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RESEARCH ARTICLE

AFLATOXICOSIS IN RABBITS WITH PARTICULAR REFERENCE TO ITS CONTROL BY N. ACETYL CYSTEINE AND PROBIOTIC

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ABSTRACT

Fungal and aflatoxins contamination in rabbit's environment and evaluation the efficacy of N-acetyl-L-cysteine (NAC) and probiotic in amelioration the toxic effects of aflatoxins were investigated. One hundred samples, including (25 each of feed, water, litter and wires of rabbit's cages) were collected from private rabbit's farms at El-Giza Governorate. The mycological examination resulted in recovering *Aspergillus* sp. at the top of fungi isolated from all examined samples(68%), followed by *Mucor sp.(35%), Penicillium sp.* (31%), *Fusarium sp. (23%), Rhizopus sp. (21%),Cladosporium sp. (6%) and Scopulariopsis sp. (4%)*. Members of genus *Aspergillus* was predominantly recovered from the present samples. *A. flavus* was isolated from (59%) of samples, followed by *A.niger* (21%) and *A.ochraceus* recovered from (19%) of samples. The isolated *A.flavus* from litter samples were detected as the most prevalence aflatoxigenic strains (100%)which produced aflatoxin B_1 and B_2 with a mean levels of (27.0±3.22). Although, the higher mean levels of AFs was obtained by *A. flavus* that recovered from feed samples (50 ± 3.5) with a lower relatively rate of incidence (87.5%). This makes the selection of proper decontamination methods that will effectively decompose aflatoxins. Thirty-five healthy New Zealand rabbits were divided into 5 groups, each including 7 rabbits. The rabbits of group 1 were kept as the negative control group. While, rabbits in the groups 2, 3, 4 and 5 were orally given AFB_1 at a dose of 50ug dissolved in 0.5 ml of olive oil/ animal daily, for 4 weeks.

On first day following the administration of AF, NAC was orally administered a dose of250 mg/kg ofb.w. for group 3 (low dose)and a dose of 500mg/kg b.w. for group 4 (high dose). While, the group 5 given probiotic in drinking water (0.5 gram dissolved in 25 liters of water).Aflatoxin induced (ROS), accompanied with significant increase in malondialdehyde (MDA) levels of lysated RBCs and a significant decrease in CAT, SOD and GPx activities. While, there was an increase in serum AST, ALT, ALP, urea and creatinine levels. The activity of pancreatic enzymes showed significant increase in serum amylase and lipase activities after the rabbit's exposure to aflatoxin B_1 .However, aflatoxin B_1 group had a decrease in concentrations of serum total protein, albumin, alpha globulin, beta globulin and gamma globulin together with A/G ratio. The administration of N-acetylcysteine or probiotic caused a decrease in malondialdehyde (MDA) levels and an increase in GSH, CAT, SOD and GPx compared to aflatoxicated group. In addition, the levels of ALT, AST, plasma amylase and lipase levels were significantly reduced due to the protective effects given by N-acetylcysteine and probiotic. The histopathological necrotic damage due to the drastic effects of AFB_1 on some vital organ as liver and kidney included hepatotoxic effects and inhibition of DNA, RNA and protein synthesis. These changes improved by administration of probiotic and N-acetyl cystein to aflatoxicated animal resulted in reduction of the aflatoxintoxic changes through the immune strengthening effect and protection of lipid and protein from oxidative damage.

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INTRODUCTION

The fungal pollution of animal and poultry feeds and human food by mycotyoxigenic fungi contributes a major health problem and is responsible for high economical losses in animal production due to decrease in milk and meat production. The toxicity to animal and poultry by feed invaded with fungi had been previously recorded by several investigators (Hassan *et al*., 2010, 2011, 2012 and Manal *et al*., 2012). Out of several flocks of poultry including turkeys affected with broader pneumonia, trachitis and air saculitis, A. fumigates and A. flavus were the most commonly isolated fungi from lesions but Penicillium and Mucor species were isolated in rare frequency

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(Singh and Clausen, 1980 and Hassan, 1998). In other cases, F. oxysporum isolated from feeds produced tibial dyschondroplasia and immune suppression in poultry (Chu *et al*., 1995 and Hassan *et al*., 2010). On the other hand, mycotoxins are formed by certain fungal species, whenever environmental factors are conductive during the growth of these frequently occurring mycomycetes on foodstuffs and animal feeds; the process takes place as a secondary metabolism. The mycotoxin inhibits cell division, RNA/ DNA synthesis and apoptosis (Rotter *et al*., 1996). Aflatoxins (AFs) are produced mainly by Aspergillus flavus and Aspergillus parasiticus (Bennett and Klich, 2003) and usually found in various agricultural commodities (Meissonnier *et al*., 2008 which are known to be very dangerous mycotoxins. The exposure to aflatoxin B1 can result in suppressed immune response, malnutrition, proliferation of the bile duct, centrilobular necrosis, fatty infiltration of the liver, hepatic lesions and even hepatomas. It is one of the most commonly found metabolites and has a highest toxigenic effects (Richard, 2007), which can induce reactive oxygen species (ROS) generation that causes oxidative stress, leading to impairment of DNA, RNA, proteins, lipids and other changes (Mary *et al*., 2012). Meanwhile, AFB1 has immunosuppressive properties and mainly exerts its effects on cell-mediated immunity that induces an inflammatory response (Meissonier *et al*., 2008). These natural toxins probably pose greater threat to human and animal health than synthetic toxins (Ames, 1983).

AFB1-mediated toxicity was also found to be related to its pro-oxidant potential. This is because reactive oxygen species (ROS) including superoxide anion (O-), hydrogen peroxide (H2O2) and hydroxyl radical (-OH) that are generated during the metabolic processing of AFB1by liver enzymes, Preston and Wiliams (2005). Also, ROS at higher concentration are important mediators of damage to cell structures, including lipids, membranes, proteins and nucleic acids oxidative stress (Poli *et al*., 2004). AFB1-DNA adduction is believed to be the source of point mutations that initiate AFB1-induced hepato-carcinogenesis (Bailey *et al*., 1996). The harmful effects of ROS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes (Hallowell, 1996). Numerous physical, chemical and biological methods had been proposed to detoxify or inactivate aflatoxins in contaminated feedstuffs. Supplementation with antioxidants, through an increased consumption in the diet either has become extremely popular as a means to improve animal and human health or increase their physical performance. The addition of chemical compounds to animal feed as N-acetyl-L-cysteine (NAC)a thiol containing anti-oxidant had been used to mitigate various conditions of oxidative stress. In addition, it reduces liver injury caused by paracetamol over dosage in human, attenuates liver injury, and prevents liver and plasma GSH depletion in mice and rabbits (Flanagan and Merediti, 1991 and Kelly, 1998). On the other hand, the biological methods by using microorganisms and their metabolites in feed and/or water to eliminate aflatoxins, can be a highly promising approach owing to its specific, efficient and environmentally friendly detoxification Some microbes, including fungal and bacterial isolates (FAO, 2001 and Nabawy *et al*., 2014). Therefore, the current study was undertaken to demonstrate the prevalence of fungi and aflatoxins in rabbit's environment and to evaluate the efficacy of N-acetyl-L-cysteine (NAC) and probiotic in amelioration the toxic effects of aflatoxins in rabbit feeds.

MATERIALS AND METHODS

Samples

One hundred samples, including (feed, water, litter and wires of rabbits cages (25 of each) were collected from diseased private rabbit's farms at El-Giza Governorate. The birds in these farms suffering from signs of toxicosis including depression, diarrhea, loss of appetite, decrease body weight gain and high mortality. The collected samples were transported to laboratory in clean sterile plastic bag for mycological investigation.

Aflatoxins standards

Standards of different types of aflatoxins (AFB1, B2, G1 and G2) and Immune-affinity columns of these types were purchased from Sigma Chemical Company (USA).

N. Acetyl Cystein

It was purchased from Sigma Chemical Company.

Probiotic

Each vial contained 1gm of powder which consisted of:
Lactobacillus plantarum 1X108 CFU,
Lactobacillus acidophilus 1X108CFU,
Saccharomyces cerevisiae 1X107 CFU ,
Carrier-skim milk up to 0.5gm.

Isolation and identification of fungi

The collected samples of feeds, litters, walls of cages and water samples were prepared and examined mycological according to the technique recommended by (APHA, 1992). The identification of isolated mould and yeast genera and species was carried out according to (Pitt and Hocking, 2009 and Refai and Hassan, 2013).

Production of aflatoxins by A. flavus on yellow corn and its estimation (Smith, 1997)

The strains of A. flavus that were recovered from the present samples were screened for aflatoxin production on yellow corn. In a flask containing 100 gm of finely ground yellow corn and 40-50 ml of distilled water was mixed and autoclaved at 121°C for one hour. The flask was shaken to prevent cooking of yellow corn. It was inoculated with spore suspension of 2 slants ofA. flavus and incubated for 4 weeks at 25-28°C. After end of incubation period, the corn was removed from flasks, dried; finely ground and 50 g of each were subjected for estimation of aflatoxins. The estimation of prepared aflatoxins was measured qualitatively by TLC (Bauer *et al*., 1983) and the positive samples for aflatoxins were measured quantitatively by fluorometeric method using specific FGis Afla test standards according to the recommended method of (AOAC, 1990 and Refai and Hassan, 2013).

Experimental Design

Thirty-five healthy New Zealand rabbits, aged 6-8 weeks and weighing between 1 and 1.5 kg were used. The rabbits were housed individually in wire cages and fed with commercial pellet feed and the water was supplied ad libitum. The animals were divided into 5groups, each including 7 rabbits. Group 1 was kept as negative control group without any treatment. Rabbits in the groups2, 3, 4 and 5wereorally given AFB1 at a dose of 50 ug dissolved in 0.5 ml of olive oil/ animal daily, for 4 weeks. On day 1 following the administration of AF, NAC was orally administered at a dose of 250 mg/kg b.w. for group 3(low dose) and a dose of 500mg/kg of b.w. for group 4(high dose). While, the group 5 given probiotic in drinking water (0.5 gram dissolved in 25 liters of water). The experimental study was extended for 4 weeks and at end of this period, blood samples were collected from each rabbit for biochemical studies and all animals were sacrificed to take their internal organs tissues for histopathological studies (Çam *et al*., 2008).

