

RESEARCH ARTICLE

Infection with *Alternaria spp.* and *Aspergillus flavus* that Isolated from Sheep's Milk on Oxidative Status in Male Rats

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ABSTRACT

The current study was designed to show some pathogenic fungi isolated from sheep milk. Milk samples were collected from 50 sheep in different regions of Anbar city, which appeared healthy and without any disease. Each sample was cultured on Sabouraud dextrose Agar at $28 \pm 2^\circ\text{C}$ for 4–7 days. Two types of isolation were used in The current study: *Alternaria spp.* and *Aspergillus flavus*. A 21 male rats were utilized and distributed as follows (seven rats in group): Group A received a normal diet only and then killed at the end of experiment. Group B administrated (intraperitoneally) of *Alternaria spp.* conidia (10^7 conidia/mL normal saline.), and then killed after infection. Group C administrated (intranasal) of *A. flavus* conidid (10^7 conidia/mL normal saline.), and then killed after infection. The result show that the percentage of mold infection was *Alternaria spp.* 2 (8.7%) and *A. flavus* 4 (17.4%), respectively.

Keywords: *Alternaria spp.*, Antioxidant enzymes, *Aspergillus flavus*, Oxidative stress.

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INTRODUCTION

The Phylum of *Alternaria* is Ascomycota and its Subdivision: Pezizomycotina, and Family: Pleosporaceae.¹⁻² Among the various diseases caused by *Alternaria*, the damage is the most common sickness and causes a medium yield loss at the rate of approximately 32–57%.³ *Alternaria spp.* has ability to produce a broad diversity of substances called toxic metabolites that possess a significant role in plant diseases. Under specific environmental statuses, various of these substances could remain and accumulate in vegetable foods (fruits) and lead to various diseases to humans and animals.⁴ *Aspergillus flavus* was found by Link in 1809 and has been called as an abiogenetic sort that lone make spores (agamic), conidia, and sclerotia. Recently, the sexual period of *A. flavus* has been notify and circulating as *Petromyces flavus*.⁵ *A. flavus* causes distinctive diaseases to creature and human either through spending of dirtied feed (that lead to aflatoxicosis or potentially malignancy of liver) or by obtrusive development (causing aspergillosis), which is frequently deadly in men who have invulnerable traded off.⁶⁻⁷ *A. flavus* was concentrated in various creature models all the more then 40 years yet I is utilizing still infrequently in contrast with another type as *A. fumigatus*. Early investigations of obtrusive aspergillosis in non-immunocompromised mice exhibited that *A. flavus* was increasingly destructive correlation practically all different

Aspergillus types.⁸ So, present study aims to show the role of saprophytic fungi to causes oxidative stress in male rats.

MATERIALS AND METHODS

Samples Collecting

Milk samples were collected from 50 sheep at different regions of Anbar city which appeared healthy and without any disease. After cleaning the teat end with 70% ethyl alcohol and discard of the first three streams of milk 5–10 mL in each test tube, the collecting process is done. All milk sample was centrifuged at 3000 rpm/ minutes for 5–10, and the sediments were cultured on Sabouraud dextrose agar (Himedia, India) and incubated at $28 \pm 2^\circ\text{C}$ for 4-7 days then examined and identified macroscopically and microscopically according to Washinton WJ, *et al.*⁹ Conidia suspension was prepared form more prevalent mold and yeast according to Washinton WJ, *et al.*,¹⁰ and adjusted to 2×10^7 conidia/mL according to Mirkov L *et al.*¹¹ by hemocytometer chamber.

Experimental Design

A total of 21 male rats were utilized and distributed as follow (seven rats in a group):

- Rats received a normal diet only and then killed at the end of the experiment.
- Rats administrated (intraperitoneally) of *Alternaria spp.* conidia (10^7 conidia/mL normal saline.), and then killed after infection.

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C. Rats administrated (intranasal) of *A. flavus* conidia (10^7 conidia/mL normal saline.) and then killed after infection.

Oxidative and Antioxidative Status

Malonedialdehyied (MDA) was estimated (in the serum of rats) according to a method based on colorimetric reaction by using acid called thiobarbituric acid (TBA)¹² glutathione (GSH) level assessed bymixed 2.3 mL cradle with 0.2 mL of the example and afterward included 0.5 mL ofDTNB. The mix was once broke down through a spectrophotometer.¹³ Catalase was estimated through utilizing the technique for Biovision-USA packs. All parameters were determined in blood serum and liver serum.

