Toxicology Reports 1 (2014) 319–329

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Quercetin inhibits the cytotoxicity and oxidative stress in liver of rats fed aflatoxin-contaminated diet

Aziza A. El-Nekeety [a], Sekena H. Abdel-Azeim [b], Aziza M. Hassan [b,c], Nabila S. Hassan [d], Soher E. Aly [a], Mosaad A. Abdel-Wahhab [a,*]

[a] Food Toxicology & Contaminants Department, National Research Center, Dokki, Giza, Egypt
[b] Cell Biology Department, National Research Center, Dokki, Giza, Egypt
[c] Biotechnology Department, Faculty of Science, Taif University, Saudi Arabia
[d] Pathology Department, National Research Center, Dokki, Giza, Egypt

ARTICLE INFO

Article history:
Received 8 February 2014
Received in revised form 17 May 2014
Accepted 24 May 2014
Available online 6 June 2014

Keywords:
Aflatoxin
Liver
Quercetin
Cytogenetic
Gene expression
Oxidative stress

ABSTRACT

This study was conducted to evaluate the protective role of quercetin (Q) against the cytotoxicity, DNA damage and oxidative stress in rats fed aflatoxin (AFs)-contaminated diet. Female Sprague–Dawley rats were divided into six groups and treated for 21 days as follows: the control group; the group fed AFs-contaminated diet (1.4 mg/kg diet); the groups treated orally with Q at low or high dose (50 and 100 mg/kg b.w.) and the groups AFs-contaminated diet plus low or high dose of Q. At the end of experiment, blood and liver samples were collected for biochemical, histological, histochemical and genetic analyses. The results indicated that animal fed AFs-contaminated diet showed significant increase in serum biochemical parameters, oxidative stress markers and DNA fragmentation accompanied with significant decrease in total proteins, GPX, SOD, DNA and RNA content and fatty acid synthase (Fas) and TNFα gene expression in the liver tissue. Q at the two tested doses succeeded to normalize the biochemical parameters, improved the content of nucleic acids in hepatic tissues, the gene expression, the histopathological and histochemical picture of the liver. It could be concluded that Q has a potential antioxidant activity, a protective action and regulated the alteration of genes expression induced by AFs.
© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

1. Introduction

Aflatoxins (AFs) are major class of mycotoxins produced mainly by two species of *Aspergillus*, *Aspergillus flavus* and *Aspergillus parasiticus*. AFs have been associated with several toxic effects in animal and human health including carcinogenic, mutagenic, teratogenic and immunosuppressive activity [2,6,23]. AFs are one of the most potent toxic substances that are found in a wide range of agricultural crops especially grains and nuts which are commonly used for the preparation of different foods [14]. Although 20 aflatoxins have been identified, only 4 of them, i.e. the aflatoxins B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2), occur naturally and are significant contaminants of a wide variety of foods and feeds. AFB_1 is the most potent carcinogenic substance and has continued to receive major research attention as the most carcinogenic and toxic mycotoxins [16,30]. It is classified by the International Agency of Research on Cancer as Group 1 human carcinogen [30]. The toxic metabolites AFB_1-8,9-epoxide resulted from biotransformation of AFB_1 by liver microsomal enzymes and the toxic effects of aflatoxins mostly arise from the binding of this particular epoxide derivative to DNA [48].

* Corresponding author. Tel.: +20 2 2283 1943; fax: +20 2 3337 0931.
E-mail address: mosaad_abdelwahhab@yahoo.com (M.A. Abdel-Wahhab).

http://dx.doi.org/10.1016/j.toxrep.2014.05.014
2214-7500/© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

It has been well documented that drug-metabolizing enzymes (phase-I and phase-II enzymes) and AFB_1-adduct formation can be changed by natural constituents of the diet, nutrients, phytochemicals and xenobiotics [1,3]. Recently, a great deal of attention has focused on the protective biochemical functions of naturally occurring antioxidants in biological systems against oxidative stress in liver tissue. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues also free radicals may act as a contributory factor in a progressive decline in the function of immune system [24]. Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes [3,53].