Biochemical Analysis

At the end of the experiment, two blood samples were collected from each group into small-labeled dry and clean vials with and without anticoagulant .Blood samples were centrifuged (3000 rpm, 10 min) and the serum was separated for biochemical parameters. The estimation of lipase and amylase levels in plasma was done as recommended by Moss and Henderson (1999). The levels of aspartate amino-transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were measured according to (Reitman and Frankel, 1957 and Tietz, 1996). While, serum urea was determined the method of Wybenga *et al*. (1971), serum creatinine level after Henry (1974). Whereas, the estimation of plasma total protein and electrophoretic pattern were carried out after SonnenWirth and Jaret (1980) and Davis (1964), respectively and calculated according Syn Gene S. No. 17292*14518 sme*mpcs

Lysate preparation and assays of antioxidant parameters

RBCs were separated from plasma by centrifugation, washed three times with saline and lysed (Tietz, 1996). The lysate was mixed with an equal volume of Drabkin`s reagent to determine hemoglobin levels by Van Kampen and Zijlstra (1965).While, the catalase activity; lipid peroxidation as malonaldehyde (MDA) and reduced glutathione (GSH) in lysated RBCs were determined according to (Aebi, 1974; Nishikimi *et al*., 1972 and Ellman, 1959), respectively. Whereas, levels of SOD and GSH-px activities were undertaken as methods as (Marklund and Marklund, 1974 and Paglia and Valentine, 1967).

Histopathological Examination

Tissue specimens from livers and kidneys were taken and preserved in 10% formalin. Then tissue preparation including dehydration with ascending grades of alcohol, clearing with xylene, paraffin shaping, molding and cutting with a microtome at a thickness of 4-5 um was performed. Sections of were stained with hematoxyline and eosin (H&E) according to Bancroft *et al*. (1996), processed as routinely for dehydration and covering then examined under bright field microscope. Another set of sections were stained with 1% ethidium bromide for 3 minutes and examination was done under fluorescent microscope using green- UV filter.

Statistical Analysis

Results were expressed as means + standard errors. Data obtained were analyzed using one way analysis of variance (ANOVA) according to, SPSS 14 (2006).Values with $P \leq 0.05$ were considered as significant.

RESULTS AND DISCUSSION

The environmental pollutions and their elimination become a worldwide problem and gain attention of all authorities. Microbial pollutants are the most dangerous factors and a wide range of diseases are caused by fungi and their toxins and constitute a major problem for animal and human health. The correlation between the environmental factors, mycosis and mycotoxicosis in animals and the role of these environmental factors in initiation of food born infections had been reported by (Hassan *et al*., 2012 and 2014). The specific qualities of climate, vegetation and land are the important factors affecting the prevalence of moulds in connection with a certain geographical locations. Mould spores can disperse in the air with the wind or in combination of wind and rain (Mikulec *et al*., 2005). Breeding factors such as animal housing, feeding on moldy hay and ventilation system or environmental factors such as temperature, wind and dew increase the potential of contracting the infection (Chihaya *et al*., 1991 and Moubasher, 1995).On the other hand, Aspergillus flavus was recorded to constitute a public health hazard due to production of aflatoxins that cause some degree of acute toxicity when consumed in high amounts and are potential carcinogen. In developing countries, it appears that there is a direct correlation between dietary aflatoxins intake and the incidence of liver cancer (FDA, 2000 and Bahtnager and Ehrlich, 2002). In addition, the outbreaks of food borne pathogens continue to draw public attention to food safety. Hassan *et al*. (2012) reported that the most common isolated moulds from feed were Aspergillus spp. (100%), Fusarium spp. (24%), Mucor spp. (28%), Pencilliumspp. (52%), Cladosporium spp.(8%) and Alternaria spp.(28%).

In addition, it has been estimated that 25% of the world's crop production is contaminated with mycotoxins. While, Pusterla *et al*. (1996) suggested that the primary infection was attributed to inhalation of spores originated from moldy hay or soil.In the present work, from rabbits farms, some animals suffered from signs of toxicosis including depression, diarrhea, loss of appetite, decreased body weight gain and high mortality, samples of feed, water, litters and wires of cages were collected for mycological investigation. The results revealed that the members of genus Aspergillus were at the top of all other fungi which recovered from (68%) of all examined samples, followed by Mucor sp.(35%), Penicillium sp. (31%), Fusarium sp. (23%), Rhizopus sp.(21%), Candida sp.(9%), Cladosporium sp. (6%) and Scopulariopsis sp. (4%),respectively (Table 1). On the other hand, aflatoxigenic A. flavus were detected in all kinds of feed samples and produced aflatoxin B1 with mean

level of (60 ± 0.1 ppb) (Adebajo *et al*., 1994 and Hassan *et al*., 2010).In another study, A. flavus was recovered from 100 samples of air, water supply and feeds including tibn, hay and processed feeds (Hassan *et al*., 2011). Whereas, (El-Ahl *et al*., 2006) illustrated that the most predominant fungi in animal and poultry feed belonged to genus Aspergillus which was isolated from all samples. Other moulds were also isolated but at low frequency as Penicillium sp., Fusarium sp, Cladosporium sp and Alternaria sp.

Currently, members of genus Aspergillus were predominantly recovered from the present samples. The species of A. flavus was isolated from (59%) of samples, followed by A.niger (21%) and A.ochraceus recovered from (19%) of samples. Other species of genus Aspergillus were also isolated in low frequencies (Table, 2). On the other hand, El-Ahl *et al*. (2006),evaluated the potential of the isolated A.flavus and A.ochraceus from feed samples for production of aflatoxin B1 and ochratoxin A (respectively). They detected that the relationship between the incidence of these moulds in samples ,colony counts and levels of produced mycotoxins were irregular. For instance in hay and layer's concentrates samples showed highest prevalence of A. flavus (100% and 60%), colony count (1.9x101±0.04) and 1.75x101±0.039),high incidence of toxogenic A. flavus (40% and 50%) and maximum levels of aflatoxin B1(2.3 and 4.1 ppm) respectively. In other study, the isolated A. flavus from tibn yielded higher mean levels of aflatoxins (2700 ± 3.7 ppb), whereas, the aflatoxigenic mould and aflatoxins were also detected in samples of feed and food included wheat and yellow corn (Hassan *et al*., 2012). They added that the levels of aflatoxins produced by the A. flavus isolated from yellow corn was at the mean level of (600 ± 6.2ppb) and 66.6% of isolates produced mean level of (300 ± 4.5 ppb) in wheat. Also, other authors (Adebajo *et al*.,1994; Kayas, 1998 and Bennett and Klich, 2003) detected significant levels of aflatoxins production by A. flavus recovered from different types of animal feeds. The International Agency for Research on Cancer had been declared that aflatoxins are potentially carcinogenic to human and animals and statistically associated with a high incidence of liver cancer in certain areas of Transkei; South Africa; China and Egypt (Chu and Li, 1994).The AFs cause significant economic losses in agriculture, morbidity and mortality in animals and immunological compromised humans, where it is capable of killing cells by causing extensive damage to cellular membrane (Ajello and Hay, 1998; Mogda *et al*., 2002 and Hassan *et al*., 2011).

Regarding the aflatoxin producing potentials in our study, the isolated A.flavus from the litter samples were detected as the most prevalence aflatoxigenic strains (100%), which produced aflatoxin BI and B2 with a mean levels of (27.0±3.22). Although, the higher mean levels of AFs was obtained by isolates that recovered from feed samples(50 ± 3.5) with a lower relatively rate of incidence (87.5%)(Table, 3).This is supported by the last awareness from use of litter as a nitrogen source as additive for animal and poultry in diet, which makes the potential mycotoxins producing fungi in feed and litter of harmful significant effect (Kubena *et al*., 1995; Hassan, 1998; Garishand Smith, 2008; Garish *et al*., 2010 and 2010 and Prasath *et al*., 2010 and Hassan *et al*, 2012)The Food and Drug Administration has established recommended maximum levels for aflatoxins in animal feed, which are 20 µg/kg of feed (FDA, 1994). Therefore, the detected levels of mycotoxins were significantly over the permissible limits in feeds because of continuous feeding of toxiocated feed. The World Health Organization , WHO (2002) had classified aflatoxin as a group 1 human and animal of carcinogenic and immunosuppressive effects that inhibit nearly cellular and humeral immunologic reactions (Pestka *et al*., 2004), which including disruption of normal cell function by inhibiting RNA, DNA, and protein synthesis; inhibition of cell division; stimulation of ribotoxic stress response; and activation of mitogen-activated protein kinesis. This resulted that the selection of proper decontamination methods that will effectively decompose aflatoxins, while retaining the nutritive quality and palatability of the treated food has continuous challenge of scientists. Many studies had shown that hepato-protective effects may be associated with an antioxidant capacity (Nayakand Sashidhar, 2010). However; certain feed additives have been successfully used to inhibit mold growth and to reduce the incidence of aflatoxicosis in animals. Therefore, intensive research has been continued out to find cost-effective and safe procedures and agents that reducethe deleterious effects of AFs (Nabawy, 2015).

Many agents have been used for the treatment as N-acetylcystein, which appears to have beneficial effects against toxicagents, neoplasia, heart disease and atmospheric pollutants (Flanaganr and Merditht, 1991 and Kelly, 1998). In addition, Valdivia *et al*. (2001) reported that NAC had a preventive effect against aflatoxinB1 intoxication in broiler chickens. Also, the probiotic enhance immune response and nutrition of host species through the production of supplemental digestive enzymes (Abdel-Kader, 2009). It was indicated that dietary probiotic had antioxidant activity and had a protective effect against dietary aflatoxin, this results agreed with (Chen *et al*., 2013a & b and Nabawy *et al*., 2014).Therefore, in this study the experimental induction of rabbits aflatoxicosis and amelioration its toxic effects by probiotic preparation and N-acetyl cystien were investigated.

In the present study, aflatoxins generate intracellular reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radicals, during its metabolic processing in the liver. The levels of MDA, a product of lipid peroxidation and endogenous scavengers GSH, CAT, SOD and GPx in lysated RBCs were measured. Aflatoxin induced (ROS), were accompanied with a significant increase in MDA levels of lysated RBCs whereas a significant decrease in CAT, SOD and GPx activities. Elevated MDA as an offense system and lowered CAT and GPx activities as a defense system play an important role in the development of cell damage (Table, 4). These results are in agreement with previous studies suggested that oxidative stress may be due to direct effect of AFs or by the metabolites formed and the free radicals generated during the formation of these metabolites (Abdel-Wahhab and Aly, 2010 and Kanbur *et al*., 2011).The reduction of protein synthesis in AFs-treated animals may affect certain metal ions (i.e., iron and copper), which play an important role in free radical production and liberation (Hamzawy *et al*., 2012 and 2013). Previous studies demonstrated that the mechanism of

AFs-induced liver injury may be due to those reactive and toxicmetabolites of AFs and the liver necrosis begins when the glutathione stores are almost exhausted (Mogda *et al*., 2014).