Statistical Analysis

The Data were examined utilizing a factual Minitab program. A measurable contrast between the methods for the test bunches was broke down utilizing one route investigation of fluctuation (ANOVA).

RESULTS

Number and Percentage of Isolates

The percentage and number of each mold type was reported as shown in Table 1. *Alternaria spp.* 2 (8.7%) and *A. flavus* 4 (17.4 %), respectively, these two types were used in the present study to show their effects on oxidative status.

Oxidative Status

MDA (increased), GSH, and catalase (decreased) levels in serum of infected rats (*Alternaria spp.* and *A. flavus*) show high difference changes ($p < 0.05$) compare with control rats as shown in Figures 1–3. Also, MDA (increased), GSH, and

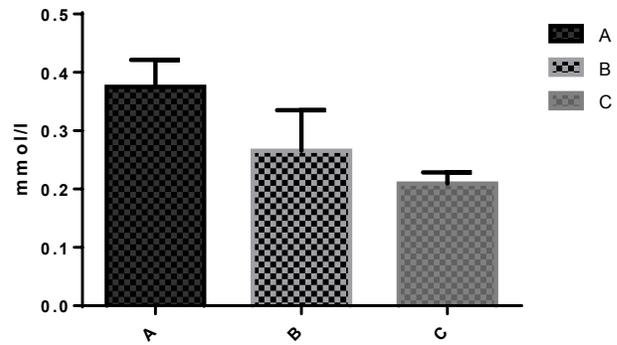


Figure 2: Levels of GSH in serum of all groups

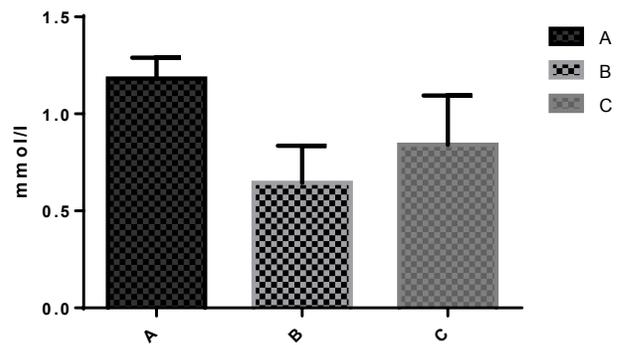


Figure 3: Levels of catalase in serum of all groups

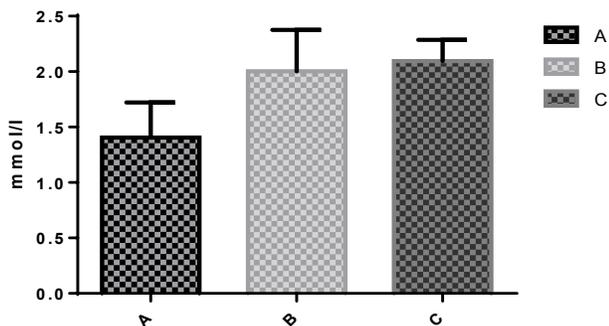


Figure 1: Levels of MDA in serum of all groups

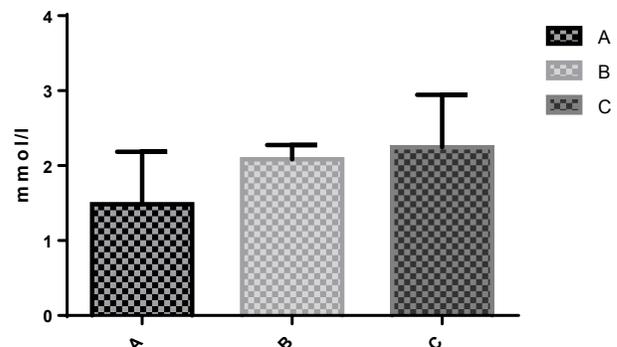


Figure 4: Levels of MDA in liver of all groups

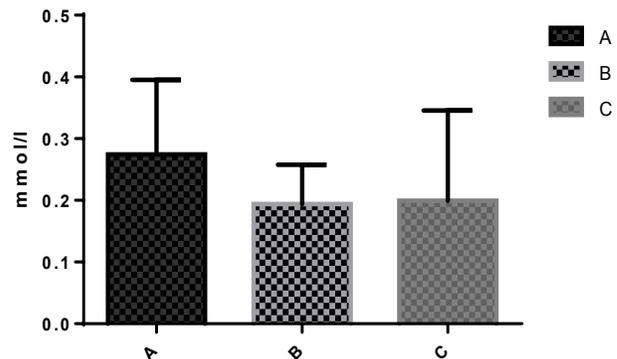


Figure 5: Levels of GSH in liver of all groups

Table 1: Prevalence of Fungal Contamination in Sheep's milk

Type of fungi	No. of isolates	Percentage %
<i>Alternaria spp.</i>	2	8.7 (%)
<i>A. flavus</i>	4	17.4 (%)
<i>A. fumigatus</i>	3	13 (%)
<i>A. niger</i>	2	8.7 (%)
<i>Acremonium spp.</i>	6	34.8 (%)
<i>Curvularia spp.</i>	2	8.7 (%)
<i>Fusarium spp.</i>	4	17.4 (%)
Total	23	100 (%)

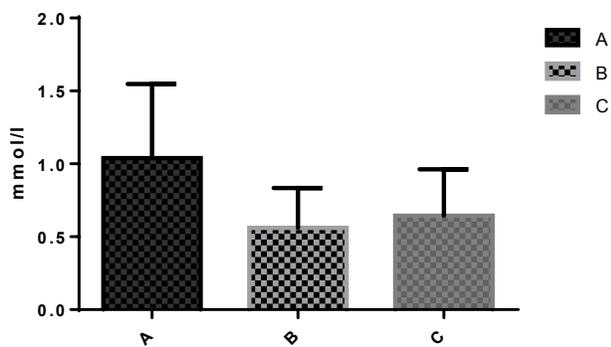


Figure 6: Levels of catalase in serum of all groups

catalase decreased levels in the liver of infected rats show high difference changes ($p < 0.05$) compare with control as shown in Figures 4-6 .

DISCUSSION

