Physiological, Immunological and biochemical effects of the fungus *aspergillus Niger* toxicity in albino male rats

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ABSTRACT

This research was planned to investigate the effect of the mycotoxin created by the fungal species *Aspergillus Niger* on experimental animals (albino male rats) to test the effects on certain biochemical and immunological variables.
The number of animals was distributed into three groups, (5) for each group of animals. The first group (T1) was the control group given the water and nutrients during the experiment's period under normal conditions. The second group (T2) received (5) ml as a single dose of Aspergillus Niger, then the animals were scarified after one week of infection. The third group (T3) received 5 ml of fungal dilution as a single dose, and vitamin E (as 1000 mg / l.) was given daily for 15 days after five days of administration the fungal solution. After 20 days of the beginning of the study, the first and third group animals are sacrificed.
It was estimated some of the immunological parameters included interleukin 10 (IL-10), interleukin 8 (IL-8), and interleukin 6 (IL-6), total protein, glutathione, malondialdehyde. The findings of the current study showed significant variations in interleukin levels that were significantly increased in the fungal therapy group relative to the other two groups ($p \leq 0.05$). The results of the current study showed a significant decrease in glutathione in fungal treatment group ($p \leq 0.05$) comparative with control group, and there are a significant increase in MAD , and peroxy nitrate ($p \leq 0.05$) in fungal treatment group, comparative with control group. While the results showed that the level of these markers begin to return to its normal value in vitamin E treatment group.

Keywords: *Aspergillus Niger, interleukins, antioxidants, vitamin E*

Introduction:

Mycotoxins are mainly formed by filamentous fungi's mycelial structure, or more specifically molds. At the end of the exponential growth cycle, these secondary metabolites are synthesized, and in fungal growth and development there seems to be no biochemical importance. "Feed and feed contamination with these mycotoxins is a significant problem for adverse effects on humans, livestock, and crops which contribute to disease and economic loss". *Aspergillus* fungi and their toxins, however, are widely distributed throughout the world where they occur in soil, crops, plant

waste, and related organic subtracts. They cause major economic losses in food, animal morbidity, and mortality and also in immunologically damaged individuals, where they can destroy cells by causing widespread damage to the cell membrane, (Mogda *et al*., 2002 and Fraga *et al*., 2008).

Aspergillus niger is one of Aspergillus genus ' most common species. This causes a disease on certain fruits and vegetables, such as grapes, onions and peanuts, called ' black mold ' and is a common food contaminant. *Aspergillus niger* may also develop such heptocarcinogenic, immunologically nephrogenic mycotoxins (Baker, 2006). The toxic effect of mycotoxin in humans, and animals depends on a number of factors including "the period of intake, period of exposure, toxin type, mechanisms of action, metabolism processes, and defense mechanisms". (Schuster et al., 2002)

Researchers have indicated that antioxidants derived from daily diets including un-enzymatic antioxidants vitamin E, vitamin C, carotenoids and, polyphenols may degrade reactive oxygen species. **(**Alpsoy, and Yalvac 2011**).** Such compounds can also be used as cofactors for antioxidant enzymes, or for cells to control the activity of antioxidants enzyme (Eboh 2014).

Materials and methods:

Animals and experimental design

Total 6 albino male rates average weight (135-160 gm) were used. Animals were caged randomly allocated into three experimental groups each of it contain 3 animals. The first group (T1) (Control) given the water and food as a normal condition.; the second group (T2) was the treated group with the fungi ***Aspergillus Niger***, as a single dose (3 ml) with the drinking water after the dilution was don for the main cultur, the animals were scarified after one week. The third group (T3) given fungal dilution (3ml) and vitamin E (as 1000 mg / l.) were given daily for 15 days after the fungal solution was given five days after administration. At the end of the experiment the animals were sacrificed. Blood samples were collected in sterile plastic test tube and, serum samples were separated and used for analysis of biochemical immunological parameters.

