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Effect of Some Isolation Fungi from Iraq / Baghdad Hospitals Apparatus on Physiology of Liver, Kidney and Testis in Albino Mice

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Abstract: ***The present study was designed to investigate the effect of Aspergillus Fumigatus and Aspergillus Flavus on liver, kidney and testosterone enzymes in albino mice male. Where 20 samples taken inside each of the Al-furat General Hospital and Yarmouk Teaching Hospital from different places including the family and hospital equipment by transport media at the rate of three replicates from each place samples were taken for the purpose of isolating and diagnosing the polluted fungi of these areas. The study found that Aspergillus was the most common fungus found in these places was the prevalence rate in the al-furat General Hospital 56% for the rest of the fungus species. In addition, A.flavus fungi was found to be 44.5%, most of which were in female lobbies and A. fumigatus 30.05%. The results of isolation from Yarmouk Hospital showed that the percentage of A. fumigatus fungi was 45.45% and 24.24%. The results showed the susceptibility of some types of A.flavus isolates to blood analysis. The results also showed the susceptibility of all fungi to keratin consumption. The study also showed a decrease in the concentration of enzymes GOT, GPT, ALP, as well as cholesterol and triglyceride decrease with increased concentration of urea and creatinine and decreased testosterone concentration.***

Keywords: Aspergillus flavus, Aspergillus fumigatus, Baghdad hospitals, GOT, GPT, ALP, urea, creatinine, cholesterol, triglyceride and testosterone

1. Introduction

Hospitals contain many sources of pollution resulting from the activities carried out. These pollutants are pathogenic bacteria, viruses, fungicides, chemical compounds, and toxin due to the many factors that occur in the presence of patients who are in the hospital, the auditors and methods of cleaning [1]. In more than a century, hospital-acquired infections have been identified as a critical problem affecting hospital-provided health care, as reported in [3, 2]. These infections include surgical, respiratory, organ, and polyp, which transmit a number of fungal diseases as a result of contamination [4]. Spontaneous spores are one of the causes that can be transmitted by contact with contaminated hospital equipment and floors, as well as through visits to patients and hospital staff.[5]. ***Aspergillus*** is one of the most important fungal species found in such places and is the most prevalent in the world and has many risks to human health, in that inhalation spores through breathing through the nose causes disease known as (Aspergillosis), and causes health problems of the lungs and highlighted ***A. flavus*** , which grows in the bronchi and acute infections is the peritoneal peritoneum caused by ***A. fumigatus,*** which invades the outer cavities of the pulmonary tissue [6], ***Aspergillus*** causes otitis media which leads to hearing loss and affects speech and IQ [7, 8, 9]. People with cancer, chemotherapy, leukemia and AIDS are the most vulnerable to this fungus, skin lesions, ulcers and pneumonia [10].

Aspergillus is produce toxins, known as aflatoxins [11, 12], are secondary metabolites of fungi, which are active biological compounds other than antigen, which do not stimulate the body to form antibodies to defense. They are toxic to humans, animals and plants and have several types B1, B2, and C1. 2 B is the most dangerous species where 2.2 milligrams are sufficient to damage the liver where the body cannot get rid of it]13], Neurotoxin and carcinogenic toxin [14] are also produced by its own species, such as ***A. flavus*** [15]. Skin infections with skin fungal infections are a high percentage of skin diseases in humans, especially in areas where the environment is suitable for growth such as moisture, heat and the availability of keratinites [16, 17].

Therefore, the study aimed at isolating and diagnosing the fungal species found in hospitals to isolate ***Aspergillus*** fungi from them, their knowledge of the most common species, and their susceptibility to humans, and studying their ability to analyze blood, keratinocytes and toxins produced by the hospital environment and the human damage caused by it.