The serum activities of aspartate transaminase(AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) had been recognized as sensitive serological indicators in the impairment of the hepatic tissues and biliary system (Abdel-Wahhab and Aly, 2003). The increase in serum AST and ALT activity indicates initial hepato-cellular damage as a result of AFB1 treatments (Abdel-Wahab *et al*., 2006). Whereas increases ALP level are associated with the hepato-biliary malfunction. Increase in urea and creatinine level observed in the current study in AFs-treated group clearly indicated the harmful and stressful effect on renal tissue (Abdel-Wahhab and Aly, 2003). These results clearly indicated that AFs had stressful effects on the hepatic and renal tissues, consistent with those reported in the literature of aflatoxicosis (Sun *et al*., 2015). Regarding the activity of pancreatic enzymes, the current data in (Table, 5) showed a significant increase in serum amylase and lipase activities after exposure of rabbits to aflatoxin B1.

Similar findings to our work were reported by (Richardson and Hamilton, 1987 and Matur *et al*., 2010) in their studies on layer chickens, where, AFB1 treatment increases the activity of pancreatic chymotrypsin, amylase and lipase. On the other hand, Osborne and Hamilton (1981) noted a lower activity of pancreatic amylase, trypsin, lipase, RNase, and DNase when broilers were exposed to AFB1. However, Osborne *et al*. (1982) suggested that AFs ingestion affect various digestive enzymatic activities that cause mal-absorption syndrome, characterized by steatorrhea, hypocarotenoidemy and lowering of bile, pancreatic lipase, trypsin and amylase. Other study showed degradation of polyunsaturated fatty acids in cell membranes by ROS resulted in the destruction of membranes and formation of MDA, which is an indicator of ROS generation (Dabrowski *et al*., 1999).In addition to the disruption of the

Table 1. Mycoflora of rabbit feed, litter, water and wires of rabbit cages

Generaof isolatedfungi	Feed(25)		Liter(25)		Wires of cages(25)		Water(25)		Total(100)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus sp.	19	76	10	40	25	100	14	55	68	68
Penicillum sp.	10	40	3	12	11	44	7	27	31	31
Fusarium sp.	5	19	8	32	-	-	-	-	23	23
Mucor sp.	15	60	8	32	10	40	2	8	35	35
Rhizopus sp.	8	32	7	22	6	24	-	-	21	21
Cladosporium sp.	1	4	4	16	-	-	1	4	6	6
Scopulariopsis sp.	1	4	3	12	-	-	-	-	4	4

Table 2. Prevalence of members of Aspergillus sp. In rabbit feed, litter, water and wires of rabbit cages

Members of Aspergillus	Feed(25)		Liter(25)		Wires of cages(25)		Water(25)		Total(100)	
	No.	%	No.	%	No.	%	No.	%	No.	%
A.flavus	16	64	10	40	21	84	12	48	59	59
A.niger	8	32	3	12	4	16	6	24	21	21
A.fumigatus	3	12	3	12	-	-	-	-	7	7
A.candidus	4	16	2	18	1	4	1	4	8	8
A.ochraceus	7	28	6	24	5	20	1	4	19	19
A.ustus	2	8	2	8	-	-	1	4	5	5
A.glaucus	1	4	1	4	1	4	-	-	3	3
A.terreus	1	4	0	0	1	4	5	20	7	7

Table 3. Aflatoxin production by isolated A.flavus from feed, litter, water and wires of rabbits cages samples

Source of tested isolates	Levels of produced aflatoxins (PPM)				
	Tested isolated A.flavus			Mean level of produced AF.(ppm) ±SE	Types of AF.
	No of tested isolates	No. of aflatoxigenic Isolates	%of aflatoxigenic Isolates		
Feed	16	14	87.5%	50 ± 3.5	AF B1, B2
Liter	10	10	100%	27.0±3.22	AFB1
Wires of rabbits cages	20	15	75%	20±0.9	AFB1
Water	10	6	60%	15.0±0.2	AFB1

Table 4. Levels (the mean ± SD) of antioxidant enzymes and non-enzyme of control and experimental rabbit's erythrocytes

Group parameter	Control	AFB1	AFB1+ Probiotic	AFB1+NAC Low dose	AFB1+ NAC High dose
MDA nmole/mg Hb	7.57c±0.97	14.20a±0.88	11.44b±0.79	10.15bc±0.58	12.71ab±0.86
GSH nmole/mg Hb	281.92a±18.29	160.85c±7.05	203.02b±8.59	221.14b±10.82	210.36b±9.04
Catalase(u/mg Hb)	180.78a±6.92	116.88b±7.44	139.98b±8.44	146.99b±11.15	128.34b±14.13
SOD(u/mg Hb)	7.45a±0.01	4.99b±0.40	5.49b±0.47	6.21ab±0.47	5.48b±0.31
GSH-px(u/mg Hb)	16.04a±0.81	11.49c±0.61	13.26bc±0.49	14.70ab±0.61	13.60bc±0.58

Each value represents mean ± S.E.; n = 5.Means with different superscripts reveal significant difference from control at $P \leq 0.05$(ANOVA).

cytoskeleton in aflatoxicated animals lead to disturbance of intracellular transport of digestive enzymes as reported by, Jungermann *et al*. (1995). The large amounts of ROS and activated pancreatic enzymes leaked from the broken cells injure capillary endothelium increased capillary permeability and edema (Dabrowski *et al*., 1999).It is suggested that aflatoxin B1potentiatedhepatocyte injury by inducing hepatocyte apoptosis as illustrated by Barlas *et al*. (2004) where, ROS plays an import role in the pathogenesis of pancreatitis-induced hepatic damage. The present data in (Table, 6), showed that aflatoxinB1 group had a decrease in concentrations of serum total protein, albumin, alpha globulin, beta globulin and gamma globulin together with A/G ratio. AflatoxinB1 was associated with other alterations in serum protein sub-fractions fractions. The significant increase in alpha-2, beta-2 and gamma-2 globulins and a significant decrease in alpha-1, beta-1 and gamma-1 globulins were detected in aflatoxicated rabbits. Some studies reported that aflatoxin had an electrophilic reactivity at the carbonyl carbon atom and could conceivably adduct amines, imidazoles and sulfhydryl groups on proteins and enzyme via the Michael carbonyl condensation reaction which can cause conformational changes that interfere with their function (Lee *et al*., 2010 and Rawal *et al*., 2010). Whereas, decreased total serum protein and complement activity may cause AFB1 to induce immunotoxicity (Azzam and Gabal, 1998).The increased level of urea and the decreased level of albumin and total protein (TP) indicated the inhibition of protein synthesis, increase of protein catabolism and/or renal dysfunction (Jindal *et al*., 1994 and Mogda *et al*., 2014).Similarly, Yunus, *et al*., (2011) suggested that the immunotoxicity of AFB1 could be attributed to the inhibition of antibody production through the toxin's effects on lymphocytes leading to enhance turnover of serum antibodies and consequently decreased antibody half-life. While, the alteration of globulin sub fractionasalpha-2, beta-2 and gamma-2 globulins and increase in alpha-2macroglobulin and beta-2 lipoprotein may results of inflammatory tissue (Kaneko *et al*., 1997).

Currently, the administration of N-acetylcysteine caused a decrease in malondialdehyde levels and an increase in GSH, CAT, SOD and GPx in comparison with aflatoxicated group. Similar findings detected that NAC inhibits apoptosis which induced by oxidative radical stress, deprivation of growth factors or a variety of cytotoxic drugs (Skrzydlewska and Farbiszewski, 2004). Other study, suggested that N-acetylcysteine may have the potential protective agent for preventing the negative oxidative parameter changes related to oxidative stress in lysated RBCs caused by aflatoxin B1(Nayak and Sashidhar, 2010) and acts as a reductant by its reducing power and by stimulating the synthesis of the major cellular GSH and enhanced GSH activity (Moldeus and Cotgreave, 1994 and Tylicki *et al*., 2003).On the other hand, NAC could inhibit and reduced the expression of inflammatory cytokines which caused the difference of serum ALT , AST and ALP levels, that considered to be one metric to assess the severity of liver injury (Wang *et al*., 2015). The reduction in levels of ALT could be attributed to the protective effects of N-acetylcysteine for hepatocyte (James *et al*., 2003) and also significantly decreased plasma amylase and lipase levels, hence, the use of N-acetlycysteine as feed additive significantly decreased histopathological damage score in both pancreas and liver (Eşrefoğlu *et al*., 2006). Therefore, treatment with a high dose of NAC may interfere with hepatic normal metabolic functions and impairs liver recovery from aflatoxin B1hepatotoxicity (Yang, *et al*., 2009). Regarding, the administration of probiotic to aflatoxicated rabbits, the results indicated that it had antioxidant activity and protective effect against dietary aflatoxin which come in parallel with that findings reported by (Chen *et al*., 2013 a).

Table 5. Levels of some enzymatic activity of control and experimental rabbit's serum

Group Parameter	Control	AFB1	AFB1+probiotic	AFB1+NAC Low dose	AFB1+ NAC High dose
Amylase(u/l)	378.95c±33.96	1065.83a±82.38	753.39b±7.31	842.12b±63.48	845.09b±67.20
lipase (u/l)	34.98e±11.87	151.31a±1.26	67.64d±1.90	77.15c±5.56	92.78b±4.35
AST (u/l)	36.00c±1.53	75.00a±4.58	54.67b±3.48	44.67bc±3.48	56.00b±3.51
ALT (u/l)	29.00b±1.52	51.66a±8.25	43.30ab±3.84	29.33b±2.72	39.33ab±3.33
ALP (u/l)	108.33c±9.60	227.00a±25.06	174.33b±15.60	161.67b±10.40	168.33b±7.84
Urea (mg/dl)	39.48ab±1.66	30.73c±1.93	36.65b±1.63	41.40a±1.04	34.89bc±2.02
Creatinine(mg/dl)	0.89ab±0.03	0.93a±0.01	0.86ab±0.02	0.81b±0.02	0.87ab±0.03

Each value represents mean ± S.E.; n = 5.Means with different superscripts reveal significant difference from control at P ≤ 0.05(ANOVA).

