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RESEARCH PAPER

Impact of Garlic and Curcumin on the Hepatic Histology and Cytochrome P450 Gene Expression of Aflatoxicosis *Oreochromis niloticus* Using RT-PCR

Manal I. El-Barbary[1,*]

[1] Fish Diseases Lab., National Institute of Oceanography and Fisheries, Egypt.

* Corresponding Author: Tel.: +90.544 5679008
E-mail: manal278@yahoo.com

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Abstract

Aflatoxin B_1 is one of the most important mycotoxins due to its hepatotoxic and carcinogenic effects on some fishes**.** This study was conducted to investigate the defensive role of curcumin and garlic on phase I of the biotransformation system in the liver of aflatoxicosis in *Oreochromis niloticus* in relation to the therapeutic properties by studying hepatic histopathology and cytochrome P450-CYP1A gene expression using real-time polymerase chain reaction (RT-PCR). Although varieties of methods have been used for gene expression quantification and are still used today, real-time RT-PCR method has achieved prominence in recent years. One hundred and eighty (180) fish were equally divided into 10 groups. T_1 was the negative control; fish in T_2 were injected intraperitonealy (IP) with AFB_1 at a rate of 6 mg kg^{-1} B.W. and fed on a basal diet. Groups T_3-T_6 were fed with garlic (T_3 & T_4) and curcumin (T_5 & T_6) at 10 & 20 g kg^{-1} diet, respectively. Groups T_7-T_{10} injected IP with AFB_1 and fed on both garlic and curcumin at the different concentrations (10 & 20 g kg^{-1} diet). AFB_1 reduced the HSI values in aflatoxicosis fish. Feeding a low dose of garlic led to significant improvement in the HSI values. Histologically, hepatic lesions were observed in the AFB_1 injected groups; damage was reduced when fish were fed on garlic and curcumin diets at a concentration 10 g kg^{-1}. The highest induction of CYP1A expression was observed in the curcumin groups followed by AFB_1. The over expression of CYP1A in T_2 was alleviated by the garlic. Therefore, it was found that garlic enhances the detoxification of AFB_1 through the suppression of CYP1A in the liver.

Keywords: Aflatoxin B_1, garlic, curcumin, cytochrome CYP1A, gene expression, Histopathology.

Introduction

One of the fastest growing worldwide food sectors is the aquaculture industry (Naylor *et al*., 2000; Subasinghe, 2005). *Oreochromis niloticus* has become the third most important fish in aquaculture, **it** has been introduced in more than 100 tropical and subtropical countries, to improve fishing productivity and facilitate the development of aquaculture (Leveque, 2002) Rapid growth rate, high resistance disease, hatching throughout the year and excellent flavour, make Nile tilapia breeding increasingly important in the world (Melo *et al*., 2006)

The major difficulty confronting the aquaculture industry in fish feeding is the occurrence of Aflatoxin B_1 (AFB_1) due to its hepatotoxic and carcinogenic properties (Wild & Turner, 2002; Williams *et al*., 2004). The International Agency for Research on Cancer classified AFB_1 as a Group I carcinogen (IARC, 1985). Aflatoxin B_1 is produced by some fungi, such as *Aspergillus flavus* and *Aspergillus parasiticus*, under ideal temperatures and humidities (Sargeant, Sheridan, O'Kelly, & Carnaghan, 1961). Aflatoxin B_1 causes reduction in body weight, behavioral abnormalities, depressed immune response, necrotic hepatocytes and other unfavorable impacts in aflatoxicated fish (Deng *et al*., 2010; Farabi,.Yousefian, & Hajimoradloo, 2006). In addition, AFB_1 has been shown to cause immunosuppressive, teratogenic and mutagenic effects in laboratory and farm animals (IPCS-WHO, 1998). Aflatoxin B_1 is also able to induce reactive oxygen species (ROS) (Adedara, Owumi, Uwaifo, & Farombi, 2010; Matur *et al*., 2011), perhaps requiring the activation of cytochrome P450. As mentioned by Santacroce *et al*. (2008), the target organ for aflatoxicosis is the liver. In the latter, aflatoxin metabolites negatively react with various cell proteins, inducing necrosis and tumor or cell death (Joner, 2000).

The toxicology of AFB_1 in aflatoxicosis, involves its biotransformation to the highly reactive AFB_1-epoxide by the CYP 450 (Guengerich *et al*., 1998), leading to carcinogenesis and mutagenesis by forming adducts with DNA (Theumer, Lopez, Masih, Chulze, & Rubinstein, 2003; Madhusudhanan, Kavithalakshmi, Radha, Shanmugasundaram, & Shanmugasundaram, 2004). In the liver, phase I biotransformation reactions are responsible for facilitating bioactivation whereas phase II

biotransformation reactions are responsible for detoxification and excretion. The pathway of biotransformation is mediated by phase I and phase II (conjugation), or a combination of both (Brandon, Raap, Meijerman, Beijnen, & Schellens, 2003). To reduce the negative effects of aflatoxicosis in fish, many plants, such as garlic and curcumin which have antioxidant properties have been evaluated (Carmia, 2001; Diab, El-Nagar, Abd-El-Hady, 2002; Chattopadhyay, Biswas, Bandyopadhyay, & Banerjee, 2004).

Garlic has shown to possess important antioxidant properties through its ability to scavenge free radicals (Carmia, 2001), inhibit lipid peroxidation and reduce oxidative stress and mutations (Shaarawy *et al*., 2009). Moreover, curcumin plays a vital role in protecting cells from oxidative stress and the resulting damage to DNA (Balu, Sangeetha, Murali, & Panneerselvam, 2006) and, it also inhibits the production of AFB_1-epoxide (Firozi, Aboobaker, & Bhattacharya,.1996) by significantly reducing CYP1A1 activity and enhancing glutathione activity to ameliorate the effects of AFB_1 (Sujatha & Sashidhar, 2010).

Curcumin has been shown to have protective effects on AFB_1-induced liver toxicity in Nile tilapia by reduced expression of CYP1A and decreased AFB_1 biotransformation (Mahfouz, 2015). The over expression in CYP1A1 and CYP2H1 was also shown to be alleviated by the supplementation of curcumin to the AFB_1 diet, which enhanced the detoxification capacity of the organism (Yarru *et al*., 2009).

The chemopreventive effect of garlic and curcumin on phase II detoxification enzymes of *O. niloticus* was studied (El-Barbary, 2016) and concluded that addition of garlic to the diet improve the detoxification of AFB_1 through the stimulation of glutathione peroxidase (GPx) in the liver. Therefore, the current study was conducted to study the effects of AFB_1 on the liver of Nile tilapia, *O. niloticus*, and to evaluate the curative and defensive role of garlic and curcumin on the phase I biotransformation system by studying cytochrome P450-CYP1A gene expression.

Materials and Methods

Preparation of Aflatoxin B_1.

Aflatoxin B_1 was produced by *A. parasiticus* (NRRL. 2999) in potato dextrose liquid medium according Reddy, Viswanathan, & Venkitasubramanian (1971). Aflatoxin B_1 was quantitatively estimated by thin layer chromatography, (AOAC, 2000). Dimethylsulphoxide (DMSO 25%) was used to dissolve the AFB_1 to the appropriate concentration before injection.