2. Materials and Methods

1) Samples were collected using medium media containing maintenance media to preserve the blackboards until transplantation. During the months (October-November-December 2015 / March 2016),(40) samples were collected from Al-Furat General Hospital and Yarmouk Teaching Hospital from different places (ECG, sonar, dialysis, pressure and diabetes). The icebox was transferred to the refrigerator for (24) hours and then transferred to the laboratory for implantation on the media.
2) The SDA center, which consists of Sabouraud Dextrose agar (OxoidCM41), 0.5gm, Chloramphenicol.

3. Prepare

a) Place 65 g of medium except for chloramphenicol in 1000 ml of distilled water.

b) Chloramphenicol is added by dissolving in acetone and mixing well.
c) Sterilize the Autoclave at 121 ° C for 15 minutes and distribute to dishes at about 50 ° C and keep in sterile conditions until planting.
d) Samples are placed on the SDA medium and placed in the incubator at a temperature of 25-30 ° C for 7-10 days.
e) The colonies of the ***Aspergillus*** were transferred to the center of czapek dox agar for classification using the taxonomic key [18, 19, 20], which was prepared as follows:
   1) Dissolve 49 g of the prepared medium in distilled water.
   2) Boil the middle until the sugar dissolves.
   3) Autoclave sterilized in 121 ml for 15 minutes.
   4) It is used to grow ***Aspergillus, penicillium*** and non-spore fungi [21].
   5) Prepare in the center of blood agars, weigh 42 grams of blood agars and then add this amount to one liter of distilled water and continue to dissolve and heat until the ingredients are homogenized and then sterilized using an autoclave device at a temperature of 121 m for 15 minutes and after sterilization cooled the center to the degree (40-45) m, then add 70 ml/liter of blood and dissolve and then add chloramphenicol, which is dissolved in acetone. The ingredients are mixed well, then poured into the precipitate, left to harden and then ready for use.[22]
   6) Take a sterile Petri dish filled to the middle with sterile soil that should be used in this technique.
   7) Spread a short strand of human hair with a diameter of 2-3 mm sterile over the surface of the soil and fertilize isolated fungi
   8) Incubate the dishes for 4 weeks at 25-30 ° C and check for observation [23].

Create animals

Thirty males were recruited from Swiss white mice at a rate of (20- 30) g and at(8) weeks of age. They were divided into three groups with 10 animals per group. The experiment lasted two weeks:

Group 1: control group was given water and food throughout the experiment.
Group 2: (0.1) ml dose of ***Aspergillus Flavus*** fungi suspension was administered orally daily by injection using insulin syringes after removal of the needle.
Group 3: (0.1) ml dose of ***Aspergillus Fumigatus*** was given daily orally by the injection method using insulin syringes after removal of the needle.

Animal sacrifice

The blood samples were collected in a stab-like manner immediately before the animals were killed. The samples were collected using insulin syringes. The blood samples were placed in sterile centrifuge tubes under 2,000 cycles per minute for 10 minutes to separate the frozen serum with C- 20 until the measurement of the concentration of enzymes and testosterone and was measured at the Center of Biotechnology and approved the principle of work on the interaction of antigen and antibody and the device of the company (Biomerieux) and using the kit of the device. [24].

4. Results

1. The fungi that were isolated from Al-Furat General Hospital after the samples were taken by swabs and at the rate of three replicates per site, the following results were observed:

Table 1: Type of fungi and place of collection collected from Al-Furat General Hospital:

No.	Apparatus	Type of fungi
1	ECG	*A.flavus, A.fumigatus ,A.granulomus ,A.parasiticus ,A.ochoratus*
2	Sonar	*Afumigatus ,A.flavus , A.ochoratus*
3	The dialysis	*A.versicolor ,A.fumigatus, A.flavus, A.ochoratus*
4	the beded	*A.niger ,A.flavus, A.parasiticus , A.fumigatus*ه
5	Stress and diabetes	A. *Fumigatus ,A.flavus,A.parasiticus*

