infiltrated with mononuclear cells (H&E bar 19.64 µm).

Fig. 4: Micrograph of spleen, dog, 2nd group. Notice focal infiltration of spleen by giant cells (arrow), macrophages, plasma cells and lymphocytes (H&E bar 19.64 µm).

Fig. 5: Micrograph of lungs, dog, 3rd group. Showing focal granulome consisted of central area of caseuos necrosis surrounded by neutrophils and mononuclear cells and encapsulated with fibrous connective tissue capsule; this granulome contains spores and hyphae of the fungus (H&E bar 19.64 µm).

Fig. 6: Micrograph of kidneys, dog, 3rd group. Notice focal areas of caseuos necrosis with mononuclear cells infiltration and fibrous connective tissue proliferation with presence of hyphae and spores (H&E bar 19.64 µm).

Fig. 7: Micrograph of lung, dog, treated group. Showing restoration of normal histological structure of lung after termination of the period of treatment (H&E bar 19.64 µm).

CONCLUSIONS

In conclusion, since aspergillosis was located mainly in the lungs and kidneys according to observed clinical findings and changes in some of the haematological and biochemical parameters supported by the histopathological findings and mycological culture, *A. fumigatus* should be considered among the diseases of the lungs and kidneys. In addition, immunosuppression has a great role in occurrence of canine asperigellosis and severity of its clinical manifestations and pathological findings.The success rate which obtained with itraconazole in the present study may indicate that a large-scale and detailed study would be of practical value.

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