Garlic and Curcumin Antioxidants and Diets

Curcumin powder was purchased from El-Doha for food stuff Co., Cairo, Egypt. While natural garlic *Allium sativum*, was dried and ground to become a powder. The selection of the concentrations of curcumin and garlic was based on previous studies performed by other researchers; Metwally (2009) fed fish on diets containing natural garlic (40 g/kg diet), Shalaby *et al*. (2006) used different levels of *Allium sativum* 10, 20, 30, and 40 g/kg diet of Nile Tilapia while Sujatha & Sashidhar (2010) used curcumin at dose 0.05%, w/w, 5g/kg diet against AFB_1. Experimental diets were prepared by individually mixing garlic and curcumin at two levels (10 and 20 g/kg diet) in corn-oil,obtained from supermarket, and adding this to the commercial fish diet, basal diet, (B.D) of 30% protein to result in the final concentration of (1 and 2%, respectively).

Experimental Design

One hundred and eighty *O. niloticus* fingerlings with an average body weight of 40±0.2 g were acclimated to aquaria and fed a control diet for 2 weeks. Fish were equally divided into 10 experimental groups (T_1-T_{10}) and each group contained eighteen fish which were stocked in three glass aquaria (70X40X30 cm). All aquaria were aerated with air stones; the fish were fed the test diets twice daily at a feeding rate of 3% of body weight. Aflatoxin B_1 (6 mg/ kg body weight) (1/6 of the LC50, according to El-Barbary (2008) was injected intraperitoneally in a single dose in aflatoxicated groups (T_2, T_7- T_{10}). Before injection, fish were anesthetized in a clove oil solution (Hamackova, Kouril, Kozak, & Stupka, 2006). Dietary and treatment groups are provide in Table 1

Hepatosomatic Index (HSI) of *O. niloticus*

At the end of the experiment (14 days), three fish were selected randomly from each group; the body and liver weight of each fish were recorded to calculate the hepatosomatic index. HSI = liver weight (g)/body weight (g) x 100 (White & Fletcher, 1985).

Histological Examination

The histological examination of Bouin's fluid-fixed liver (three individuals per group) was performed (Bernet, Schmidt, Meier, Brkhardt-Holm, & Wahli, 1999), and photographed using a ICC50 HD camera and a Leica LAS EZ microscope.

Liver sampling, RNA isolation and cDNA synthesis

At the end of the experiment, three samples of liver from each group were isolated surgically and frozen instantly in liquid nitrogen (-80ºC) until gene expression analysis. Total RNA was isolated from homogenized liver (Chomczynski, & Sacchi, 1987;

Table 1. Experimental design

Group	Aflatoxin B1 (AFB_1)	Diet
T_1	DMSO (25%)	Basal Diet
T_2	AFB1+ DMSO (25%)	Basal Diet
T_3	DMSO (25%)	Basal Diet + garlic 10 g/kg
T_4	DMSO (25%)	Basal Diet + garlic 20 g/kg
T_5	DMSO (25%)	Basal Diet + curcumin 10 g/kg
T_6	DMOS (25%)	Basal Diet + curcumin 20 g/kg
T_7	AFB1 + DMSO (25%)	Basal Diet + garlic 10 g/kg
T_8	AFB1 + DMSO (25%)	Basal Diet + garlic 20 g/kg
T_9	AFB1 + DMSO (25%)	Basal Diet + curcumin 10 g/kg
T_{10}	AFB1 + DMSO (25%)	Basal Diet + curcumin 20 g/kg

Boom *et al*., 1990) using the GeneJET™ RNA Purification Kit. For real time PCR reactions, total cDNA was generated using Revert Aid First Strand cDNA Synthesis Kit as (Wiame, Remy, Swennen, & Sagi, 2000).

Primer Design and Quantitative Real Time PCR

The level of CYP1A expression was assessed in groups T_1, T_2, T_3, T_5, T_7 and T_9, using RT- PCR. All PCR reactions were performed in a Veriti® 96- Well Thermal Cycler (Applied Biosystemem Catalog Number 4479071). These were.performed using 10µl of Maxima SYBR Green/ROX qPCR Master Mix (2x) (Thermo Scientific #K0223). The PCR reaction mix was denatured at 95°C for 10 min before the first PCR cycle. The thermal cycle profile steps were denaturation, annealing, extension and 40 PCR cycles were used for amplification of the samples. The primer sequences of the housekeeping gene (β- actin) were used as an internal control for normalization and CYP1A gene and their accession numbers are shown in Table 2. For each group, the expressions of CYP1A target gene were analyzed using Mx3000P real-time PCR system (Stratagene, La Jolla, CA, USA).

Statistical Analysis

One-way analysis of variance was used to compare the means of different groups using the SAS statistical software package (SAS, 1996). When the F-test was positive, the least significant difference (Duncan, 1955) was calculated for the comparisons among means. The analysis of the gene expression of CYP1A was performed using Comparative C_T Method, also referred to as the $\Delta\Delta C_T$ Method. The experimental values presented are taken from User Bulletin #2, Relative Quantitation of Gene Expression page 14.

Results

Hepatosomatic Index

Data presented in Table 3 shows that the *O. niloticus* injected with AFB_1(Group T_2) demonstrated a significant decrease in the HSI values when compared to control group T_1. Fish in group T_4 exhibited a lower value of HSI than the value of those with supplemented treatments (T_3-T_6). However, the results showed that the addition of both garlic and curcumin did not increase the HSI levels in aflatoxicated *O. niloticus*.in comparison to T_2,.with the exception of the low level of garlic in (T_7) that reflected the highest significant improvement in the HSI value when compared to the control groups. However, the two levels of curcumin with AFB_1 led to the lowest HSI values among all groups. Generally, garlic and curcumin did not improve the negative effects of AFB_1 on HSI.

Histopathology

No significant alterations were recorded in the hepatic histology of *O. niloticus* control (Figure 1a). However, livers of *O. niloticus* that were treated with AFB_1 (T_2) showed severe lesions in the form of central veins surrounded by inflammatory cells, focal areas of necrosis between the hepatocytes, severe hemolysis between clearly necrotic hepatocytes and some of the hepatocytes even showed pycnosis (Figure 1b). However, fish fed on garlic alone at the two levels 10 and 20 g /kg diet (T_3 , T_4 groups, Figure 1c and 1d respectively) showed some changes in the liver structure such as dilation in blood sinusoids (T_3, Figure 1c) and severe hemolysis in blood vessels and vacuolar degeneration in the hepatocytes T_4 (Figure 1d). Group T_5 (10 g/kg curcumin) showed slight hemorrhage between hepatocytes with vacuolar degeneration and dilation in the sinusoids (Figure 1e,). These changes were more apparent in T_6 with the higher dose of (20g/kg) curcumin as hemosiderin could be observed accumulated around blood vessels with vacuolar degeneration in hepatocytes (Figure 1f). Fish in group T_7 showed hepatocytes with prominent vacuolization and lacking normal polygonal structure with vacuolar degeneration (Figure 2a). While fish in group T_8 showed severe hemolysis diffused between clearly necrotic hepatocytes, some of which showed pycnosis (Figure 2b). The effect of curcumin (groups T_9 and T_{10}) showed accumulation of hemosiderin

Table 2. Real-time PCR primers of *O. niloticus* CYP1A and β –actin genes

Gene	Accession number	Gene Sequence (5-'3')	Location	Product size bp
CYP1A	FJ389918.2	F- GCAAATGGCTGCTGCTTGTCA	1912-1932	546
	Hal (2013)	R-GTGTATCAAGGGTTCATGCCCT	2479-2458	
β actin	EU887951.1	F- GGGTCAGAAAGACAGCTACGGTT	42 – 63	143
		R- CTCAGCTCGTTGTAGAAGGTGT	164 - 185	