Table 2: The percentage of species recurrence and the rate of appearance of the types of ***Aspergillus*** in Al-Furat General Hospital:

No.	Type of fungi	Percentage of frequency of type	The percentage of the appearance of the species
1	*A.flavus*	44.5 %	25.6 %
2	*Afumigatus*	30.05 %	22.75 %
3	*A.parasiticus*	15.1 %	19.37 %
4	*A.ochoratus*	6.78 %	18 %
5	*A.versicolor*	3.4 %	13.2 %

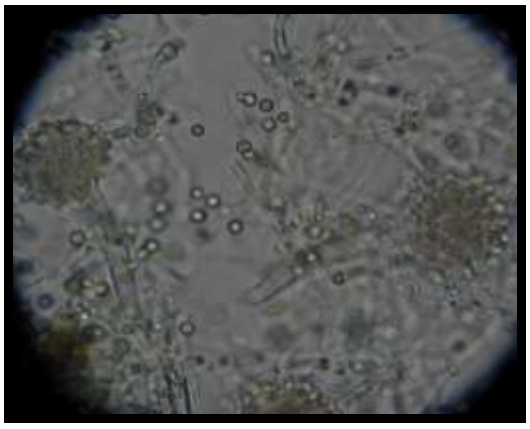
2. The fungi that was isolated from Yarmouk Teaching Hospital

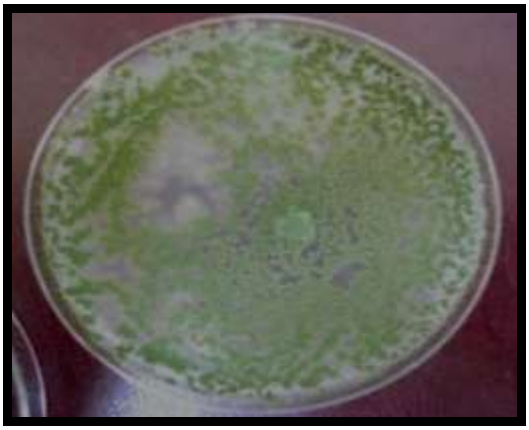
Table 3: Type of fungus and collection site taken from Yarmouk Teaching Hospital

No.	Apparatus	Type of fungi
1	ECG	***Afumigatus ,A.flavus , A.niger***
2	Sonar	***Afumigatus ,A.flavus , A.niger***
3	The dialysis	***A.granulomus ,A.fumigatus***
4	the beds	***A.flavus, A.fumigatus, A.granulomus***
5	Stress and diabetes	***A. Fumigatus ,A.flavus***

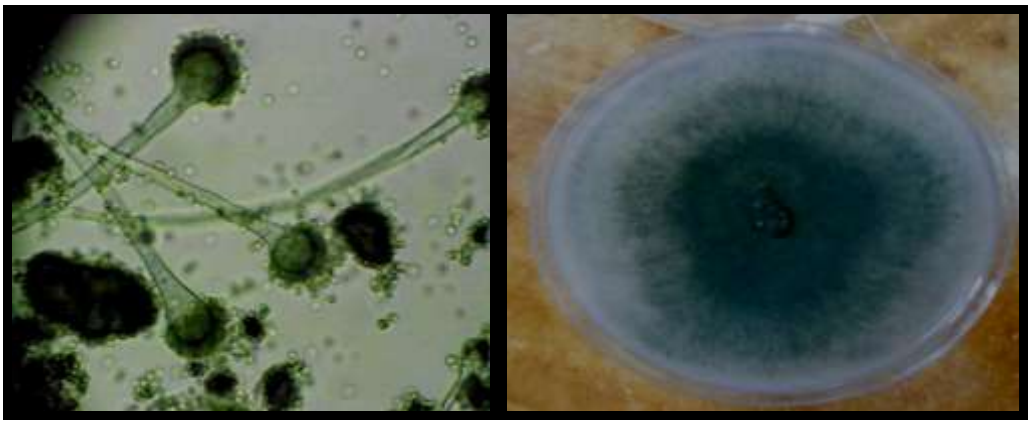
Table 4: The percentage of the type of recurrence of the emergence of sparlic species in Yarmouk Teaching Hospital:

No.	Type of fungi	Percentage of frequency of type	The percentage of the appearance of the species
1	*Afumigatus*	45. 45 %	22.4 %
2	*A.flavus*	24.24 %	44.2 %
3	*A.granulomus*	21.21 %	18.2 %
4	*A.niger*	9.09 %	15.3 %





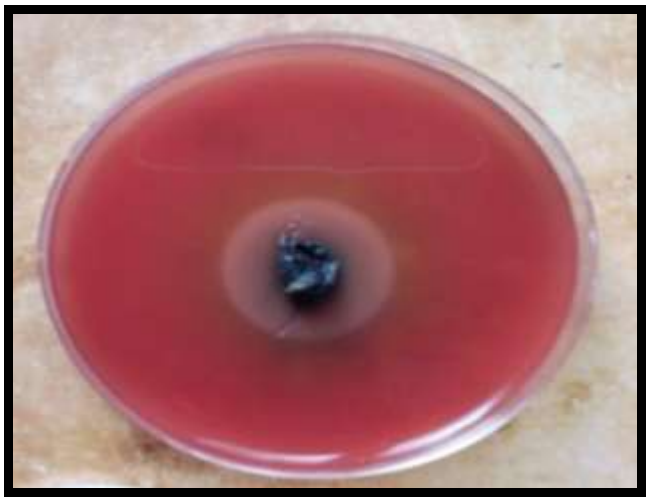
A-



B-

Picture (1): Some isolated spragalus species from hospitals. Right and left colonies showing fungi A -*A.flavus* , B- *A.fumigatus*

3. The results of isolated fungus transplantation on the blood agar center showed a complete decomposition of the blood cells and the fungus analyzer was of the same type as the picture (2). *A.flavus.*



Picture (2): Portability ***A. flavus.*** On blood analysis on the blood agar medium

4 . The results of the incubation of fungus with the hair has been shown consumption of karacene material by all the fungal species that have been isolated from the hospital, which indicates the susceptibility of fungi to cause skin diseases for humans.

Table 5: Effect of ***Aspergillus*** fungi on GOT, GPT, ALP

Groups	GOT (IU/ml) Mean±SD	GPT (IU/ml) Mean±SD	ALP (IU/ml) Mean±SD
Control	A 205.71±2.16	A 66.703±2.553	A 78.005±1.655
Flavus	B 178.81±1.80	B 48.645±2.505	B 65.235±2.785
Fumigatus	C 183.71±3.50	B 50.560±2.262	B 63.687±3.196
LSD	3.90	3.68	3.96

The results showed a significant decrease (P <.05) in the concentration of liver enzymes (GOT, GPT, ALP) and ***Aspergillus*** treatment compared with the control group, as shown in Table (5).

Table 6: Effect of ***Aspergillus*** on Urea and Creatinine in albino mice male:

The results showed a significant increase (p <.05) in the concentration of urea and creatine and the treatment of ***Aspergillus*** compared to control group, as shown in Table (6).

Groups	Urea mg/dl Mean±SD	Creatinin mg/dl Mean±SD
Control	A 15.320±0.933	A 0.7650±0.0342
Flavus	B 19.110±1.026	B 1.5625±0.0330
Fumigatus	C 24.605±2.124	B 1.5950±0.0370
LSD	2.205	0.052

Table 7: Effect of ***Aspergillus*** on Cholesterol and Triglyceride in White Mice.