Table 6. Levels (the mean ± SD) of total protein and its fractionation (g/dl) in control and experimental rabbit's serum

Group Parameter	Control	AFB1	AFB1+Probiotic	AFB1+NAC Low dose	AFB1+ NAC High dose
T. protein	7.20a±0.15	5.31c±0.22	6.11bc±0.28	6.56ab±0.11	5.98bc±0.37
Albumin	2.43a±0.04	1.20d±0.15	1.65c±0.14	2.04b±0.10	1.98bc0.04
Alpha1	0.90a±0.05	0.65c±0.03	0.79ab±0.04	0.83ab±0.03	0.76bc0.02
Alpha2	0.42a±0.03	0.47a±0.01	0.45a±0.01	0.43a±0.01	0.45a0.003
T. alpha	1.33a±0.03	1.12b±0.02	1.25ab±0.05	1.26a±0.04	1.21ab±0.02
Beta1	0.77a±0.05	0.46b±0.01	0.60ab±0.09	0.63ab±0.04	0.64ab±0.02
Beta2	0.62a±0.01	0.69a±0.01	0.65ab±0.02	0.63ab±0.01	0.65ab±0.01
T. beta	1.40b±0.06	1.16b±0.03	1.16ab±0.03	1.27ab±0.05	1.29ab±0.01
Gamma1	1.65a±0.07	1.31b±0.10	1.55ab±0.09	1.59a±0.02	1.50ab±0.04
Gamma2	0.38a±0.03	0.47a±0.03	0.47a±0.03	0.38a±0.03	0.43a±0.04
T. gamma	2.03a±0.07	1.79a±0.07	1.95a±0.09	1.95a±0.09	1.93a±0.08
T. globulin	4.77a±0.11	4.08b±0.08	4.46ab±0.18	4.51ab±0.01	4.00b±0.37
A/G ratio	0.50a±0.006	0.30b±0.03	0.30ab±0.03	0.45ab±0.02	0.50b±0.05

Each value represents mean ± S.E.; n = 5.Means with different superscripts reveal significant difference from control at P ≤ 0.05(ANOVA).

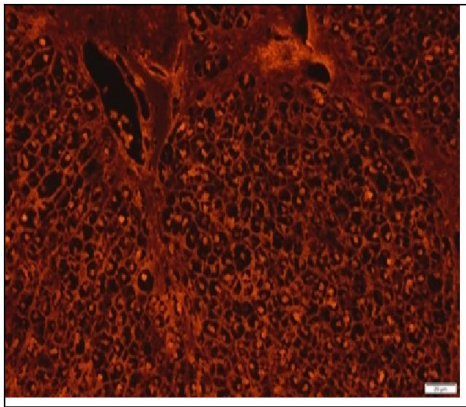

Fig. 1. Photomicrograph of liver AF/B1 group, reveals unhealthy nuclear membrane. Ethidium bromide X400

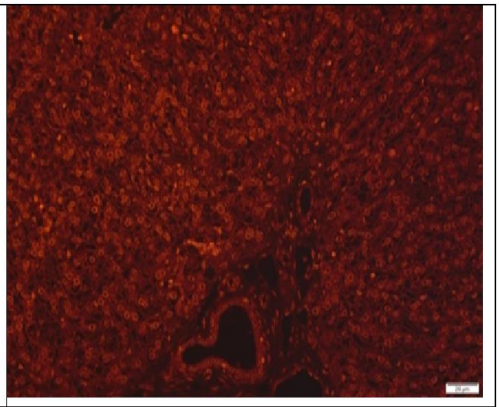

Fig. 2. Photomicrograph of liver of treated groups reveals exclusion of ethidium bromide by intact nucealr membrane. Ethidium bromide X 400

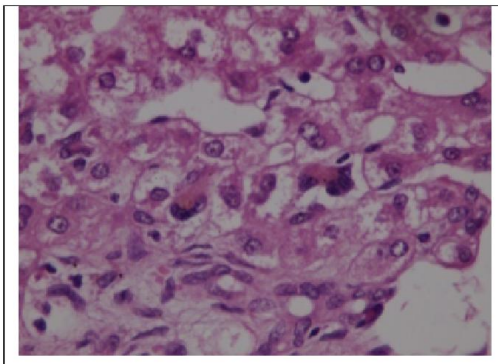

Fig. 3. Photomicrograph of liver of probiotic/ group reveals high incidence of multi-nucleated giant cells formation. H&E X1000

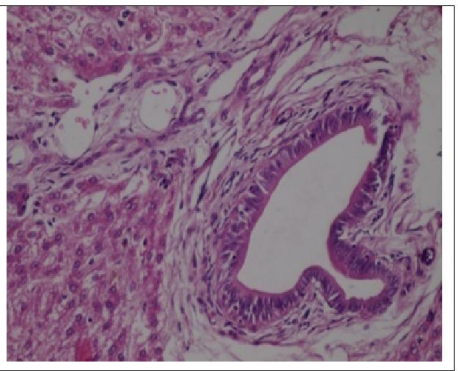

Fig. 4. Photomicrograph of liver, showing marked proliferation of bile ductlues in both AF/B1 and probiotic groups. H&E X400

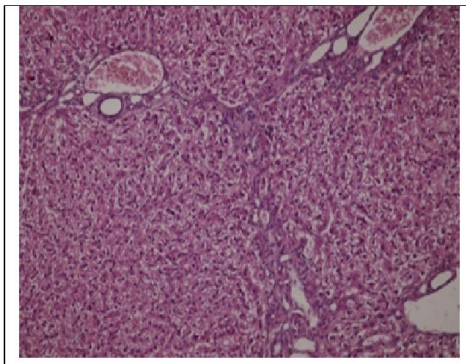

Fig. 5. Photomicrograph of liver, with clear portal-portal bridging formation, in both AF/B1 and probiotic groups. H&E X200

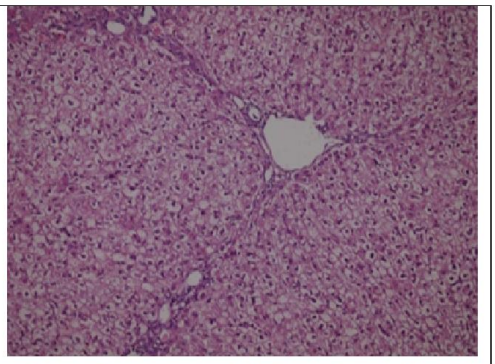

Fig. 6. Photomicrograph of liver, with remetion in portal- portal bridging formation, as detected in NAC treated groups. H&E X200

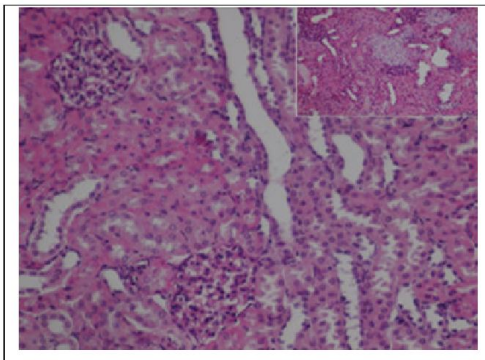

Fig.7. Photomicrograph of kidneys, revealed severe nephrotoxic effect of AF/B1 and protiotic treatments. H&E X200

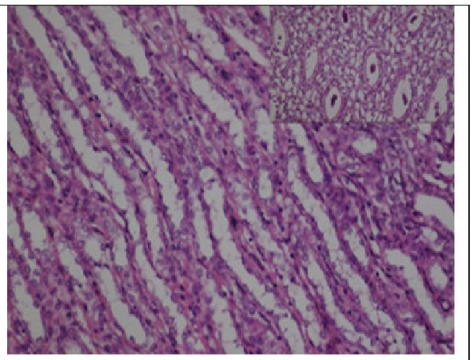

Fig. 8. Photomicrograph of kidneys, revealed severe nephrotoxic effect of NAC at high dose, which appeared in form of dequamation of tubular lining epithelia with cellular cast formation in collecting ducts. H&E X200

Also, it were reported that probiotic had been reduced oxidative stress and inflammatory response (Chen *et al*., 2013b and Zhao *et al*., 2013). Whereas, some authors detected that probiotic had antioxidant activity and protective effect against sperm damage induced by high-fat diet, which bind with microbial toxins (Aluwong *et al*., 2013). In addition, the probiotic could be enhanced immune response and nutrition of host species through the production of digestive enzymes (Abdel-Kader, 2009).Currently, our study detected that the probiotic administration for aflatoxicated rabbits helped the animals to overcome the drastic toxic effects of AFB1on some vital organ as liver, where, it prevented the hepatotoxic effect and inhibition of DNA, RNA and protein synthesis that could be resulted from aflatoxicosis. Similar findings to our work were reported by (Marzouk *et al*., 1994 and Mehrim *et al*., 2009). The probiotic had the ability to enhance the humeral and cellular immunity (Takeshi and James, 2000). Here, in our study, the dietary probiotic detected to have antioxidant activity and had a protective effect against dietary aflatoxin this results agreed with (Chen *et al*., 2013 a) and reduced oxidative stress and inflammatory response (Chen *et al*., 2013 band Zhao *et al*., 2013).

In the present study the histopathological examination of liver and kidney tissues of rabbits in treated groups were investigated (Fig. 1-8). The sensitivity of different tissues to the toxic effect of aflatoxin depend mainly on the bio-distribution of the certain enzymes which responsible for the metabolic transformation of AFs into toxic active intermediate metabolites (Singh and Clausen, 1980). The present recorded criteria are related to the toxic changes of aflatoxicosis and the degree of its mitigation using different investigated therapeutic agents. Aflatoxicosis leads to damage of all component of the cell that includes lipids, proteins and DNA through induction state of oxidative stress (Chandra *et al*., 2015).The ability of aflatoxin to reduce cellular antioxidant capacity induces hepatic and renal lipid peroxidation toxic effect (Hathout *et al*., 2011). Additionally when aflatoxins enter the cell, it binds with DNA and thereby inhibits the action of RNA polymerase, followed by inhibition of mRNA which reflected by marked reduction in the protein synthesis (Rees, 1966), so aflatoxin act as an inhibitor of protein synthesis at some specific stages (Wogan, 1966).