Table 3. Effects of AFB_1 on hepatosomatic index (HSI) of fish injected with AFB_1 with or without feeding on garlic and curcumin dietaries (M ±SE)

Treatments	Liver weight (g)	Body weight (g)	HSI
T_1	0.47^{ab}±0.01	39.8^{a}±0.6	1.18^{ab}±0.01
T_2	0.34^{b} ±0.00	38.5^{a}±0.5	0.88^{b} ±0.03
T_3	0.40^{ab} ±0.01	38.5^{a} ±0.9	1.04^{ab}±0.01
T_4	0.38^{b} ±0.02	38.3^{a} ±0.8	1.00^{b} ±0.06
T_5	0.48^{ab}±0.07	39.0^{a} ±1.8	1.23^{ab} ±0.1
T_6	0.45^{ab}±0.07	40.0^{a} ±2.3	1.10^{ab} ±0.1
T_7	0.55^{a} ±0.06	41.1^{a} ±1.5	1.33^{a} ± 0.1
T_8	0.38^{b} ±0.02	40.3^{a} ±1.5	0.94^{b}±0.04
T_9	0.29^{c} ±0.02	43.1^{a} ±1.9	0.67^{c}±0.03
T_{10}	0.34^{b} ±0.03	40.4^{a} ±1.6	0.84^{b} ±0.07

Within each column, means superscript with different letters (a, b, c) are significantly different at P<0.05.

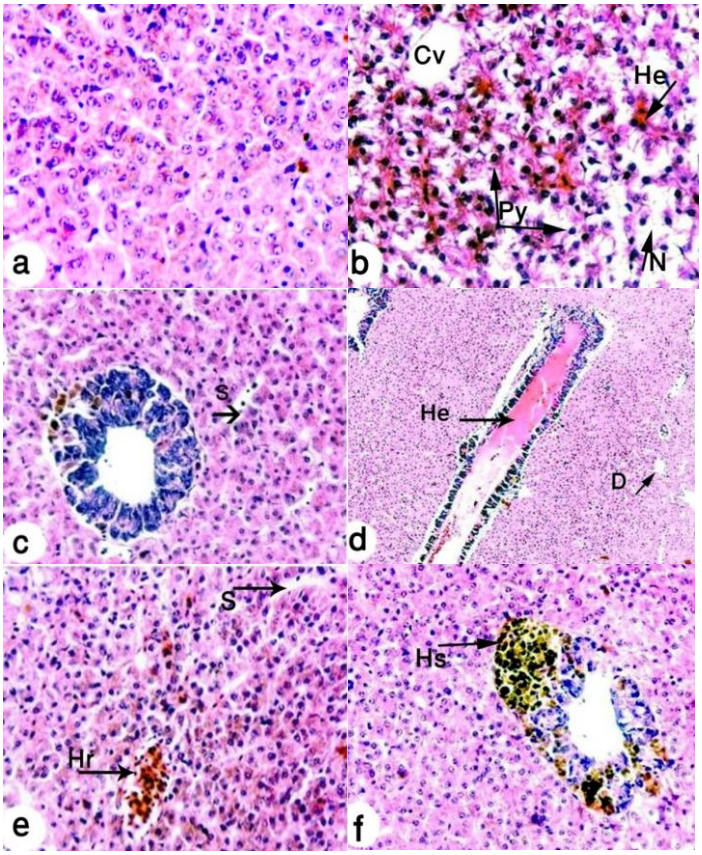

Figure 1. Histopathological changes in the liver of *O niloticus* groups (T_1-T_6) stained with H&E.; (a) T_1 normal structure (x300). (b) T_2 (AFB_1 control) central veins surrounded by inflammatory cells, focal areas of necrosis between the hepatocytes, very severe hemolytic diffusion between clearly necrotic hepatocytes, some of the hepatocytes showing pycnosis (x300). (c) T_3 dilation in blood sinusoids (x200). (d) T_4 intravascular hemolysis in blood vessels and vacuolar degeneration of hepatocytes (x100). (e) T_5 slight hemorrhage between hepatocytes with dilation in sinusoids and vacuolar degeneration in hepatocytes (x250). (f) T_6 hemosiderin accumulation around blood vessels with vacuolar degeneration in hepatocytes (x200). Cv= central veins, N=necrosis, Py= pycnosis, He= hemolysis, S= sinusoids, D= vacuolar degeneration, Hr= hemorrhage, Hs= hemosiderin.