A highly promising candidate for the prevention of adverse health effects in humans is quercetin (3,3′,4′,5,7-pentahydroxyflavone), one of the most abundant flavonoids in the human diet, which is found in fruits and vegetables such as blueberries, onions, curly kale, broccoli, and leek [37]. It is well documented that quercetin has broad bioactivity, such as antioxidative, hypolipidemic properties [9,10], ROS scavenging, anti-inflammatory and anti-fibrotic properties [29,36]. Moreover quercetin exhibits hepatoprotective effect against ethanol hepatotoxicity by counteracting oxidative stress *in vivo* [18,39,59] and *in vitro* [35,63]. The aims of the current study were to evaluate the protective role of aqueous extract of quercetin against oxidative stress, the cytotoxicity and DNA damage in liver tissue of rats fed AFs-contaminated diet.

2. Materials and methods

2.1. Chemicals and kits

Aflatoxins (AFs) standards and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) kits were purchased from Spectrum-diagnostics Co. (Cairo, Egypt). Dimethyl sulfoxide (DMSO) was supplied by Merck (Germany). Triglycerides, total proteins, glutathione peroxidase (GPx), superoxide dismutase (SOD), alkaline phosphatase (ALP), nitric oxide (NO), lactate dehydrogenase (LDH), carcinoembrionic antigen (CEA), total lipid, cholesterol and lipid peroxide formation as malondialdehyde (MDA) were determined using kits purchased from Biodiagnostic Co. (Giza, Egypt). All other chemicals used throughout the experiments were of the highest analytical grade available.

2.2. Aflatoxins production

The aflatoxins (AFs) were produced through the fermentation of maize by *Aspergillus parasiticus* NRRL 2999 as described by [54]. The fermented maize was autoclaved; ground to a fine meal, and the AFs content was measured by the use of HPLC [28]. The AFs within the maize meal consisted of 45% B_1, 12% B_2, 30% G_1, and 13% G_2 based on total AFs in the maize powder. The maize meal was incorporated into the basal diet to provide the desired level of 1.4 mg of total AFs/kg diet. The diet containing AFs was analyzed and the presence of parent AFs was confirmed by HPLC. The safety measures recommended by [61] were taken when handling the AFs-contaminated diet.

2.3. Experimental animals

Three-month-old female Sprague–Dawley rats (100–150 g each) were purchased from Animal House Colony, National Research Centre, Dokki, Giza, Egypt. Animals were maintained on the specified diet and housed in filter-top polycarbonate cages in a room free from any source of chemical contamination, artificially illuminated (12 h dark/light cycle) and thermally controlled (25 ± 1 °C) at the Animal House Lab., National Research Centre. All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre, Dokki, Giza, Egypt.

2.4. Experimental design

Animals were divided into six groups (8 rats/group) and were maintained on their respective diet for 3 weeks as follow: Group 1, normal control fed on basal diet and received 0.5% DMSO at a dose of 1 ml/kg b.w. by gastric tube; Group 2, animals fed AFs-contaminated diet (1.4 mg/kg diet); Group 3, animals treated daily with low dose of quercetin (50 mg/kg b.w.) dissolved in 0.5% DMSO by gastric tube; Group 4, animals treated daily with high dose of quercetin (100 mg/kg b.w.) dissolved in 0.5% DMSO by gastric tube; Groups 5 and 6, animals fed AFs-contaminated and treated orally with low and high dose of quercetin, respectively. The animals were observed daily for signs of toxicity during the experimental period. At the end of the treatment period (i.e. day 21), all animals were fasted for 12 h, then blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia. Sera were separated using cooling centrifugation and stored at −20 °C until analysis. The sera were used for the determination of ALT, AST, ALP, LDH, NO, CEA, total protein, cholesterol, triglycerides and total lipid according to the kits instructions using Jenway spectrophotometer 6715 (Staffordshire, UK).