The cytopathic effect of aflatoxicosis occurs in 2 forms, both of necrosis and apoptosis. Apoptosis could be attributed to the ability of AFB1 to increase the expression of pro apoptotic proteins p53 and bax and decreased the expression of bcl2 (Brahmi *et al*., 2011), while necrosis begins when the glutathione stores are almost exhausted as a result of aflatoxin metabolism (Abdel-Wahhab *et al*., 2010).In the present study AFB1 attacks the soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis (Berg *et al*., 2004), that appeared in form of severe vacuolar degeneration, reflect impairment of cellular membranes. Depending on the fact that endonucleases recruited during primary necrosis and introduce nuclear alterations (Lecoeur, 2002), so unhealthy nuclear membrane permit the ethidium bromide stain to pass and stain nuclear material, while intact nuclear membrane execute the ethidium bromide, so the nuclei appeared unstained. We use this modification of viability assay, in our study, to illustrate the pathogenic and the beneficial effects of the AFB1 and the therapeutic agents respectively. The unhealthy nuclear membrane of the aflatoxicated liver showed high incidence of nuclear material staining using ethidium bromide (Fig. 1). Regarding to NAC, as a biological precursor of glutathione, which increase the intracellular GSH and restore its levels following depletion through elevation in the glutathione peroxidase and glutathione reductase activities, total glutathione, and total reactive antioxidant potential levels and cause a reduction in the thiobarbituric acid reactive substances, lipid hydroperoxides, carbonyl protein and hydrogen peroxide levels (Tirouvanzaim, *et al*., 2006). This beneficial effect of NAC was detected as a marked improvement of hepatic features manifested by mitigation of the vacuolar degenerative changes at both low and high doses and nuclear membrane integrity, as detected by ethidium bromide; this is also detected at the same level in probiotic treated group (Fig. 2).

The ability of probiotics, as a natural antioxidant, to compete the aflatoxin pathogenic effect through the immune strengthening effect and protection of lipid and protein from oxidative damage, was investigated. The known immune effect of probiotics was detected in our study in form of marked increase in the number of mutli-nucleated giant cells (Fig. 3) which are the end result of macrophage activation and fusion, these finding was supported by the suspension given by Ouwehand, *et al*. (2002) who explored the immune- modulator effect of probiotics to improve phagocytosis by increasing the number of natural killer cells. Biliary changes which represent the hallmark feature of aflatoxicosis was clearly identified in the present work inform of extensive bile ductules proliferation intermingled with varying degrees of fibrosis (Fig. 4), which in accordance with Bastianello, *et al*. (1987). In addition to portal-potral bridging formation; this is in agreement with Hyde, *et al*. (1977).

The observed portal- portal fibrosis due to effect of AFs was previously explained by the over production of ROS. ROS activate hepatic ito cells proliferation with enhancement of the extracellular matrix production and end result of fibrogenesis (Wu and Zern, 2000). Despite of the coumarin-like anticoagulant effect of AFs, the detected intravascular thrombus formation, in our work, could be the attributed to the release of thromboplastic substances from damaged hepatocytes (Bastianello *et al*.,1987).These numerous thrombus interrupt the blood flow, lead to development of ischemic condition which further activate kupffer cells (KC) and represent another main source for ROS (Selzner *et al*., 2003). In the probiotic treated group there was characteristic feature of fat droplet storage in the hepatocyte stellate cells (ito cells), providing energy required for HSC activation (Hernández-Gea and Friedman, 2012) and proliferation into fibrogenic cell type, as a principle event in liver fibrosis. These enhanced inflammatory and immune-mediated responses can promote hepatocyte necrosis and apoptosis and thereby strengthen and perpetuate the stimuli for fibrogenesis (Cohen-Naftaly*et al*., 2011), that explain the high incidence of fibrogenesis in portal area's and high level of portal-portal bridging formation in this group (Fig. 5). In NAC both of low and high dosed groups, the significant decrease in the incidence of hepatic fibrosis; could

be attributed to the recorded anti-inflammatory effects via inhibition of NF-κB and modulating cytokine synthesis (Berk *et al*., 2014) with subsequent inhibition of hepatic stellate cells proliferation (Takashima *et al*., 2002) (Fig.6).

Regarding to renal changes, despite of nephropathy in aflatoxicosis is less commonly studied (Hayes and Williams, 1978), the increase in urea and createnine level detected by Abdel-Wahhab and Aly (2010), clearly indicated the possible harmful and stressful effects on renal tissue. The stated stressful effects on the renal tissues in our study are in consistent with those reported by Abdel-Wahhab *et al*. (2007) and Abdel-Wahhab and Kholif (2008). Our study detected enlarged hypercellular golermuli, these changes explained by Rubin and Strayer (2008) to an overload on podocytes. So the glomerular injury may be direct due to injury of podocytes or indirect, secondary to tubular necrosis (Kumar*et al*., 2003) (Fig.7). Regarding to renal medulla Arora *et al*.(1978) reported that as the kidneys also excreted AFB1, so the renal medulla was quite sensitive to this mycotoxin. We had detected renal medulla pathogenic effect in form of hyaline cast formation and coagulative necrosis of collecting tubules lining epithelia, while, El- Mahalaway (2015)reported as loughing of epithelial lining of the tubules which resulted in cellular casts. However, we could not detect any improvement of the renal pathological features in probiotic treated group.In our study, there are marked dose-dependent effects of NAC on the renal tissues, which in consistent with Dickey *et al*. (2008) who mentioned that the protective properties of NAC are affected by the dose and route of administration, that later on stated by Sandilands and Bateman. (2009). N-acetylcysteine has proven to be renal protective in experimental models of both toxic, and ischemic acute renal failure (Takhtfooladi *et al*., 2012), using the developed mesangial enlargement and the extensive distal and proximal tubular cells necrosis, sloughing of epithelial cells into the tubular lumen, with cast formation in tubular lumen as a hallomarks, which in consistent with the benefit effects recorded in NAC/low dosed group in the present study.Inthe present study high dose of NAC have been demonstrated to frequently cause nephrotoxic effects, which in agreement with (Sandilands and Bateman; 2009), we also reported cellular cast formation (Fig.8) as a result to lining epithelial damage and desquamations. As NAC up regulate the cellular level of GSH, early hypothesis was given by Monks, *et al*. (1990) who explain that, although GSH protects cells from potentially toxic electrophiles formed via the metabolism of xenobiotics, and such reactions have long been associated with the process of detoxification. The compounds that form GSH conjugates are processed and are usually excreted in urine as their corresponding mercapturic.

However, evidence indicating that GSH conjugation plays an important role in the formation of toxic metabolites from a variety of chemicals has accumulated. Thus, several classes of compounds are converted, via conjugation with GSH, into either cytotoxic, genotoxic, or mutagenic metabolites. The specificity and rate of mercapturic acid de-acetylation determine its toxicity; hence the mercapturate metabolites of aflatoxin may be in relation with their cyto- and nephrotoxicity (Stocker *et al*.,2012). Hence, the use of feed additives of natural origins to avoid the toxic effect of mycotoxins and certain biological preparation as probiotic and N.acetylcysteinhave been successfully used to inhibit mold growth and to reduce the incidence of aflatoxicosis in animals. Nowadays, feed additives may add to feed in very small quantities to improve the growth performance and economic efficiency animal products. Additives is essential for biological functions of the animal including growth promoters, digestion, absorption, antimicrobial agent, metabolic modifiers,probiotic and prophylactics, amelioration the toxic effects of mycotoxins if existed in feed (Namur *et al*.,1998 and Nabawy, 2015). In general, mycotoxicosis can be controlled by improving the methods of harvesting management, storing and transportation of feed in good environmental conditions. The frequent testing program of feed for mycotoxins contamination should be adapted for the control of mycotoxins in the food chain. It was investigated that the potential production of aflatoxins by toxigenic fungi in feed could be expected hazard to animal and poultry health which consumed these feeds (Fraga *et al*., 2008 and Hassan *et al*., 2008; 2010 and 2011).

Conclusion

In this study moulds were recorded to produce aflatoxins caused some degree of acute toxicity when consumed in high amounts and are potential carcinogens in rabbits. In developing countries, it appears that there is a direct correlation between dietary aflatoxin intake and the incidence of liver cancer. It also resulted in significant losses in animal health, which is an important contributor to the country's economy in the form of meat, milk, wool and leather. The results today suggest that N-acetylcysteine may be a potential protective agent for preventing the negative oxidative parameter changes caused aflatoxin B1. In addition, the probiotic enhance the immune response and nutrition of host species through the production of supplemental digestive enzymes. Therefore, frequent testing program of the animal feeds and other environmental factors for fungi and mycotoxin contamination and use of feed additives as probiotic and N.acetylcystein are a critical demand today to safe the animal and human health.

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REFERENCES

Abd El-Kader, M. A.A. 2009. The role of probiotics in the control of some diseases of cultured fresh water fish. MVSC.Thesis, Kafr El-Sheikh University.

Abdel-Wahhab, M. A., Omara, E. A., Abdel-Galil, M.M., Hassan, N. S., Nada, S. A., Saeed, A., and El Sayed, M. M. 2007. Zizyphus Spina-Christi Extract Protects Against Aflatoxin B1-Intitiated Hepatic Carcinogenicity. Afr. J. Tradit. Complement. *Altern. Med*., 4(3): 248–256.

Abdel-Wahhab, M., Ahmed, H. and Hagazi, M. 2006. Prevention of aflatoxin B1-initiated hepatotoxicity in rat by marine algae extracts. *J. Appl. Toxicol*., 26: 229–238.

Abdel-Wahhab, M.A. and Aly, S.E. 2003. Antioxidants and radical scavenging properties of vegetable extracts in rats

fed aflatoxin-contaminated diet. *J Agric Food Chem*., 2003 Apr 9; 51(8):2409-14.

Abdel-Wahhab, M.A. and Aly, S.E. 2010. Antioxidant property of Nigella sativa (black cumin) and Syzygiumaromaticum (clove) in rats during aflatoxicosis. *J. Appl. Toxicol*., 25, 218–223.

Abdel-Wahhab, M.A., Hassan, N. S., El-Kady, A. A., Khadrawy, Y.A., El-Nekeety, A.A., Mohamed, S.R., Sharaf, H.A. and Mannaa, F.A. 2010. Red ginseng extract protects against aflatoxin B1 and fumonisins induced hepatic pre-cancerous lesions in rats". *Food and Chemical Toxicology*, vol.48, no.2, pp.733–742.