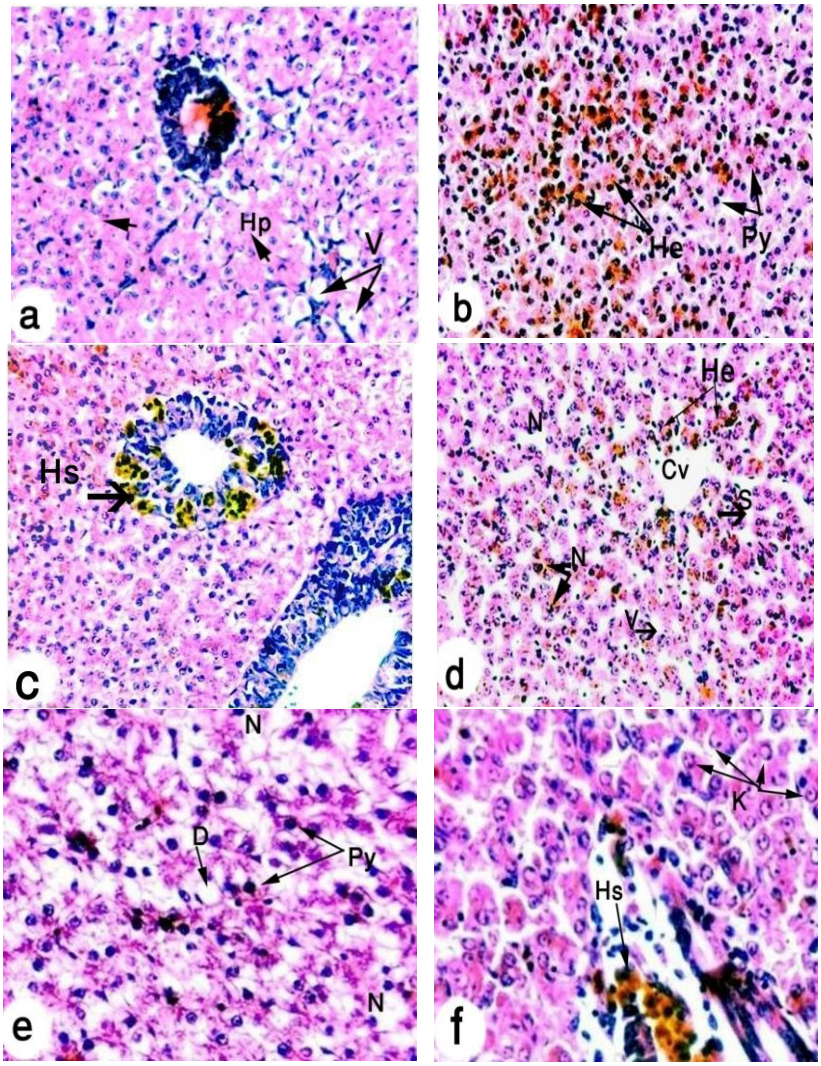

Figure 2. Histopathological changes in the liver of *O niloticus* groups (T_7-T_{10}) fed on either garlic or curcumn supplemented diet with AFB_1 injection stained with H&E. (a) T_7 hepatocytes have lost normal polygonal structure and show prominent vacuolization (x300). (b) T_8 severe hemolysis between clearly necrotic hepatocytes, some of which pycnotic (x200). (c) T_9 hemosiderin accumulation around blood vessels and pancreatic acini (x200). T_{10} (d) central vein surrounding by necrosis hepatocytes with hemolysis besides increased vacuolation in hepatocytes and dilation in blood sinusoids (x200). T_{10} (e) pycnotic nuclei in the necrotic hepatocytes and vacuolar degeneration (x300).T_{10} (f) disappearance of hepatocyte wall, karyolitic necrosis with fibrosis and hemosiderin accumulation (x300).). Cv= central veins, N=necrosis, Py= pycnosis, He= hemolysis, S= sinusoids, D= vacuolar degeneration, V= vacuolation, Hs= hemosiderin, Kr= karyolitic necrosis.

around blood vessels and pancreatic acini with vacuolar degeneration in hepatocytes (Figure 2c).

Group T_{10} (Figure 2d-f) with the highest concentration of curcumin showed central vein surrounded by necrotic hepatocytes with severe hemolysis. It also revealed increased vacuolation in hepatocytes and congestion and dilation in blood sinusoids with a sever hemolysis between necrotic hepatocytes (Figure 2d). In addition, fish in group T_{10} exhibited pycnotic nuclei and vacuolar degeneration in the hepatocytes (Figure 2e) with the disappearance of the hepatocyte wall with karyolitic necrosis and fibrosis (Figure 2f). In general, fish in the garlic treatment (groups T_7- T_8) showed a reduction in the damage compared to that of the fish in the curcumin diet groups (T_9- T_{10}) and the low concentrations of garlic and curcumin (groups T_7, T_9) showed a reduction in the damage better than the high doses.(T_8, T_{10}).

CYP1A Gene Expression

The results of the histological study and measurements of the HSI indicated that low concentrations of garlic and curcumin were better than the high ones. Therefore, CYPA1 gene expression was measured in liver samples of six groups (T_1, T_2, T_3, T_5, T_7 and T_9) which were only fed on low concentrations of either garlic or curcumin; the groups also contained the positive and negative

control fish. Highest CYPA1 expression was observed in the curcumin fed groups (T_5 and T_9), followed by the AFB_1 only group (T_2) when compared to control (T_1). The greatest significant reduction in CYPA1 expression was observed in the garlic fed groups (T_3 and T_7) (Figure 3).

Discussion

Aflatoxin B_1 induced a significant decrease in the HSI values of *O. niloticus* compared to control (T_1) (Table 2) similar to that reported in other studies (Usanno, Chaisilapasung, Sukrakanchana, & Supamattaya, 2005; El-Barbary & Mohamed, 2014). Singh & Venkitasubramanian (1975) reported that the weight of the liver is positively related to its liver content. However, HSI values significantly decreased in animals exposed to high levels of AFB_1 due to the inhibition of lipid mobilization in the adipose tissue. Deng *et al*. (2010) also observed that AFB_1 could decrease lipid content in liver lesions which could contribute to the abnormal growth in aflatoxicated livers. Also, the results showed significant decrease in HSI in the fish injected with 6ppm AFB_1 which was related to damage in the liver, (a). Lower HSI and histopathological changes in the liver suggest a progression towards AFB_1-induced heptocarcinogenesis (Zychowski *et al*., 2013). The liver histology of fish fed the basal diet (T_1) did not show significant alterations. However, sever lesions were observed in the liver of *O. niloticus* injected with AFB_1 (T_2) with infiltration of inflammatory cells, focal areas of necrosis between the hepatocytes and diffusion of severe hemolysis between vacuolated and necrotic hepatocytes (1b,). This was in agreement with El-Barbary and Mehrim (2009) where *O. niloticus* injected with AFB_1 (9mg /kg body weight) showed severe hemolysis, congestion and thrombosis in the blood vessels along with an accumulation of melanomacrophages. In addition, livers of *O. niloticus* injected with 6 mg AFB_1 /kg body weight showed severe lesions such as thrombosis in blood vessels, focal areas of necrosis between the hepatocytes, which had prominent vacuolization with pycnosis (El-Barbary, 2010). Vacuolar degeneration was also observed in the liver of tilapia injected with AFB_1 Huang *et al*. (2010).

Similarly, feeding AFB_1 exhibited many pathological changes in the liver of tilapia including infiltration by eosinophilic cells and numerous inflammatory cells (Deng *et al*., 2010). Tilapia fed 0.05-0.2 mg AFB_1/kg diet showed vacuolization with necrosis in the hepatocytes (El-Banna, Teleb, Hadi, & Fakhry, 1992). Additionally, hepatic necrosis, fibrosis and carcinogenesis were also reported in fish and animals fed on AFB_1 (Cummings & Macfarlane, 1997; Klein, Van Vleet, Hall, & Coulumbe, 2000). On the other hand, some histological changes were observed in fish liver fed a basal diet including 10 g/kg of either garlic or curcumin (T_3 and T_5 c and 1e). However, histological changes in the liver of the fish fed the higher concentration (20 g/kg) of garlic and curcumin (T_4 and T_6) appeared to be greater than in the group fed the lower concentration. The severe hemolysis in blood vessels and vacuolar degeneration of hepatocytes in group T_4 may be due to the properties of garlic (*Allium sativum*) .Whether garlic is fresh, aged, oiled and powdered it has been shown.to have anti-platelet effects (Bordia, 1978). Garlic is also known as a mild anticoagulant (Agarwal, 1996; Makheja & Bailey, 1990).

Garlic (*Allium sativum*) juice, equivalent to 2 g/kg body weight, caused cytoplasmic vacuolation with dilation and congestion in blood vessels of the liver cells of *Chrysichthys auratus*. In addition, some nuclei of the hepatocytes retained their normal size but others were pycnotic (Al-Salahy & Mahmoud, 2003). In the present study, fish fed the high dose of curcumin (20 g/kg diet) exhibited degeneration vacuoles in hepatocytes and large accumulation of hemosiderin around blood vessels (T_6, 1f). That disagrees with the study by Manju (2012) who, reported that in the curcumin-treated *Anabas sp*., there were no histological changes in the liver after feeding two doses of curcumin at 5 and 10 g/kg.