The present results show the percentage and number of each mold type and the types used for infection, including *Alternaria spp.* 2 (8.7%) and *A. flavus* 4 (17.4 %), respectively, and the results show effect of the two type on oxidative status. Zhou *et al.*,¹⁴ The type of milking using in this area is the manual milking which participates in introducing the conidia into the udder through the contaminated teat and the teat canal by the workers' contaminated hands. The previous study found that the fungal contamination of milk could reach 46.15% with the manual milking with partially lower 44.70% percentage by machine milking¹⁵ estimations of MDA, is broadly utilized as pointer of lipid peroxidation, and expanded degrees of lipid peroxidation items have been related with an assortment of ceaseless illnesses in both human and model framework.¹⁶ Roughly 30 metabolites with conceivable harmfulness are known from different types of *Alternaria*,¹⁷⁻¹⁸ and the capacity of *A. flavus* creating mycotoxin can be harmful for creatures and human wellbeing,¹⁹ Aflatoxin here was also actuate noteworthy increment in MDA level. The consolidated impacts of AF and form could build the age of free radicals, which may surpasses the cell protections bringing about oxidative pressure.²⁰ Additionally, Liu *et al.*²¹ alluded Aflatoxin to increment receptive oxygen species (free radical) for that to prompt an elevated in intracellular lipid peroxide levels in their investigation on the oxidative worry of Aflatoxin on hepatocytes in rodents. Comparative investigation performed by Souza *et al.*²² alluded Aflatoxin to cause lipid peroxidation in rodents liver. At long last, Shen *et al.*²³ alluded Aflatoxin to raised MDA level in rodents liver. That may clarify the impacts of *Alternaria spp.* also, *A. flavus* on oxidative status in the current investigation.

CONCLUSION

Levels of MDA, GSH and catalase show high significant changes ($p < 0.05$) in an infected (with *Alternaria spp.*) group and in an infected (with *A. flavus*) group compared with control. It was concluded that *Alternaria spp.* and *A. flavus*

lead to induce oxidative stress and decrease in the antioxidant enzymes levels.

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