Abdel-Wahhab, M.A., and Kholif, A.M. 2008. Mycotoxins in animal feeds and prevention strategies: A review. *Asian Journal of Animal Sciences*, 2(1):7–25.

Adebajo, L.O., Idowu, A.A. and Adesanya, O.O. 1994. Mycoflora and mycotoxins production in Nigerian corn and corn-based snacks. *Mycopathologia*, 126 (3): 183-192.

Aebi, H. 1974. Catalase. In: Bergmeyer, HU (ed.), Methods of Enzymatic Analysis, Chemic Academic Press Inc, Verlag, NY, 2: 673–85.

Ajello, L., R.J. Hay. 1998. Medical Mycology, Vol. 4, 9th Ed. Co-Published in the USA, Oxford University Press, Inc, New York, Lon-don, Sydney, Auckland.

Aluwong, T., Kawu, M., Raji, M., Dzenda, T., Govwang, F., Sinkalu, V. and Ayo, J. 2013. Effect of Yeast Probiotic on Growth, Antioxidant Enzyme Activities and Malon-di-aldehyde Concentration of Broiler Chickens. Antioxidants, 2:326-339.

Ames B.N. 1983. Dietary carcinogens and anti-carcinogens oxygen radicals and degenerative diseases. *Science*, 221:1256–1264.

APHA, 1992. Standard Methods for the Examination of Water and Wastewater.18th edition. American Public Health Association, Washington, D. C.Beach watch.

Arakawa, K. 2007. Studies on infectious and toxic metabolite activities of saprophytic fun-gi in domestic animal breeding environment. Bulletin of Nippon Veterinary and Life Sci-ence University, 56: 75-77.

Arora, R.G., Appelgren, L.E. and Bergman, A. 1978. Distribution of [14C]-labelled aflatoxin B1 in mice. ActaPharmacolToxicol (Copenh). Oct; 43 (4):273-9.

Association Official Analytical Chemists AOAC, 1990. Official Methods of Analysis. 15th Ed., Assoc. of official Analytical chemists, Washington, D. C.

Awaad, M.H.H., Atta, A., Wafaa, A. AbdelGhany, Elmenawy, M., Ahmed, K., Hassan, A.A., Nada, A.A. and Abdelalem 2011. Effect of a Specific Combination of Mannan-Oligosaccharides and β-Glucans Ex-tracted from Yeast Cells Wall on the Health Status and Growth Performance of Ochratoxi-cated Broiler Chickens. *J. of American Sci-ence*, 2011, 7 (3), 82-96.

Azzam, A. H. Gabal, M. A. 1998. Aflatoxin and immunity in layer hens. Avian Pathology. 27: 6, 570-577

Bahtnager, D., Yu, J. and Ehrlich, K. 2002. Toxins of filamentous fungi. In:

Bailey, E.A., Iyer, R.S., Stone, M.P., Harris, T.M., Essigmann JM. 1996. Mutational properties of the primary aflatoxin B1-DNA adduct. Proc. Natl. Acad. Sci. U.S.A.; 93:1535–1539. Application. Churchill Livingstone, Edinburgh, England.

Bancroft, J.D., Stevens, A. and Turner, D.R. 1996. Theory and Practice of Histological Techniques. 4thEd. New York, Churchill, Livingstone.

Barlas A, Cevik H, Arbak S, Bangir D, Sener G, Yeğen C, Yeğen BC. 2004. Melatonin protects against pancreaticobiliary inflammation and associated remote organ injury in rats: role of neutrophils. *J Pineal Res*., 37:267–275.

Bastianello, S.S., Nesbit, J.W., Williams, M.C. and Lange, A.L. 1987. Pathologicafindins in a natural outbreak of aflatoxicosis in dogs. *Onderstepoort J. Vet. Res*., 54: 635-640.

Bauer, J., Montgelas, A.V. and Gedek, B. 1983. Aflatoxin B1 production in presenceof preservatives antimicrobial agents. Proc. Int. symp. Mycotoxins, Cairo, Egypt., PP.249-255.

Bennett, J. W. and Klichm, 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497-505.

Berg, D., Youdim, M.B., Riederer, P. 2004. Redox imbalance. *Cell Tissue Res*., 318: 201– 213.

Berk M, Dean OM, Cotton SM, Jeavons S, Tanious M, Kohlmann K, Hewitt K, Moss K, Allwang C, Schapkaitz I, Robbins J, Cobb H, Ng F, Dodd S, Bush AI, Malhi GS.2014. The efficacy of adjunctive N-acetylcysteine in major depressive disorder: a double-blind, randomized, placebo-controlled trial. *J Clin Psychiatry*, 75(6):628-36.

Brahmi, D.1., Bouaziz, C., Ayed, Y., Ben Mansour, H., Zourgui, L., Bacha, H. 2011. Chemopreventive effect of cactus Opuntiaficusindica on oxidative stress and genotoxicity of aflatoxin B1. NutrMetab (Lond). 2011 Oct 18;8:73.

Breitenbach, M., Crameri, R., Lehrer, S. (Eds.). Fungal Allergy and Pathogenicity Chem. Immunology, 81. Karger, Basel, pp. 167– 206.

Çam,Y., G. Eraslan, 2, A. Atasever, M. Eren, B.C. Liman, N. Şeybek, 2008. Efficacy of N-Acetylcysteine on Aflatoxicosisin Rabbits. *Polish J. of Environ. Stud*., Vol. 17, No. 2, 189-197.

Chandra, K., Salman, A.S., Mohd, A., Sweety,R., Ali, Kh. N. 2015. Protection against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of Costusspeciosus Rhizome Extract. *International Journal of Pharmacognosy and Phytochemical Research*, 7(2); 383-389.

Chen, L., Gong, L. Z. and Xu, J. X. 2013 b. Antioxidative activity and protective effect of probiotics against high-fat diet-induced sperm damage in rats. *Animal*, 7(2):287-92.

Chen, L., Liu, W., Li, Y.,Luo, S., Liu, Q., Zhong, Y., Jian, Z. and Bao, M. 2013 a. Lactobacillus acidophillus ATCC 4356 attenuates the atherosclerotic progression through modulation of oxidative stress and inflammatory prosess. Int. Imunopharmacol.,17(1):108-115.

Chihaya,Y., Furusawa, Y., Okada, H., Matsukawa, K. and Matsui,Y. 1991. Pathological studies on systemic mycoses in calves. *Journal of Veterinary Medical Sci-ence*, 53(6). 1051-1058.

Chowdhury, S. R., and T. K. Smith 2005. Effects of feeding grains naturally contami-nated with Fusarium mycotoxins on hepatic fractional protein synthesis rates of laying hens and turkeys. *Poult.Sci*., 84:1671–1674.

Chu, F. S. and Li, G. Y. 1994. Simultaneous occurrence of fumonisin B1 and other myco-toxins in moldy corn

collected from the people's of republic of China in regions with high incidence of oesophageal cancer. *Appl. Environ. Microbiol*., 60, 847-852.

Chu, Q., Wu, W. D., Cook, M. E. and Smally, E. B. 1995. Induction of tibial dyschondroplasia and of cell mediated immunity in chickens by F. oxysporum grown on sterile corn. Avian Dis. (USA), 39 (1): 100-107.

Cohen-Naftaly, M, Friedman, S.L.2011. Current status of novel antifibrotic therapies in patients with chronic liver disease. *Therap. Adv. Gastroenterol*., 4:391–417.

Dabrowski, A., Konturek, S.J., Konturek, J.W., Gabryelewicz, A. 1999. Role of oxidative stress in the pathogenesis of caerulein-induced acute pancreatitis. *Eur J Pharmacol*., 377:1–11.

Davis, B. 1964. Disk electrophoresis. II Method and application to human serum protein. *Ann. N.Y. Acad. Sci*., 121: 404-427.

Dickey, D.T.1, Muldoon, L.L., Doolittle, N.D., Peterson, D.R., Kraemer, D.F., Neuwelt, E.A. 2008. Effect of N-acetylcysteine route of administration on chemoprotection against cisplatin-induced toxicity in rat models. CancerChemother. Pharmacol. Jul; 62(2):235-41.

El- Ahl, Rasha, H. Sayed, Refai, M.K. and Hassan, A.A. 2006. "Prevalence of fungiandtoxigenicity of A. flavusand A. ochraceusisolated from single and compoundfeed with particular references to the elimination of these contaminants." *Egypt. J. Agric. Res*., 86: 500- 510. (10).

Ellman, G. 1959. Tissuesulphydrylgroups. Arch Biochem. Biophys.; 82: 70–73

El-Mahalaway, A.M. 2015. Protective effect of curcumin against experimentally induced aflatoxicosis on the renal cortex of adult male albino rats: a histological and immunohisochemical study. *Int J Clin. Exp. Pathol*., Jun 1; 8(6):6019-30.

Eşrefoğlu, M., Gül, M., Ateş, B., Batçıoğlu, K., Selimoğlu, M.A. 2006. Antioxidative effect of melatonin, ascorbic acid and N –acetylcysteine on caerulein-induced pancreatitis and associated liver injury in rats. *World J Gastroenterol.*, 12(2):259-264.

F.D.A., Food and Drug Administration 1994. Action levels for poisonous or delete-rious substances in human food and animal feed. Washington, Department of Health and Human Services, 1125-126.

Flanaganr, J. and Merditht, J. 1991. Use of n-acetylcysteinein clinical toxicology. *Am. J. Med*., 91, 131-140.

Food and Agriculture Organization of the United Nations FAO, 2001. Manual on the Application of the HACCP System in Mycotoxin Prevention and Control; FAO: Rome, Italy.

Food and Drug Administration (FDA) 2000. Conference on mycotoxins in animalfeeds, grains and food related to human and animal health. Rockville, Maryland.

Fraga, M. E., Direito, G. M., Gatti, M. J., Moraes, A. M. L., Cavaglieri, L. R., Dalcero, A. M., Rosa, C. A. da R. 2008. Revaluation of aflatoxin production by Aspergillus candidus and Eurotiumchevalieri isolated from poultry feed in Brazil. RevistaBrasileira de MedicinaVeterinaria. 30: 2, 86-90.

Girish, C. K. and Smith, T. K. 2008. Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on small intestinal morphology of Turkeys. *Poultry Science*, 87: 6, 1075-1082

Girish, C. K., Smith, T. K., Boermans, H. J., Kumar, P. A., Girgis, G. N. 2010. Effects of dietary Fusarium mycotoxins on intestinal lymphocyte subset populations, cell proliferation and histological changes in avian lymphoid organs. *Food and Chemical Toxicology*, 48: 10, 3000-3007.