The role of both garlic and curcumin on ameliorating AFB_1 toxicity in Figure 2,.showed that severe histopathological changes in liver structure are caused by AFB_1 alone and may become less severe when these aflatoxicated fish are fed on garlic or curcumin especially at concentrations of 10 g/kg/ body weight. This is in agreement, with Alnaqeeb *et al*. (1992) who observed that histopathological changes in hepatic animals, pretreated with garlic,

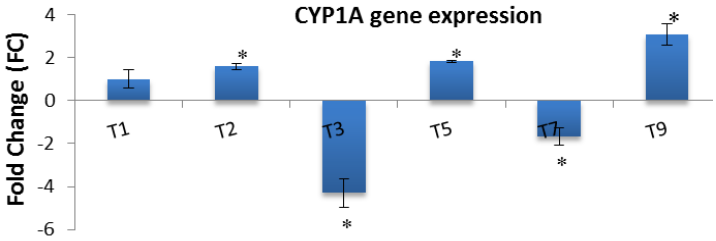

Figure 3 Gene expression patterns for CYP1A in different *O. niloticus* treatments, values are shown as means±SE and asterisks indicate significant difference (P≤0.05) between fish treatments.

showed a significant decrease in the damage compared to controls. The chemopreventive effect and antiaflatoxigenic activity of garlic and curcumin have previously been recorded (Guengerich & Shimadam, 1991; Ferreira *et al*., 2013; Lee *et al*., 2001). Mahfouz (2015) recently, reported that in fish, curcumin exhibited protective impacts on aflatoxicosis in *O. niloticus* by reducing the oxidative stress and hence resulting in enhanced growth performance. The histopathological findings revealed that garlic and curcumin tested at the highest concentration (20 g/kg diet) did not overcome the negative effects of AFB_1 (6 mg/kg/body weight) on the hepatic histology. Recently, the assessments of gene expression modulations, in response to both negative and positive factors on fish have become very important (Qiang, Yang, He, Wang, Zhu, & Xu, 2014; Jing, Li, Zhang, Yuan, Wang, & Gong, 2017).

Cytochrome P450 enzymes are expressed in numerous tissues but the highest levels are found in the liver (Vrzal, Ulrichova, Dvorak, 2004: Mori *et al*., 1992). In hepatocytes, the levels of cytochrome P450 was reduced during cancerous conditions (Gregus, Watkins Thompson, & Klaassen, 1982) and CYP 450 played a vital role in the formation of mutagenic electrophilic intermediates and carcinogenic from naturally occurring dietary compounds (Guengerich, Gillam, & Shimada, 1996). Therefore, studying gene expressions of cytochrome P450 in fish liver influenced by AFB_1, has become very important in the uncovering of the pathogenetic mechanism of AFB_1 and overcoming its negative effects. Where the target of using garlic and curcumin in the present study was to inhibit the gene expression of CYPA1 as a defensive mechanism against that the AFB_1 is initiated upon the reduction of CYP1A in the liver.

In this study the hepatic expression of cytochrome P450 was up-regulated in fish injected with AFB_1 (Figure 3). This increasing may be due to the cell damages caused by AFB_1 in liver where AFB_1 can cause cell damages by affecting hepatic cytochrome P450s-mediated bioactivation (Yarru *et al*., 2009; Sumit, & Roger, 2010).

Administrating a curcumin diet to fish IP injected with AFB_1 (T_9) showed up-regulation of CYP1A compared to group T_2. Yarru *et al*., (2009) also showed increased expression of CYP1A1 which was attributed to AFB_1, but which was significantly decreased after the addition of curcumin to the AFB_1 diet. The protective effects of curcumin on AFB_1 in Nile tilapia by reducing expression of the CYP1A were also recorded by Mahfouz, (2015). In the present study, increasing CYP1A expression in groups T_5 and T_9 may be responsible for the side effect of curcumin on histopathological liver. As suggested by Hari Kumar & Kuttan (2006) and Yarru *et al*. (2009), it has been revealed that the over expression of the CYP gene generates more ROS, thus inducing an oxidative stress that might lead to a hepatocellular injury. Administration of garlic in the diet showed an inhibition of CYP1A expression in aflatoxicosis in fish (T_7) when compared to the control (group T_2) (Figure 3). Natural organosulfur compounds, such as garlic, are considered potent chemopreventive compounds as they inhibit CYP1A1 and CYP1A2 directly (Skupinska, Misiewicz-Krzeminska, Stypulkowski, Lubelska, & Kasprzycka-Guttman, 2009; Guengerich & Shimada, 1991). Garlic appears therefore, to decrease the AFB_1 toxicity there by alleviating the hepatic lesions and protecting the liver from damage induced by AFB_1.

Recently, inhibition of CYP1 enzymes has been suggested to be a tumor chemoprevention strategy, due to its direct relationship with the formation of carcinogens (Badal *et al*., 2011). The over expression in CYP1A in control fish (group T_2) due to AFB_1 was decreased by supplementation of garlic (T_7) to the diet, which improved the detoxification of AFB_1 through the suppression of CYP1A in the liver. These results agree with El-Barbary (2016) who concluded that the garlic reflected the highest induction of expression of Glutathione Peroxidase, GPx, in T_7 group due to addition of garlic to the diet could improve the detoxification of AFB_1 in the liver.

Conclusion

Nile Tilapia injected with AFB_1 had greater liver damage than control fish (no AFB_1). Aflatoxin B_1 administered IP at a dose of 6 mg/kg body weight had a negative effect on HSI, liver histology and CYP1A production. A degree of protection was observed in some *O. niloticus* aflatoxiced groups that fed on garlic and curcumin supplemented diets. Expression of CYP1A in the garlic supplemented groups was significantly down regulated when compared to the control, suggesting detoxification of AFB_1 through the suppression of CYP1A in the liver. In addition, inclusion of garlic in the diet improved the HSI and liver structure more so than curcumin supplementation. Curcumin didn't appear to reduce the negative effects of AFB_1 the highest expression of CYP1A was observed in the curcumin groups followed by the AFB_1 group compared to the control. The optimal level of curcumin as a dietary antioxidant for the detoxification of the negative effects of AFB_1 may need further investigation.

References

Adedara, I.A., Owumi, S.E., Uwaifo, A.O., & Farombi, E.O. (2010). Aflatoxin B1 and ethanol co-exposure induces hepatic oxidative damage in mice. *Toxicology and Industrial Health*, 26, 717–724. http://dx.doi.org/10.1177/0748233710377772

Agarwal, K.C. (1996). Therapeutic actions of garlic constituents. *Medicinal Research Reviews*, 16, 111-24. http://dx.doi.org/10.1002/(SICI)1098-1128(199601)16:1<111::AID-MED4>3.0.CO;2-5

Alnaqeeb, M.A., Ali, M, Thomson, M., Khater, S.H.,

Gomes, S.A., & Al-Hassan, J.M. (1992). Histopathological evidence of protective action of garlic against collagen and arachidonic acid toxicity in rabbits. *Prostaglandins Leukot Essent Fatty Acids*, 46, 301- 6. PMID: 1409768

Al-Salahy, M.B., & Mahmoud, A.B. (2003). Metabolic and histological studies on the effect of garlic administration on the carnivorous fish *Chrysichthys auratus*. *Egyptian Journal of Biology*, 5, 94-107

AOAC, (2000). Official Method of the Association of Official Analytical Chemists. 17th Edn., AOAC, Washington, DC., USA., pp: 21-447.