After the collections of blood samples, animals were sacrificed by cervical dislocation and samples of the liver of each animal were dissected, weighed and homogenized in phosphate buffer (pH 7.4) to give 20% (w/v) homogenate according to [34]. This homogenate was centrifuged at 1700 rpm and 4 °C for 10 min; the supernatant was stored at −70 °C until analysis. This supernatant (20%) was used for the determination of hepatic lipid peroxide formation as malondialdehyde (MDA) and it was further diluted with phosphate buffer solution to give 2% and 0.5% dilutions for the determination of hepatic glutathione peroxidase (2%) and superoxide dismutase (0.5%) activities.

Other samples of the liver from all animals were fixed in 10% neutral formalin and paraffin embedded. Sections (5 μm thickness) were stained with hematoxylin and eosin (H & E) for the histological examination. Other sections from liver were stained with Bromophenol blue for the determination of protein content in liver tissue [22]. Another sample of the liver of each animal within different treatment groups was fixed in 3% glutaraldehyde,

dehydrated and embedded in resin [26]. Semi-thin sections (1 μm thickness) were cut by Leica ultramicrotome and stained with 1% toluidine blue. Ultrathin sections (60–100 nm thickness) were also prepared and stained with uranyl acetate and lead citrate to be examined under JOEL EM 1005 transmission electron microscope using an accelerated voltage of 60 kV.

2.5. Genetic analysis

2.5.1. Determination of nucleic acids in hepatic tissue

Nucleic acids were determined in hepatic tissues according to the method described by [11]. In brief, the hepatic tissues were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol. The levels of DNA were determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

2.5.2. DNA fragmentation assays for apoptosis

Apoptotic changes in the liver were evaluated colorimetrically by DNA fragmentation and by agarose gel electrophoresis according to the procedure of [45]. Liver samples were homogenized in 700 μl hypotonic lysis buffer and centrifuged for 15 min at 11,000 rpm. The supernatants containing small DNA fragments were separated; one-half of the volume was used for gel electrophoresis and the other half together with the pellet containing large pieces of DNA were used for quantification of fragmented DNA by the diphenyl amine (DPA) assay. The samples were treated with equal volumes of absolute isopropyl alcohol and NaCl to precipitate DNA. Extracted DNA was electrophoresed on 1% agarose gels containing 0.71 μg/ml ethidium bromide. At the end of the runs, gels were examined using UV transillumination. The diphenyl amine (DPA) assay reaction suggested by Burton [13] and modified by Perandones et al. [45] was applied. The colorimetric reaction was measured spectrophotometrically at 575 nm and the percentage of DNA fragmentation was calculated.

2.5.3. RNA isolation

Hepatic tissue was ground in liquid nitrogen and total RNA was extracted from all groups of the experiment (five samples from each group). The extraction of total RNA was performed using BioZOL reagent according to the manufacturer's procedures. The concentration and purity of RNA were measured at 260/280 nm using ultraviolet spectrophotometer (ratios fell between 1.75 and 1.9, indicating very pure RNA in all cases). Equal amounts of RNA isolated from individual rat of each group were prepared for the semi-quantitative RT-PCR [12,27].

2.5.4. Semi quantitative RT-PCR

Semi quantitative RT-PCR was performed in a 20-μl volume that contained 5 μM oligo dT12-18, 2 μg total hepatic RNA, 200 U Superscript TM II reverse transcriptase (Life Technologies) at 42 °C for 10 min followed by 42 °C for 1 h. In a total volume of 20 μl, the PCR mixture contained 150 μM dNTPs, 1 μM antisense and sense primers for fatty acid synthase (Fas) or TNFα, 1 μl reverse-transcribed cDNA, and 2 U Taq polymerase (PE Applied Biosystems, Foster City, CA). The sequences of oligonucleotide primers were: FAS: sense 5′-CTCCAGACATTGTTC-3′, antisense 5′-CGCCTATGGTTGTTGACC-3′; TNFα: sense 5′- ACA GAA AGC ATG ATC CGC- 3′, antisense 5′-CTC GGA CCC CTG GAC GTA-3′; β-actin: sense 5′-CGTGACATCAAAGAGAAGCTGTGC-3′, antisense 5′-GCTCAGGAGGAGCAATGATCTTGAT-3′. Amplification conditions were (94 °C 15 s, 54 °C 1 min, 72 °C for 30 s) for 15–35 cycles. The expected amplicon lengths were 477 bp for Fas; 692 bp for TNFα and 376 bp for β-actin [1]. An aliquot of the semi quantitative RT-PCR reaction (10 μl) was separated on a 1.2% agarose gel containing ethidium bromide, visualized under UV light, and analyzed using NIH Image software.