Halliwell, B. 1996. Antioxidants in human health and disease. *Ann Rev Nutr*., 16:33-50.

Hamzawy, M. A., El-Denshary, E. S., Hassan, N. S., Mannaa, F. A. and Abdel-Wahhab, M. A. 2012. "Antioxidant and hepatorenoprotective effect of thyme vulgaris extract in rats during aflatoxicosis," *Global Journal Pharmacology*, vol. 6, no. 2, pp. 106–117, 2012.

Hamzawy, M.A., El-Denshary, E.S., Hassan, N.S., Mannaa, F.A. and Abdel-Wahhab, M.A. 2013. Dietary Supplementation of Calendula officinalis Counteracts the Oxidative Stress and Liver Damage Resulted from Aflatoxin. ISRN Nutr. 2013 Feb 12: 538427.

Hassan, A, A., Rashid M.A. and KoratumKh. M. 2008. Measurement of mycotoxins in feeds and sera of cattle and sheep and eval-uation of its effect on some fertility related hormones in male rats. *Egypt. J. Comp. Path. & Clinic. Path*., 21:340-358

Hassan, A. A. 1998. Mycosis in turkeys. 5th Scientific Congress proceeding, Fac. of Vet. Med., Cairo University, *Vet. Med. J. Giza*, 46(4B). 857-865.

Hassan, A.A., El-Barawy, A.M. and El-Mokhtar, M., Nahed 2011. Evaluation of biological compound of streptomyces species for control of some fungal diseases. *J. of American Science*, 7 (4), 752-760.

Hassan, A.A., Howayda, M. El Shafei, Noha, H. Oraby, Rasha, M.H. Sayed El Ahland Mogeda, K. Mansour 2012. Studies on mycosis and mycotoxicosis in cattle. First Conf. of An. Health Res. Inst. Assoc., December 2012. pp. 216 – 227.

Hassan, A.A., Noha, H. Oraby, Aliaa A. E. Mohamed and Mahmoud, H.H. 2014. The possibility of using Zinc Oxide nanoparticles in controlling some fungal and bacterial strains isolated from buffaloes. *Egypt. J. of Appl. Sci*., 29 (3) 2014, 58-83

Hassan, A.A., Wael, M. Tawakkol and Elbrawy, A.M. 2010. The hepato protective effect of dimethyl 4,4-dimethoxy 5,6,5,6-dimethylene dioxy-biphenyl- dicarbxylate (D.D.B.) against liver injury induced by aflatoxin B1 in rates. *Int. J. Life Sciences*, 7: 148-153.

Hathout, A.S., Mohamed, S.R., El-Nekeety, A.A., Hassan, N.S.; Aly, S.E. and Abdel-Wahhab, M.A. 2011. Ability of Lactobacillus casei and Lactobacillus reuteri to protect against oxidative stress in rats fed aflatoxins-contaminated diet. Toxicon. Aug; 58(2):179-86.

Hayes, A.M. and Williams, W.L. 1978. Acute toxicity of aflatoxin B1 and rubratoxin B in dogs. *J. Environ. Pathol. Toxicol*., 1: 59-70.

Henry, R.J. (1974): "Clinical chemistry, principles and techniques." 2ndEd., Harport and Rowhogerstown, M.D. 862.

Hernández-Gea, V. and Friedman, S.L. 2012. Autophagy fuels tissue fibrogenesis. Autophagy. (May 1; 8(5): 849–850.

Hyde, W., Kiessy, J., Ross, P.F. and Stahr, H.M. 1977. General gas chromatography. Analytical methods of toxicology, pp.: 104-106. John Wiley and Sons Inc., New York.

James, L.P., McCullough, S.S., Lamps, L.W. and Hinson, J.A. 2003. Effect of N-acetylcysteine on acetaminophen toxicity in mice: relationship to reactive nitrogen and cytokine formation. *Toxicol Sci*., 75(2):458-67.

Jindal, N.; S. K. Mahipal, and Mahajan, N. K. 1994. "Toxicity of aflatoxin B1 in broiler chicks and its reduction by activated charcoal," *Research in Veterinary Science*, vol. 56, no. 1, pp. 37– 40.

Jungermann J, Lerch MM, Weidenbach H, Lutz MP, Krüger B, Adler G. 1995. Disassembly of rat pancreatic acinar cell cytoskeleton during supramaximal secretagogue stimulation. *Am J Physiol*., 268:G328–G338.

Kelly, G. S. 1998. Clinical applications of n-acetylcysteine. AlternativeMed. Rev. 3, 114.

Kanbur, M.; Eraslan, G.; Sarica, Z. S and Aslan, O. 2011. "The effects of evening primrose oil on lipid peroxidation induced by subacute aflatoxin exposure in mice," *Food and Chemical Toxicology*, vol. 49, no. 9, pp. 1960–1964.

Kaneko, J. Jerry; Harvey, J.W. and Bruss, M.L. 1997. Clinical biochemistry of domestic animals, fifth Ed., academic press.

Kayas, 1998. Mikotoksinlervemikotoksinzehirlenmeleri. In: Veteriner Hekimliginde Toksikoloji KAYA S., PIRINÇÇI I.,BILG ILI A. (eds.), I. Baski, Medisan, Ankara. pp. 341-375.

Kubena, L.F., Edrington, S., Kamps,C., Harvery, R.B., Eliscalde, M.H. and Rattingt, G.E. 1995. Influence of FBI present in F. monillform culture material and T-2 toxin in turkey poults. *Poult. Sci*., 74:306-313

Kumar, V., Cortan, R.S., Robbins, S.L. 2003. Robbins basic pathology.7th edition. WB Saunders Company. pp. S16–S32.

Lecoeur, H. 2002. Nuclear apoptosis detection by flow cytometry: influence of endogenous endonucleases. *Exp Cell Res*. Jul 1; 277(1): 1-14.

Lee, JK.; Chang, HJ. and Chun, HS. 2010. Protective effect of volatile extract from Pinusdensiflora against aflatoxin B1-induced oxidative Stress in HepG2 Cells. *Food Sci. Biotechnol*., 19: 229-233.

Manal, A. Hassan; Howayda , M. El Shafei; Noha, H. Oraby; Maha , El Kholy and Ragaa, Abdel Sattar 2012. Efficiency of use of yeast extract and acetic acid in detoxifications of aflatoxin contaminated chicken feeds. New Yourk Science J. VOL.5, N. 6. 216-225.

Marklund S. and Marklund G. 1974. Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*., 47(3):469–474.

Mary V.S., Theumer M.G., Arias S.L., Rubinstein H.R. 2012. Reactive oxygen species sources and biomolecular oxidative damage induced by aflatoxin B1and fumonisin b1 in rat spleen mononuclear cells. *Toxicology*, 302:299–307.

Marzouk, M.S., M.M. Bashandi, R. El-Banna, M. Moustafa and M.A. Eissa (1994): Hematological studies on Oreochromisniloticus exposed to chronic dietary aflatoxicosis. Egypt. *J. Comp. Pathol. Clin. Pathol*., 7: 497-504.

Matur, E., Ergul, E., Akyazi, I., Eraslan, E., Cirakli, Z.T. 2010. The effects of Saccharomyces cerevisiae extract on the weight of some organs, liver, and pancreatic digestive enzyme activity in breeder hens fed diets contaminated with aflatoxins. *Poult. Sci*., 89, 2213–2220.

Mehrim, A.I. 2009. Effect of dietary supplementation of Biogen® (Commercial probiotic) on mono-sex Nile tilapia Oreochromisniloticusunder different stocking densities. *J. Fish. Aquat. Sci*., 4: 261-273.

Meissonnier G.M., Pinton P., Laffitte J., Cossalter A.-M., Gong Y.Y., Wild C.P., Bertin G., Galtier P., Oswald I.P. 2008. Immunotoxicity of aflatoxin B1: Impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. *Toxicol. Appl. Pharmacol*., 231:142–149.

Mikulec, S.D., Stojanović, L. and Jovanović, L. 2005. Moulds associated with milk de-pending on Macroclimate and geographical location. *Applied Ecology and Environmental Research*, 3(2). 61-65.

Mogda, K. Mansour, Hanan, A. Tag El- Din, Safaa, M. Abou El Soud and Abo Norag M, A. 2014. A trial to ameliorate the toxic effect of Aflatoxin in rabbits. *J. Vet. Sci*., 15-25.

Mogda, K. Mansour, Hassan, A.A. andRashed M.A. 2002. The fungi recorded in imported feed samples with reference to control of T-2 toxicosis by antioxidant substances in chicks. *Vet. Med. J., Giza*, 50 (4). 485-499.

Moldeus, P andCotgreave, I.A. 1994. Nacetylcysteine. *Methods Enzymol*., 234: 482–492.

Monks, T.J.1, Anders, M.W., Dekant, W., Stevens, J.L., Lau, S.S., van Bladeren, P.J. 1990. Glutathione conjugate mediated toxicities. *Toxicol. Appl. Pharmacol*., Oct; 106(1):1-19.

Moss, D.W. and Henderson, E.R. 1999. Digestive enzyme of pancreatic organ.Tietztext book of clinical chemistry 3rd Ed p. 689-708.

Moubasher, A.H. 1995. Soil fungi are an active patner of our ecosystem. Their biodi-versity and activities should be appreciated. Qatar Univ. Sci. J., 15:239-247

Nabawy, Gehan A., Hassan, A. A., El- Ahl, Rasha, H. S. and Refai, M. K. 2014. Effect of metal nanoparticles in comparison with Commercial antifungal feed additives on the growth of Aspergillus flavus and aflatoxin b1 production. *Journal of Global Biosciences*, Volume 3, Number 6, 2014, pp. 954-971

Nabawy, Gehan, A. 2015. Effect of metal element nanoparticles in the growth of aflatoxogenicA. flavusand aflatoxin production in feed, Master thesis in veterinary science (Microbiology), Faculty of Veterinary Medicine, Cairo University.

Namur, A.P., J.Morel and H.Bichek 1998. Compound animal feed and feed additives.InDebour, F.H.Bichel, Livestock feed resources and feed evaluation in Europe. Elsevier. Sci.Publ.Amsterdam

Nayak, S and Sashidhar, R.B. 2010. Metabolic intervention of aflatoxin B1 toxicity by curcumin. *J. Ethnopharmacol*., 17; 127 (3):641-4.