Badal, S., Williams, S.G., Huang, G., Francis, S., Vedantam, P., Dunbar, O., & Delgoda R. (2011). Cytochrome P450 1 enzyme inhibition and anticancer potential of chromene amides from *Amyris plumier*. *Fitoterapia*, 82, 230-236. http://dx.doi.org/10.1016/j.fitote.2010.10.003

Balu, M., Sangeetha, P., Murali, G., & Panneerselvam, C. (2006). Modulatory role of grape seed extract on agerelated oxidative DNA damage in central nervous system of rats. *Brain Research Bulletin*, 68, 469-73. http://dx.doi.org/10.1016/j.brainresbull.2005.10.007

Banerjee, S.K., Maulik, M., Manchanda, S.C., Dinda, A.K., Das, T.K., & Maulik, S.K. (2001). Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. *Food and Chemical Toxicology*, 39, 793-797. http://dx.doi.org/10.1016/S0278-6915(01)00018-7

Bernet, D., Schmidt, H., Meier, W., Brkhardt-Holm, P., & Wahli T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases*, 22, 25-34. http://dx.doi.org/10.1046/j.1365-2761.1999.00134.x

Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Dillen, P.M.E.W., & van der Noordaa, J. (1990). Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology*, 28, 495–503. PMID: PMC269651

Brandon, E.F.A., Raap, C.D., Meijerman, I., Beijnen, J.H., & Schellens, J.H.M. (2003). An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. *Toxicology and Applied Pharmacology*, 189,233–246. PMID: 2791308

Borek, C. (2001). Antioxidant health effects of aged garlic extract. *Journal of Nutrition*. 131, 1010–1015. PMID: 11238807

Chattopadhyay, I., Biswas, K., Bandyopadhyay, U., & Banerjee, R.K. (2004). Turmeric and curcumin: biological actions and medicinal applications. *Current Science*, 87, 44–53.

Chavez-Sanchez, M.C., Palacios, C.A.M., & Moreno, I.O. (1994). Pathological effects of feeding young Oreochromis niloticus diets supplemented with different levels of Aflatoxin B1. *Aquaculture*, 127, 49–60.

Chomczynski, P., & Sacchi N. (1987). Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. *Analytical Biochemistry*, 162, 156-159. http://dx.doi.org/10.1006/abio.1987.9999

Clark, D.J, George, S.G., & Burchell, B. (1991). Glucuronidation in fish. *Aquatic Toxicology*, 20, 35-56. http://dx.doi.org/10.1016/0166-445X(91)90040-G

Cummings, J.H., & Macfarlane, G.T. (1997). Colonic microflora: nutrition and health. *Nutrition*, 13, 476–478. http://dx.doi.org/10.1016/S0899-9007(97)00114-7.

Davila, J., Marcial-Martinez, L.M., Viana, M.T., & Vazquez-Duhalt, R. (2010). The effect of broccoli in diet on the cytochrome P450 activities of tilapia fish *(Oreochromis niloticus*) during phenol exposure. *Aquaculture*, 304, 58–65. http://dx.doi.org/10.1016/j.aquaculture.2010.03.020

Deng, S.X., Tian, L.X., Liu, F.J., Jin, S.J., Liang, G.Y., Yang, H.J., & Liu Y.J. (2010). Toxic effects and residue of aflatoxin B1 in tilapia (*Oreochromis niloticus×O. aureus*). *Aquaculture*, 307, 233–240. http://dx.doi.org/10.1016/j.aquaculture.2010.07.029.

Diab, A.S., El-Nagar, G.O., & Abd-El-Hady, Y.M. (2002). Evaluation of *Nigella sativa* L (black seeds; baraka), *Allium sativum* (garlic) and BIOGEN as feed additives on growth performance and immunostimulants of *O. niloticus* fingerlings. *Suez Canal Veterinary Medicine Journal*, 745–775.

Duncan, D.B. (1955). Multiple range and multiple F. test. *Biometrics*, 11, 1 – 42.

Egen-Schwind, C., Eckard, R., & Kemper, F.H. (1992). Metabolism of garlic constituents in the isolated perfused rat liver. *Planta Medica*, 58, 301-305. http://dx.doi.org/10.1055/s-2006-961471

El-Banna, R., Teleb, H.M., Hadi, M.M., Fakhry, F.M., 1992. Performance and tissue residue of tilapias fed dietary aflatoxin. *Veterinary Medicine Journal*, 40, 17– 23.

El-Barbary, M.I. (2016). Detoxification and antioxidant effects of garlic and curcumin in *Oreochromis niloticus* injected with aflatoxin B_1 with reference to gene expression of glutathione peroxidase (GPx) by RT-PCR. *Fish Physiology and Biochemistry*. 42, 617–629. http://dx.doi.org/10.1007/s10695-015-0164-4.

El-Barbary, M.I. (2010). Antiaflatoxigenic activity of chemical antioxidants (glutathione and glutathione enhancer) on *Oreochromis niloticus*. *Egyptian Journal of Aquatic Research*, 36, 203-215.

El-Barbary, M.I. (2008). Aflatoxin B1 induced-changes in protein electrophoretic pattern and DNA in *Oreochromis niloticus* with special emphasis on the protective effect of rosemary and parsley extracts. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 4, 381-390

El-Barbary, M.I., & Mehrim, A.I. (2009). Protective effect of antioxidant medicinal herbs, rosemary and parsley, on subacute aflatoxicosis in *Oreochromis niloticus. Journal of Fisheries and Aquatic Sciences*, 4,178-190. http://dx.doi.org/10.3923/jfas.2009.178.190

El-Barbary, M.I., & Mohamed, M.H. (2014). Chemoprevention and therapeutic efficacy of glutathione against aflatoxicosis in Nile tilapia (*Oreochromis niloticus*) *Global Vetrinaria*,13, 1111-1121. http://dx.doi.org/10.5829/idosi.gv.2014.13.06.91169

Farabi, S.M.V., Yousefian, M & Hajimoradloo, A. (2006). Aflatoxicosis in juvenile *Huso huso* fed a contaminated diet. *Journal of Applied Ichthyology*, 22 (Suppl. 1), 234–237. http://dx.doi.org/10.1111/j.1439-0426.2007.00958

Ferreira, F.D., Kemmelmeier, C., Arroteia, C.C., da Costa, C.L., Mallmann, C.A., Janeiro, V., & Jr, M.M. (2013). Inhibitory effect of the essential oil of *Curcuma longa* L. and curcumin on aflatoxin production by *Aspergillus flavus* Link. *Food Chemistry*, 136, 789–793. http://dx.doi.org/10.1016/j.foodchem.2012.08.003

Firozi, P.F., Aboobaker V.S., & Bhattacharya, R.K. (1996). Action of curcumin on the cytochrome P450-system catalyzing the activation of aflatoxin B1. *Chemico-Biological Interactions*, 100, 41-51. PMID: 8599854

Gregus, Z., Watkins J.B., Thompson T.N. & Klaassen, C.D. (1982). Resistance of some phase II biotransformation pathways to hepatotoxins. *Journal of Pharmacology and Experimental Therapeutics*, 222(2), 471–9.

Guengerich, F.P., Gillam, E.M., & Shimada, T. (1996). New applications of bacterial systems to problems in toxicology. *Critical Reviews in Toxicology*, 26, 551–583. http://dx.doi.org/10.3109/10408449609037477

Guengerich, F.P., Johnson, W.W., Shimada, T., Ueng, Y.F., Yamazaki, H. & Langouet, S. (1998). Activation and detoxication of aflatoxin B1. *Mutation Research.* 402, 121–128. PMID: 9675258

Guengerich, F.P.M., & Shimada, T. (1991). Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chemical Research in Toxicology*, 4, 391-407.

Gusman, J., Malonne, H., & Atassi, G. (2001). A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. *Carcinogenesis*, 22, 1111–7. PMID: 11470738

Hal, A.M.A. (2013). Comparative study on protein expression of drug–metabolizing CYP1 family genes from Lake Manzala. Ph.D. Thesis, Fac. Agric., Ain Shams University, Cairo, Egypt.