The isolation of fungi

We obtained ready isolation from the microbiology laboratory, the veterinary collage/ Tikrit university, then subculture distributed in plates contained the PDA medium and added 50 mg / kg of chlorophenicol antibiotic in order to prevent the growth of bacteria, the new culture of this fungus is collected for 72 hours, the 10 dilution of this culture is prepared, and the dilution number 4 is used. For the purpose of identifying the possibility of producing fungus for aflatoxin, following steps were donning:

1. The fungal isolation was planted on a medium containing a PDA by taking the disc from the fungal colonies and incubating the dishes for a week.
2. Turn the dishes upside down after the incubation period so that the lid of each dish is set down and 0.1 ml of the ammonia solution with filter paper in the lid of each dish and returned to the incubator for three days at 25 ° C.
3. Then the plates were withdrawn and a change was found in the colonial bases. The orange or yellow color implies that aflatoxins are produced by the fungus.

Serum analysis

1. The estimation of interleukins -10 (IL-10), interleukin 8 (IL-8), and interleukin 6 (IL-6), don by ELISA method, according to the instruction of the ready kits.
2. Serum glutathione was calculated using a method described by Tietz (1999) (…)And serum Malondialdehyde as the final oxidized lipid component as mentions by Wysocka (1995).
3. Serum peroxy nitrate radical was estimated using a modified method reported by Vanuffelen et al., (1998).
4. Vitamin E was measured according to method which based on the reaction of Emmerie – Engel .

Statistical analysis

The variables are evaluated as means ± Standard Deviation, and determined by ANOVA's one way, followed by t-testing.

Significant statistical differences are considered when p≤0.05.

Results and discussion:

Table (1): Levels of interleukins (IL-10, IL-8, IL-6) (pg/ml)

Groups/ variables	IL-10 pg/ml	IL-8 pg/ml	IL-6 pg/ml
T1	5.22±0.43	41.32±12.2	115.3±48
T2	8.99±1.80	88.45±18.3	190.8±31
T3	7.45±0.79	58.51±15.6	132.2±45

The present study showed a significant increase in the level of interleukins (IL-10, IL-8, IL-6) compared to the control group in the fungal treatment group, whereas the amount would return to its natural ratio in the treatment group for vitamin E (T3).

The ability of a fungal pathogen to cause disease requires the ability of the host to survive. Host survival depends on the host's immune system resistance, including the phagocyte microbial killing mechanisms (Neofytos, et al., 2009). The innate immune system is made up of macrophages and neutrophils that phagocytize harmful microorganisms and also use reactive oxygen, nitrogen and chlorine products to support the host defense. (Yen *et al*., 5006). The adaptive immune system produces antibodies that can enhance the "respiratory burst" of the immune effector cells, "these reactive species can destroy pathogens and have potent immunomodulatory effects on the immune system by quickly altering or inactivating proteins, lipid membranes and DNA that affect the efficacy of the host response"(Neofytos, et al., 2009). Direct anti-fungal response resulting in; either a phagocytic, or microbicidal compound secretion process, and secondly, promoting function by generating proinflammatory mediators such as (cytokines and chemokines) In the course of antifungal responses, and this is what we obtained from this research.

"The generation of reactive oxygen species (ROS) through a process known as the respiratory burst is a major antimicrobial defense mechanism installed by these phagocytes". After cytokine stimulation, phagocytic cells activate the NADPH oxidase complex assembly, resulting in superoxide (O2−•) generation. Given the potency of the ROS produced by NADPH oxidase, (Patin et al., 2018).