Nishikimi, M., Roa, N.A. and Yogi, K. 1972. The occurance of super oxid anion in the reaction of reduced phenazinemethosulphate and molecular oxygen. *Biochem. Bioph. Res. Common*., 46:849 – 854.

Osborne, D. J., Huff, W. E., Hamilton, P. B. and Burmeister, H. R. 1982. "Comparison of ochratoxin, aflatoxin, and T-2 toxin for their effects on selected parameters related to digestion and evidence for specific metabolism of

carotenoids in chickens," *Poultry Science*, vol. 61, no. 8, pp. 1646–1652.

Osborne, D.J.; Hamilton, P.B. 1981. Decreased pancreatic digestive enzymes during aflatoxicosis. *Poult. Sci*., 60, 1818–1821.

Ouwehand, A.C.,Salminen, S. andIsolauri, E. 2002. Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek. Aug; 82(1-4):279-89.

Paglia, D.E. and Valentine W.N. 1967. Determination of GSH-px J. Lab. Clin.Med.70: 158-169.

Pestka, J.J., Zhou, Y. Moon and Chung 2004. Cellular and molecular mechanisms for immune modulation by deoxynivalenon and other trichothecenes: Unraveling a paradox. *Toxicol. Lett*., 153:61–73.

Pitt, J.I. and Hocking, A.D. 2009. Fungi and food Spoilage Book. 3rd Ed., Chapter 4-10, pp. 29. Academic Press, New York, London, Toronto, Montreal, Tokyo.

Poli, G., Leonarduzzi, F., Biasi, E. 2004. Chiarpotto Oxidative stress and cell signalling. Curr Med Chem,11:1163-82.

Prasath, N. B., Rao, G. V. S.; Balachandran, C.; Raj, G. D.; Manohar, B. M. 2010. T-2 toxin induced apoptosis in thymus and spleen of turkey poults. *Indian Veterinary Journal*, 87(5): 499-500.

Preston, R.J. and Williams, G.M. 2005. DNA-reactive carcinogens: mode of action and human cancer hazard. Criterion Rev.

Pusterla, N., Ossent, P., Braun, U. 1996. A case of acute disseminated mycotic pneumo-nia in a cow. Schweizer-Archiv-fur-Tierhei-lkunde., 138(4). 189-194.

Rawal, S, Yip, S.S., and Coulombe, R.A. Jr 2010. Cloning, expression and functional characterization of cytochrome P450 3A37 from turkey liver with high aflatoxin B1 epoxidation activity. *Chem Res Toxicol*., 16; 23(8):1322-9.

Rees, K.R. 1966. Plant toxins and human disease. The mechanism of action of aflatoxin in producing acute liver necrosis. *Proc R Soc Med*., Aug; 59(8):755-7.

Refai, M.K. and Hassan, A.A. 2013. Monograph on Mycotoxigenic Fungi and Mycotoxins in food and feeds with synopsis of the authours done on Mycotoxigenic Fungi and Mycotoxins in Foods and Feeds. http:// Cairo academic edu./Egypt, Atefhassan/ Monograph.

Reitman, S. and Frankel, S. 1957. "Acolorimetric determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminase." *Am. J. Clin. Path*., 28: 56-58.

Richard, J.L. 2007.Some major mycotoxins and their mycotoxicoses—An overview. *Int. J. Food Microbiol*., 119:3–10

Richardson, K.E., Hamilton, P.B. 1987. Enhanced production of pancreatic digestive enzymes during aflatoxicosis in egg-type chickens. *Poult. Sci*., 66, 640–644.

Rotter, B.A., Prelusky, D.B., and Pestka, J.J. 1996. Toxicology of deoxynivalenol (vomitoxin). *J Toxicol Environ Health*, 48: 1-34.

Rubin, R., Strayer, D.S. 2008. Clinicopathologic foundations of medicine.5th edition. Baltimore, MD: Wolters Kluwer Health; Lippincott Williams & Wilkins; 2008. Rubin pathology; pp. 702–705.

Sandilands, E.A, and Bateman, D.N. 2009. Adverse reactions associated with acetylcysteine. ClinToxicol (Phila). Feb; 47(2):81-88.

Sayin, V.I., Ibrahim, M.X., Larsson, E., Nilsson, J.A., Lindahl, P, Bergo, M.O. 2014. "Antioxidants accelerate lung cancer progression in mice". *SciTransl Med*., 6 (221): 221ra15

Selzner, N., Rudiger, H., Graf, R. and Clavien, P.A. 2003. Protective strategies against ischemic injury of the liver. *Gastroenterology*; 125: 917-936.

Shen, H.,Liu, J., Wang, Y.,Lian, H., Wang, J., Xing, L., Yan, X.,Wang, J. andZhang, X. 2013. Aflatoxin G1-induced oxidative stress causes DNA damage and triggers apoptosis through MAPK signaling pathway in A549 cells. *Food Chem. Toxicol*., Dec; 62:661-669.

Singh, N. and Clausen, J. 1980. Different tissue response of mixed function oxidases and detoxifying enzymes to aflatoxin B1administrated in the rat. *Br. J. exp. Path*., 61, 611.

Skrzydlewska, E. andFarbiszewski, R. 2004. Protective effect of Nacetylcysteine on reduced glutathione, reduced glutathione-related enzymes and lipid peroxidation in methanol intoxication. Drug and Alcohol Dependence, Volume 57, Issue 1, 1 November 1999, Pages 61-67.

Smith, J.E. 1997. Aflatoxins. In Handbook plant and fungal toxicant." Edited by J.P. Fle-xinx, D'Mello, CR (Press, Boca Raton), New York, pp., 269-285.

SonnenWirth, A. and Jareet, L. 1980. "Garduals Clinical Laboratory Methods and Diagnosis ."Vol.1,8th Ed., Mosby.

SPSS 14 2006. "Statistical Package for Social Science, SPSS for windows Release 14.0.0, and 12 June, 2006." Standard Version, Copyright SPSS Inc., 1989-2006, All Rights Reserved, Copyright ® SPSS Inc

Stocker, P., Brunel, J.M., de Rezende, L., do Amaral, A.T., Morelli, X., Roche, P., Vidal, N., Giardina, T. and Perrier, J. 2012. Aminoacylase 1-catalysed deacetylation of bioactives epoxides mycotoxin-derived mercapturates; 3,4-epoxyprecocenes as models of cytotoxic epoxides. Biochimie. Aug; 94(8):1668-1675.

Sun, Y., Park, I., Guo, J., Alexandra C., and Kim, S. 2015. Impacts of low level aflatoxin in feed and the use of modified yeast cell wall extract on growth and health of nursery pigs. *Animal Nutrition*, 1-7.

Takashima, T., Kawada, N., Maeda, N., Okuyama, H., Uyama, N., Seki, S. Arakawa, T. 2002. Pepstatin A attenuates the inhibitory effect of N-acetyl-L-cysteine on proliferation of hepatic myofibroblasts (stellate cells). *Eur J Pharmacol*., 2002 Sep 20; 451(3):265-70.

Takeshi, M. and James, C. 2000. Modulating immune responses with probiotic bacteriaImmunology and Cell Biology, 78: 67–73.

Takhtfooladi, M.A., Jahanshahi, A., Jahanshahi, G., Sotoudeh, A, Takhtfooladi, H.A., Khansari, M. 2012. Protective effect of N-acetylcysteine on kidney as a remote organ after skeletal muscle ischemia-reperfusion. Acta Cir Bras. Sep; 27(9):611-615.

Tietz, N. W. 1996. Fundamentals of clinical chemistry 4th Ed., Vol 9, (Moss, D.W and Hendersson, A. R.) W. B. Saunders Company.

Tirouvanziam, R., Conrad, C.K., Bottiglieri, T., Herzenberg, L.A., Moss, R.B. and Herzenberg, L.A. 2006. High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. PNAS, March 21, vol 103, No. 12: 4628- 4633.

Tylicki, L., Rutkowski, B., Horl, W.H. 2003. Antioxidants: a possible role in kidney protection. Kidney Blood Press Res 26: 303–314.

Valdivia, A. G., Martineza, Damianf J. Quezada, T., Ortizr, Martinezc, Liamas, J., Rodriguezm, L., Yamamotal, Jaramillof, Loarca Pinam G., Reyesj, L. 2001. Efficacy of n-acetylcysteineto reduce the effects of aflatoxin B1 intoxication inbroiler chickens. Poultry Sci., 80, 727.

Van Kampen, E.J., and Zijlstra, W.G. 1965. Determination of hemoglobin and its derivatives. *Adv. Clin. Chem*., 8:140–187.

Wang, C. Xia,Y., Zheng, Y., Dai, W., Wang, E.,Chen, K., Li, J., Li, S., and Zhu, R. 2015. Protective Effects of N-Acetylcysteine in Concanavalin A-Induced Hepatitis in Mice. Mediators of Inflammation. 1-17.

WHO Technical Report series 2002. Evaluation of certain mycotoxins in food fifty sixth report of the joint FAO/WHO Expert Committee on food additive" Geneva. pp. 11, 14, 28, 30 and 68.

Wogan, G.N. 1966. Chemical nature and biological effects of the aflatoxins. Bacteriological Reviews, June, Vol. 30, No.2: 460-468.

Wu, J. and Zern, M.A. 2000. Hepatic stellate cells: a target for the treatment of liver fibrosis. J. Gastroent.; 35: 665-671.

Wybenga, D.R., Digigorgio, J. and Piliggi, V.J. 1971. "Automated method for urea measurement in serum." Clin. Chem., 97: 891-903.

Yang, R., Miki, K., He, X., Meaghan E Killeen and Fink, M. 2009. Prolonged treatment with N-acetylcystine delays liver recovery from acetaminophen hepatotoxicity. Critical Care Vol 13 No 2, 1-7.

Yunus, W., Fazeli, E. and Bohm, J. 2011. Aflatoxin B1 in Affecting Broiler's Performance, Immunity, and Gastrointestinal Tract: A Review of History and Contemporary Issues Toxins, 3, 566-590.

Zafarullah, M., Li, W.Q., Sylvester, J., Ahmad, M. 2003. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci.; 60:6–20.

Zhao, Y., Zhao, L., Zheng, X., Fu, T., Guo, H. and Ren, F. 2013. Lactobacillus salivarius strain FD 889 induced longevity in caenorhabditiselegans by dietary restriction. *J. Microbiol*., 51(2):183-8.

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