Halver, J.E. (1969). Aflatoxicosis and trout hepatoma. In: Goldblatt, L.A. (Ed.), Aflatoxin: Scientific Background,Control, and Implications. Academic Press, NewYork,.pp. 265–306.

Hamackova, J., Kouril, j., Kozak, P., & Stupka, Z. (2006). Clove oil as an anaesthetic for different freshwater fish species. *Bulgarian Journal of Agricultural Science*, 12,185-194

Hari Kumar, K.B., & Kuttan, R. (2006). Inhibition of drug metabolizing enzymes (cytochrome P450) in vitro as well as in vivo by *Phyllanthus amarus* Schum & Thonn. *Biological and Pharmaceutical Bulletin*, 29, 1310–1313. PMID: 16819159

Hendricks, J.D. (1994). Carcinogenicity of aflatoxins in nonmammalian organisms. In: Eaton D.L., Groopman J.D. (eds) Toxicology of aflatoxins: human health. Veterinary and agricultural significance. Academic Press, San Diego pp 103–136

Huang, Y., Han, D., Zhu, X., Yang, Y., Jin, J., Chen, Y., & Xie, S. (2011). Response and recovery of gibel carp from subchronic oral administration of aflatoxin B1. *Aquaculture*, 319, 89–97

IARC, (1985). Aflatoxin. IARC Monographs , Suppl. 7:83–7.

IPCS-WHO, (1998). Aflatoxins. Available at: http://www.inchem.org/documents/

Jantrarotai, W., Lovell R.T., & Grizzle, J.M. (1990). Acute toxicity of aflatoxin B1 to channel catfish. *Journal of Aquatic Animal Health*, 2, 237–247. http://dx.doi.org/org/10.1577/1548-8667(1990)002<0237:ATOABT>2.3.CO;2

Jing, D., Li, M., Zhang, Y., Yuan, .L., Wang, R., Gong, Y. (2017). Differential Induction of Enzymes and Genes Involved in Oxidative Stress in Gill and Liver Tissues of Mudskipper *Boleophthalmus pectinirostris* Exposed to Lead. *Turkish Journal of Fisheries and Aquatic Sciences*, 17:437-443. http://dx.doi.org/10.4194/1303-2712-v17_2_23

Joner, A. (2000). Mycotoxins. http://www.ansci.cornell.edu/courses/as625/1999term/toner/aflatoxins.html.

Kim, K.M., Chun, S.B., Koo, M.S., Choi,.W.J., Kim,.T.W., Kwon, Y.G., & Kim, Y.M. (2001). Differential regulation of NO availability from macrophages and endothelial cells by the garlic component S-allyl cysteine. *Free Radical Biology and Medicine*, 30, 747–756. PMID: 11275474

Klein, P.J., Van Vleet, T.R., Hall, J.O., & Coulumbe, R.A. (2003). Effects of dietary butylated hydroxytoluene on aflatoxin B1-relevant metabolic enzymes in turkeys. *Food and Chemical Toxicology*, 41:671–678. PMID: 12659720

Kunnumakkara, A.B., Ichikawa, H., Anand, P., Mohankumar, C.J., Hema, P.S., Nair M.S., & Aggarwal, B.B. (2008). Coronarin D, a labdane diterpene, inhibits both constitutive and inducible nuclear factor-kappa B pathway activation, leading to potentiation of apoptosis, inhibition of invasion, and suppression of osteoclastogenesis. *Molecular Cancer Therapeutics*, 7, 3306 - 3317. http://dx.doi.org/10.1158/1535-7163.MCT-08-0432.

Langeswaran, K., Jagadeesan, A.J., Revathy, R., Balasubramanian, M.P. (2012). Chemotherapeutic efficacy of limonin against Aflatoxin B1 induced primary hepatocarcinogenesis in *Wistar albino* rats. *Biomedicine & Aging Pathology*, 2, 206–211. http://dx.doi.org/10.1016/j.biomag.2012.08.002

Lee, S., Campbell, B.C., Russell, J., Molyneux, R.J., Hasegawa, S., & Lee H.S. (2001). Inhibitory effects of naturally occurring compounds on aflatoxin B1 biotransformation. *Journal of Agricultural and Food Chemistry*, 49, 5171–5177. PMID: 11714299

Leveque, C. (2002). Out of Africa: The success story of tilapias. *Environmental Biology of Fishes*, 64, 461-464. http://dx.doi.org/10.1023/A:1016190529697

Madhusudhanan, N., Kavithalakshmi, S.N., Radha, Shanmugasundaram, K., & Shanmugasundaram, E.R. (2004). Oxidative damage to lipids and proteins induced by aflatoxin B (1) in fish (*Labeo rohita*)-protective role of *Amrita Bindu. Environmental Toxicology and Pharmacology*, 17, 73–77. http://dx.doi.org/10.1016/j.etap.2004.03.002

Mahfouz, M.E. (2015). Ameliorative Effect of Curcumin on Aflatoxin B1 Induced Changes in Liver Gene Expression of *Oreochromis niloticus*. *Journal of Molecular Biology*, 49 (2), 275–286. PMID:26065259

Makheja A.N. & Bailey J.M. (1990). Antiplatelet constituents of garlic and onion. *Agents Actions.* 29, 60 -3. PMID: 2111084

Manju, M., Akbarsha, M.A., & Oommen, O.V. (2012). In vivo protective effect of dietary curcumin in fish *Anabas testudineus* (Bloch). *Fish Physiology and Biochemistry.* 38, 309–318. http://dx.doi.org/10.10.1007/s10695-011-9508-x

Manning, B.B., Li, M.H.,.& Robinson, E.H. (2005). Aflatoxins from moldy corn cause no reductions in channel catfish *Ictalurus punctatus* performance. *Journal of World Aquaculture Society*, 36, 59-67.

Matur, E., Ergul, E., Akyazi, I., Eraslan, E., Inal, G., Bilgic, S., & Demircan, H., (2011). Effects of *Saccharomyces cerevisiae* extract on haematological parameters, immune function and the antioxidant defence system in breeder hens fed aflatoxin contaminated diets. *British Poultry Science*, 52 (5), 541–550. http://dx.doi.org/10.1080/00071668.2011.617726.

McLean, M., & Dutton, M.F., (1995). Cellular interactions

and metabolism of aflatoxin: an update. *Pharmacology and Therapeutics*, 65, 163–92. PMID: 7540767

Melo, D.C., Oliveira, D.A.A., Ribeiro, L.P., Teixeira, C.S., Souza, A.B., Coelho, E.G.A., Crepaldi, D.V. & Teixeira, E.A., (2006). Caracterizacao genetica de seis planteis comerciais de tilapia (*Oreochromis*) utilizando marcadores microssatelites [Genetic characterization of six commercial stocks of tilapia (*Oreochromis* spp) using microsatellite markers]. *Brazilian Journal of Veterinary and Animal Science*, 58, 87-93. http://dx.doi.org/org/10.1590/S0102-09352006000100013.

Metwally, M.A.A., (2009). Effects of garlic (*Allium sativum*) on some antioxidant activities in Tilapia nilotica (*Oreochromis niloticus*). *World Journal of Fish and Marine Sciences*,1 (1), 56–64.