2.6. Statistical analysis

All data were statistically analyzed by analysis of variance (ANOVA) using the General Linear Model Procedure of the Statistical Analysis System [49]. The significance of the differences among treatment groups was determined by Waller–Duncan *k*-ratio [60]. All statements of significance were based on probability of $P \leq 0.05$.

3. Results

The results of the current study revealed that various liver enzymes were seriously affected by AFs treatment while upon treatment with quercetin whether in low or high doses, a significant improvement was achieved. Significant increases ($P \leq 0.05$) in AST, ALT, ALP, LDH, total lipids, cholesterol, triglycerides, CEA and NO accompanied with a significant decrease in total protein were detected in animals fed AFs-contaminated diet (Table 1). Animals received quercetin at the two tested doses plus AFs showed a significant improvement in all biochemical parameters toward the normal value of the controls. Moreover, treatment with AFs resulted in a significant decrease ($P \leq 0.05$) in GPX and SOD activities in liver accompanied with a significant increase in MDA. Treatment with quercetin succeeded to induce a significant improvement in antioxidant parameters and oxidative stress markers (Table 2).

The results also indicated that rats fed AFs-contaminated diet showed a significant cytotoxicity as indicated by the depletion in DNA and RNA content in the hepatic tissue (Table 3). The results also revealed that AFs induced DNA damage as evaluated by measuring the level of fragmented DNA calorimetrically using diphenylamine (DPA) and by comparing DNA profiles on agarose gel electrophoresis. These results showed that AFs caused marked DNA fragmentation in the liver (39.40%) compared to untreated control rat (7.80) as indicated by DPA assay (Table 4). Treatment with quercetin significantly brought down the percentage of the DNA damage to 15.93% and 10.15% at the low and high doses, respectively. Moreover, the DNA fragmentation detected by gel electrophoresis as a DNA ladder representing a series of fragments that are multiples of 180–200 bp (Fig. 1) showed a significant

Table 1
Effect of oral treatment with quercetin on serum biochemical parameters in rats fed AFs-contaminated diet for 3 weeks.

Parameters	Groups					
	Control	AFs	QL	QH	AFs + QL	AFs + QH
AST (U/l)	153.77 ± 5.89[a]	234.6 ± 4.79[b]	157.59 ± 4.95[a]	156.35 ± 2.11[a]	170.22 ± 2.16[e]	155.81 ± 1.74[a]
ALT (U/l)	67.15 ± 1.42[a]	98.90 ± 1.76[b]	66.92 ± 1.95[a]	66.26 ± 1.33[a]	58.02 ± 0.55[c]	58.02 ± 0.88[c]
ALP (U/l)	164.17 ± 15.16[a]	231.06 ± 13.29[b]	161.54 ± 3.97[a]	164.63 ± 2.78[a]	168.68 ± 3.64[a]	166.63 ± 3.49[a]
LDH (IU/l)	2013.17 ± 162.53[a]	8024.52 ± 60.74[b]	2365.50 ± 5.70[c]	2795.21 ± 60.65[d]	4313.15 ± 73.70[f]	2557.39 ± 98.99[d]
TP (mg/dl)	6.08 ± 0.08[a]	3.84 ± 0.27[b]	7.19 ± 0.11[c]	7.32 ± 0.13[c]	7.41 ± 0.16[c]	7.66 ± 0.34[c]
TL (mg/dl)	148.05 ± 6.06[a]	185.34 ± 9.11[b]	136.09 ± 6.35[c]	150.39 ± 5.47[a]	133.12 ± 10.99[c]	129.55 ± 10.45[c]
TG (mg/dl)	34.35 ± 2.80[a]	92.05 ± 2.28[b]	31.13 ± 4.86[a]	32.06 ± 5.12[a]	49.91 ± 3.31[d]	39.53 ± 1.90[e]
Cho (mg/dl)	79.99 ± 5.45[a]	157.35 ± 3.96[b]	63.58 ± 1.90[c]	56.14 ± 2.10[d]	83.1 ± 1.26[e]	70.44 ± 1.82[f]
CEA (ng/ml)	5.09 ± 0.39[a]	14.31 ± 0.18[b]	4.1 ± 0.52[a]	4.30 ± 0.36[a]	5.46 ± 0.51[a]	5.66 ± 0.39[a]
NO (μmol/l)	18.20 ± 0.61[a]	29.31 ± 1.07[b]	17.17 ± 0.54[a]	15.07 ± 0.58[c]	16.72 ± 0.48[c]	15.11 ± 0.51[c]