Groups	Chole.(U/L) Mean±SD	T.G (U/L) Mean±SD
Control	A 160.54±1.14	A 144.76±2.61
Flavus	B 136.20±3.69	B 118.93±1.21
Fumigatus	B 133.72±2.02	B 120.17±2.27
LSD	3.79	3.19

The statistical results showed a significant decrease ($p < .05$) in cholesterol concentration and triglyceride in male white mice and treated with ***Aspergillus*** compared to control group, as shown in Table (7)

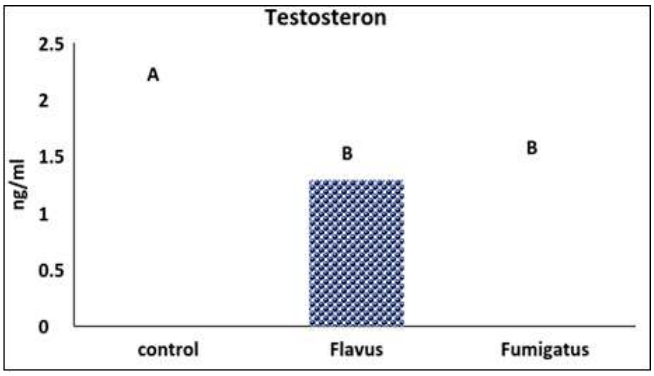


Diagram (1): The effect of ***Aspergillus*** fungi on the concentration of testosterone in male white mice

The results showed a significant decrease ($P < .05$) in the testosterone concentration in male white rats and treated with ***Aspergillus*** compared to the control group, as shown in Figure (1).

5. Discussion

It was noticed that the isolated fungi of Al-Furat General Hospital and Al-Yarmouk Teaching Hospital are mostly belonging to the genus ***Aspergillus***. They are isolated from the following places: beds, baths, corridors, devices and containers. The tables show the fungi isolated from the General al-furat Hospital. ***Aspergillus*** is 65% In the world, as can be seen from Table (1, 2), the ***A.flavus*** fungi is more prevalent, at 44.5%. [7], ***A. fumigatus*** was 30.05% as shown in Table 2 and most areas are rich in organic matter (sources of carbon dioxide) that are suitable for this fungus In addition to the remnants of the tissue and the fallen cornea materials, as shown in Table (4).