Mori, T., Kitamura, R., Imaoka, S., Funaet, Y., Kitada, M., & Kamataki, T., (1992). Examination for lipid peroxidation in liver microsomes of guinea pigs as a causal factor in the decrease in the content of cytochrome P450 due to ascorbic acid deficiency. *Research communications in chemical pathology and pharmacology*, 75, 209–220

Nakagawa, S., Masamoto, K., Sumixyoshi, H., Kunihiro, K., & Fuwa, T. (1985). Effect of raw and extracted aged garlic on growth of young rats and their organ after per oral administration. *Journal of Toxicological Sciences*, 5, 91-112. PMID: 7431442

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature.* 405, 1017–1024. http://dx.doi.org/10.1038/35016500

Pinlaor, S., Yongvanit, P., Prakobwong, S., Kaewsamut, B., Khoontawad, J., Pinlaor, P., & Hiraku, Y. (2009). Curcumin reduces oxidative and nitrative DNA damage through balancing of oxidant–antioxidant status in hamsters infected with *Opisthorchis viverrini*. *Molecular Nutrition & Food Research*, 53, 1316–1328. http://dx.doi.org/10.1002/mnfr.200800567.

Qiang, J., Yang, H., He, J., Wang, H., Zhu, Z., Xu, P. (2014). Comparative Study of the Effects of Two High-Carbohydrate Diets on Growth and Hepatic Carbohydrate Metabolic Enzyme Responses in juvenile GIFT tilapia (*Oreochromis niloticus*). *Turkish Journal of Fisheries and Aquatic Sciences*, 14: 515-525. http://dx.doi.org/10.4194/1303-2712-v14_2_23

Reddy, T.V., Viswanathan, L., & Venkitasubramanian, T.A., (1971). High aflatoxin production on chemically defined medium. *Applied and Environmental Microbiology*, 22, 393-396. PMID: PMC376320

Sahoo, P.K., & Mukherjee, S.C. (2001). Immunosuppressive effects of aflatoxin B1 in Indian major carp (*Labeo rohita*). *Comparative Immunology, Microbiology & Infectious Diseases.* 24 (3), 143–149. PMID: 11440188

Santacroce, M.P., Conversano, M.C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G., & Crescenzo, G. (2008). Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Reviews in Fish Biology and Fisheries.* 18, 99–130. http://dx.doi.org/10.1007/s11160-007-9064-8

Sargeant, K., Sheridan, A., O'Kelly, J., & Carnaghan, R.B.A., (1961). Toxicity associated with certain samples of groundnuts. *Nature.* 192, 1096 –7. http://dx.doi.org/10.1038/1921096a0

SAS, (1996). SAS Users Guid: Statistics. Version 2, 5 Edition. SAS Institute, Inc., Cary, NC.

Shaarawy, S.M., Tohamy, A.A., Elgendy, S.M., Abd-Elmageed, Z.Y., Bahnasy, A., Mohamed, M.S., Kandil,.E., Matrougui, K. (2009). Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. *International Journal of Biological Sciences*, 5, 549– 557. http://dx.doi.org/10.7150/ijbs.5.549

Shalaby, A.M., Khattab, Y.M., & Abdel rahman, A.M. (2006). Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile Tilapia (*Oreochromis niloticus*). *Journal of Venomous Animals and Toxins including Tropical Diseases.* 12, 172–201. http://dx.doi.org/org/10.1590/S1678-91992006000200003.

Sharma, C.P., & Nirmala, N.V. (1985). Effects of garlic extract and of three pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure comments. *Thrombosis Research.* 37, 489-90. PMID:3992531

Singh N., & Venkitasubramanian, T.A. (1975). Effect of aflatoxin B1 on lipids of rat tissues. *Environmental Physiology & Biochemistry*, 5, 147–157. PMID: 1149724

Skupinska, K., Misiewicz-Krzeminska, I., Stypulkowski, R., Lubelska, K., & Kasprzycka-Guttman, T., (2009). Sulforaphane and its analogues inhibit CYP1A1 and CYP1A2 activity induced by benzo[a]pyrene. *Journal of Biochemical and Molecular Toxicology*, 23(1), 18–28. http://dx.doi.org/10.1002/jbt.20259

Srivastava, K.C. (1984). Aqueous extracts of onion, garlic and ginger inhibit platelet aggregation and alter arachidonic acid metabolism. *Biomedica biochimica acta*. 43, 335-46. PMID:6440548

Subasinghe, R.P. (2005). Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Preventive Veterinary Medicine*, 67, 117–124. http://dx.doi.org/10.1016/j.prevetmed.2004.11.004

Sujatha, N., & Sashidhar, R.B., (2010). Metabolic intervention of aflatoxin B1 toxicity by curcumin. *Journal of Ethnopharmacology*, 127, 641–644. http://dx.doi.org/10.1016/j.jep.2009.12.010

Sumit, R.J.E.K., & Roger, C.J. (2010). Aflatoxin B1 in poultry: toxicology, metabolism and prevention. *Research in Veterinary Science*, 89, 325–331. http://dx.doi.org/10.1016/j.rvsc.2010.04.011.

Theumer, M.G., Lopez, A.G., Masih, D.T., Chulze, S.N., & Rubinstein, H.R. (2003). Immunobiological effects of AFB1 and AFB1–FB1 mixture in experimental subchronic mycotoxicoses in rats. *Toxicology.* 186, 159–170. PMID: 12604180

Tuan, N.A., Grizzle, J.M., Lovell, R.T., Manning, B.B., Rottinghaus, G.E., (2002). Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B1. *Aquaculture.* 212, 311–319. http://dx.doi.org/ 10.1016/S0044-8486(02)00021-2.

Usanno, O., Chaisilapasung, S., Sukrakanchana, N., Supamattaya, K. (2005). Effects of. aflatoxin B1 on sex reversed red tilapia (*Oreochromis niloticus Linn.*

× *O. mossambicus* Peters). *Songklanakarin Journal of Science and Technology*, 27 (Suppl. 1), 187–197.

Vrzal, R., Ulrichova, J., & Dvorak, Z., (2004). Aromatic hydrocarbon receptor status in the metabolism of xenobiotics under normal and pathophysiological conditions. Biomed Papers. 148, 3–10. http://dx.doi.org/10.5507/bp.2004.001.

White, A., & Fletcher, T.C. (1985). Seasonal changes in serum glucose and condition of the plaice *Pleuronectes platessa* L. *Journal of Fish Biology*, 26,755–764. http://dx.doi.org/10.1111/j.1095-8649.1985.tb04316

Wiame, I., Remy, S., Swennen, R., & Sagi L. (2000). Irreversible heat inactivation of DNase I without RNA degradation. *Bio Techniques*. 29, 252-256 PMID: 10948426

Wild, C.P., Turner, P.C., (2002). The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis.* 17, 471–81. PMID: 12435844

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure,potential health consequences, and interventions. *The American Journal of Clinical Nutrition*, 80, 1106–1122. PMID: 15531656

Yarru, L.P., Settivari, R.S., Gowda, N.K., Antoniou, E., Ledoux, D.R., & Rottinghaus G.E., (2009). Effects of turmeric (*Curcuma longa*) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. *Poultry Science*, 88, 2620–2627. http://dx.doi.org/10.3382/ps.2009-00204.

Zychowski, E., Pohlenz, C., Mays, T., Romoser, A., Hume, M., Buentello, A., Gatlin, D.M., & Phillips, T.D. (2013). The effect of NovaSil dietary supplementation on the growth and health performance of Nile tilapia (*Oreochromis niloticus*) fed aflatoxin-B1 contaminated feed Katherine. *Aquaculture*, 376-379, 117–123. http://dx.doi.org/10.1016/j.aquaculture.2012.11.020