Data are expressed as mean ± SE; $n = 8$.
Within each row, means superscripts with different letters are significantly different at $P < 0.05$ compared to the control group (a), AFs-treated group (b) and quercetin-treated groups (c and d).

Table 2
Effect of oral treatment with quercetin on hepatic GPX, SOD and MDA of rats fed AFs-contaminated diet for 3 weeks.

Parameters	Groups					
	Control	AFs	QL	QH	AFs + QL	AFs + QH
GPX (U/mg protein)	23.64 ± 0.72[a]	8.07 ± 1.55[b]	31.83 ± 0.51[c]	45.23 ± 1.03[d]	19.24 ± 1.50[e]	22.80 ± 2.48[a]
SOD (U/mg protein)	2.87 ± 0.13[a]	1.53 ± 0.11[b]	2.76 ± 0.16[a]	3.24 ± 0.17[c]	2.93 ± 0.12[a]	3.01 ± 0.17[c]
MDA (mol/mg protein)	11.53 ± 1.43[a]	28.27 ± 0.38[b]	11.73 ± 1.81[a]	11.86 ± 1.28[a]	14.23 ± 1.51[c]	13.52 ± 1.41[c]

Data are expressed as mean ± SE; $n = 8$.
Within each row, means superscripts with different letters are significantly different at $P < 0.05$ compared to the control group (a), AFs-treated group (b) and quercetin-treated groups (c and d).

increase in the group fed AFs-contaminated diet compared to the control and quercetin-treated groups. Treatment with quercetin resulted in a significant improvement in DNA fragmentation percentage in AFs-treated animals. This improvement was more pronounced in the group received the high dose of quercetin although this dose did not normalize the percentage of DNA fragmentation.

In the current study, bands produced from amplifying cDNA of fatty acid synthase (Fas), tumor necrosis factor (TNF) and the house keeping gene β-actin as a control were analyzed and the results of gene expression were based on quantifying the signal intensities in each band. The results were expressed as the ratio between maximum optical

Table 3
Effects of oral treatment with quercetin on nucleic acid contents in hepatic tissues of rats fed AFs-contaminated diet for 3 weeks.

Treatments	DNA (mg/g)	RNA (mg/g)
Control	0.356 ± 0.012[a]	0.189 ± 0.003[a]
AFs	0.170 ± 0.013[b]	0.109 ± 0.006[b]
QL	0.382 ± 0.015[a]	0.157 ± 0.009[c]
QH	0.308 ± 0.027[a]	0.187 ± 0.006[a]
AFs + QL	0.220 ± 0.011[c]	0.141 ± 0.010[c]
AFs + QH	0.277 ± 0.012[c]	0.190 ± 0.020[a]

Data are expressed as mean ± SE; $n = 8$.
Within each column, means superscripts with different letters are significantly different at $P < 0.05$ compared to the control group (a), AFs-treated group (b) and quercetin-treated groups (c and d).

Table 4
Effects of oral treatment with quercetin on DNA fragmentation in liver of rats fed AFs-contaminated diet for 3 weeks.