The fungi isolated from the Yarmouk hospital were ***A. fumigatus*** (45.45%) followed by ***A.flavus*** (24.24%). This wide spread of the fungus was due to the appropriate conditions in addition to the production of large numbers of breeding units in these areas. [25,26]. These blackboards have a great ability to high morbidity in the case of weak immune system and have the ability to grow at temperatures of 37 m, such as normal flora, as mentioned in [27,28]. The results shown in the susceptibility of these fungi to the analysis of blood, where tested the species of ***Aspergillus*** has been found that the types capable of analyzing the blood is ***A.flavus*** in the center of blood agar and that this is due to the possession of these fungi factors such as fermentation of enzymes and the production of toxins fungal These toxins have the ability to break down the body's various tissues, where this susceptibility is an indicator of its morbidity. When a defense defect occurs, the fungi take advantage of it and cause various diseases when appropriate conditions are found[29]. ***Aspergillus*** species are the most efficient fungi in the decomposition process, in between Other fungi produce toxins leading to the destruction of the liver, spleen, kidneys and lungs [30]. The results indicate a significant decrease in the concentration of enzymes GOT, GPT, ALP compared to the control group due to the treatment of ***Aspergillus*** fungi. The cause of the decrease is the damage caused by the liver and kidneys by the effect of fungi causing a decrease in the rate of release of enzymes [31]. The studies indicated that hepatocellular damage occurred with the appearance of hepatocellular hepatic cells and hepatic necrosis. In the kidney, treatment with AFlatoxin (AF) caused renal degeneration and proliferative cell proliferation [32]. The liver is one of the most important members of the AF toxin. Exposure a small amount of it causes liver cirrhosis and liver cancer [33]. The metabolic outcomes of ***A.flavus*** resulted in liver congestion These are the toxins AF (the secondary metabolites of fungus) and are produced from the fungus ***A. flavus*** and these toxins on several types, including B1, B2, G1, G2 and the most common AFB1 toxins of the species mentioned [34]. More serious than B1 on the liver. B1 is more dangerous than G1 in the kidney [35,36]. It was observed that oral administration of G1 poisoning caused liver cancer and renal nephropathy caused by these toxins [37]. The cause of cancer is that AF toxins induce oxidative damage and cause the creation of free radicals that interact with cell components such as fat, DNA and RNA and then cause damage to the liver and kidneys [38]. Treatment with these toxins has reduced the effectiveness of antioxidant enzymes including Catalase, Superoxide dismutase, Glutathione peroxidase and Glutathione reductase [39]. G1 toxins cause liver cell cavities with the death of some cells, the accumulation of thrombocytopenic cells in the liver tissue, and the accumulation of leukocytes around the central vein. These toxins also cause renal cell degeneration, atrophy and congestion of the capillaries of the kidney [40]. The results of the treatment of male rats with AF toxins showed changes in the concentration of liver enzymes GOT, GPT [41]. It was observed that treatment with these toxins cause increased concentration of creatinine compared to the control group where creatinine is built in the liver and then through the blood circulation, it is taken to the skeletal muscles by converting it to Creatine phosphate, which is a source of energy to contract skeletal muscle [42]. A study showed that AF toxins cause degeneration of the kidneys, causing increased creatinine concentration as a result of increased muscle release and decreased renal insufficiency [43]. Another study showed that treatment with these toxins increased the concentration of creatinine [44]. Where the kidney is the release of creatinine and the treatment of AF toxins cause the dissolution of the cells of the kidney causing the increase of concentration [45]. The results of our research showed a significant increase in urea in the treated groups of ***Aspergillus*** compared to control. The increased concentration of urea was observed due to damage to the

kidney function due to the treatment of AFB1 and AFB2, which is caused by the interaction of these toxins with normal formation [46]. The results showed a decrease in concentration of cholesterol and triglyceride when treated with ***Aspergillus*** as a result of the toxins released from these fungi, which cause bile duct necrosis and accumulation of fat in the liver [47]. A study indicated that fat accumulation and hypertrophy in the liver resulted from the treatment of Aflatoxin, which causes a decrease in body weight, because the food is not taken by animals, including rats [48,49]. Where AF toxins cause impaired metabolism of carbohydrates, fats, and proteins in liver cells, causing the accumulation of fat in the liver and decrease in blood [50]. The results of our study showed a decrease in the concentration of testosterone compared with the control group, as treatment with AF toxins caused a decrease in the thickness of the germicidal layer of sperm. [51] These toxins break down the Sertoli cells, causing a decrease in the secretion of Inhibin, which is produced by the cells of Sertoli, which affects the pituitary gland Causing inhibition or decrease in FSH secretion [52]. AF toxins caused the destruction of LIDC cells, causing a decrease in the concentration of testosterone in the blood [53]. The cause is the accumulation of these toxins in the pituitary gland, which leads to a decrease in the secretion of LH, which affects the cells of LIDK to stimulate the secretion of testosterone [54].

6. Conclusion

The results of isolation form Al-furat and Yarmouk Hospital showed that the percentage of ***A. fumigatus*** fungi was 45.45% and 24.24%. The results showed the susceptibility of some types of ***A.flavus*** isolates to blood analysis. While the al-furat General Hospital 56% for the rest of the fungus species. In addition, ***A.flavus*** fungi was found to be 44.5%, most of which were in female lobbies and ***A. fumigatus*** 30.05%. The results also showed the susceptibility of all fungi to keratin consumption. The study also showed a decrease in the concentration of enzymes GOT, GPT, ALP, as well as cholesterol and triglyceride decrease with increased concentration of urea and creatinine and decreased testosterone .

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