Many researchers have been found in recent study that; this group of cytokines is the initial response to pathogen identification. (Palencia *et al*., 2010). It remains their most important task to mediate the recruitments of additional immune cells at the infection site, and members include: interlukin ligands such as interleukin-1 (IL-1) family (IL-1β, and tumor necrosis factor-α (TNF-α)). (Jhingran et al.,, 2015)

Table (2) Mean + SD serum peroxynitrite, malondialdehyde and gluthathione (µmol/gm)

Groups / variables	glutathione	Malondialdehyde	peroxy nitrate
T1	6.74± 0.44a	2.85 ± 0.15a	0.057 ± 0.05a
T2	3.25± 0.27b*	3.93 ± 0.47b*	0.163 ± 0.043 b*
T3	5.79±0.59a	2.45 ± 0.16a	0.038 ± 0.08a

The results of the current study showed in table (2) there are a significant decrease in glutathione in fungal treatment group (p≤ 0.05) comparative with control group, and there are a significant increase in MAD , and peroxy nitrate (p≤ 0.05), in fungal treatment group, comparative with control group.

Findings suggest that ingestion of *A. Niger*, mediated serum antioxidant reduces and increased of MDA and peroxide nitrate confirming Ishihura's (2010) results. For animals exposed to oxidative stress, this researcher reported a negative correlation between serum glutathione and MDA.

The fungus produces oxalic acid (oxalate); a "characteristic that is rarely mentioned in other Aspergillus species as part of its leavening process"; the Pyruvate, which is the ultimate glycolysis product, converted first to oxaloacetate, next, the oxaloacetate acetyl hydrolase, which is present in the cytoplasm of *A. niger*, lead to hydrolysis of oxaloacetate to oxalate, and acetate, as a result, oxalate is deposited in the body with calcium, forming calcium oxalate crystals (Thorpe et al., 23004). These deposits result in tissue damage and necrosis due to blockage in the blood vessels, and cell toxicity, due to oxidative stress. "These events have the effect of raising malondialdehyde and peroxy nitrate and decreasing glutathione" (Kayano et al., 2013). Glutathione is an important tripeptide-like thiol-containing molecule that is needed to maintain redox homeostasis and metal ion detoxification. Because of the damaging nature of A-produced calcium oxalate; pulmonary necrosis rapidly develops. (Verweii and Brandt, 2007), and acute renal failure due to deposition of calcium oxalate crystal were reported (Wong Sak et al., 2013).

The cellular antioxidant status specifies the sensitivity to oxidative damage in response to oxidative stress, and is typically modified. (Gutteridge and Halliwell 1999).

In the current study, we investigated the role of vitamin E in oxidative stress and inflammation. We found that vitamin E reduced the formation of reactive oxygen species (ROS), and prevent inflammation. Vitamin E improve the immune response at many levels, "such as induction of AMPs, skewing of T-cells from Th1 to Th17" , "as well as general anti-inflammatory effects" (Abdala-Valencia et al., 2012).

A number of "non-enzymatic and enzymatic" detoxification pathways collectively known as antioxidants keep the balance in the cell between beneficial and harmful effects of ROS. (Sies, 1993; Bast and Haenen, 2015). An imbalance between the pro-oxidant and/or antioxidant components is at the source of a dynamic physiological status known as "oxidative stress"

In addition, "a-tocopherol has a remarkable anti-inflammatory role by inhibiting, for example, cyclooxygenase-2 (COX2)" (O'Learyetal., 2004), Vitamin E shows other effects in addition to its anti-inflammatory and anti-oxidant activities, "such as regulation of the expression of genes encoding proteins involved in signaling" (Cardenas and Ghosh, 2013).

Some investigations, conducted in animal models as well as in humans, have shown that vitamin E supplementation benefits (Iuliano et al., 2000; Abdala-Valencia et al., 2012), While others had found a negative effects (Bjelakovic et al., 2012), or no effect at all (Morley and Trainor, 2001; Hemilä and Kaprio, 2011).

vitamin E "induce peroxidation inhibition, elimination of free oxygen radicals and breakdown of peroxidation chain reactions" **(**Murray et al**.,** 2000**)** by "Protein Kinase C (PKC) inhibition, and calcium metabolism by glutathione peroxide inhibition" **(**Das & King 2007**)**.

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