Treatments	DNA fragmentation (%)	Changes
Control	7.80 ± 1.43[a]	–
AFs	39.40 ± 1.91[b]	+31.60
QL	7.40 ± 1.21[a]	−0.40
QH	9.0 ± 0.84[c]	+1.20
AFs + QL	19.60 ± 1.12[d]	+11.80
AFs + QH	12.40 ± 1.21[e]	+4.60

Data are expressed as mean ± SE; $n = 8$.
Within each column, means superscripts with different letters are significantly different at $P < 0.05$ compared to the control group (a), AFs-treated group (b) and quercetin-treated groups (c and d).

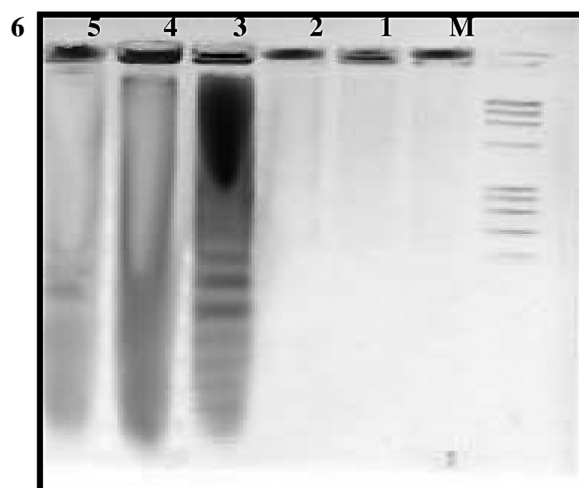

Fig. 1. Effects of oral treatment of quercetin on DNA fragmentation of hepatic tissue in rats fed AFs-contaminated diet for 3 weeks. Agarose gel electrophoretic pattern of DNA isolated from liver tissue of different groups. Lane M: phi x marker, Lane 1: Control, Lane 2: QL, Lane 3: QH, Lane 4: AFs, Lane 5: AFs + QL and Lane 6: AFs + QH.

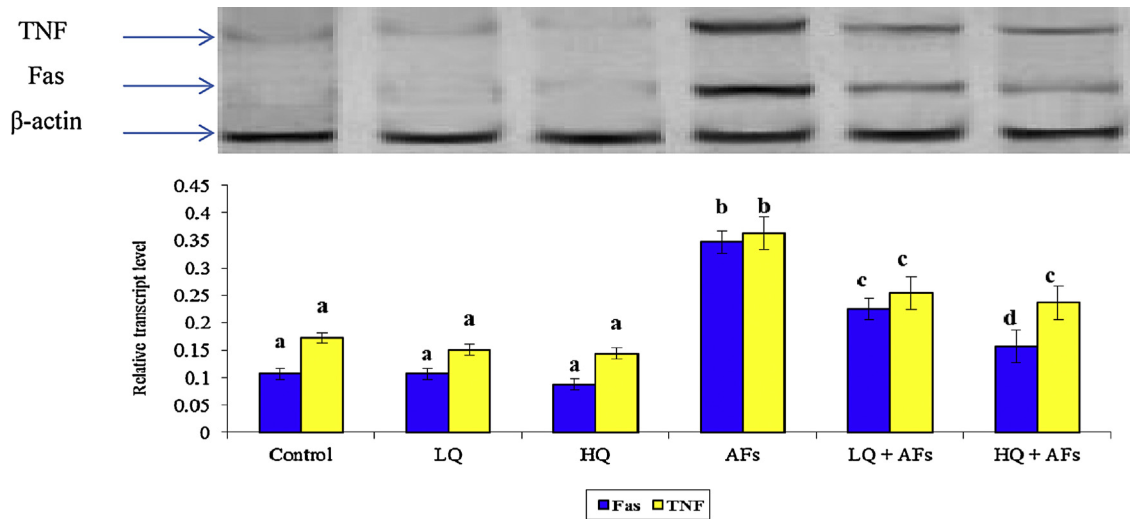

Fig. 2. Effects of oral treatment of quercetin on RNA gene expression of fatty acid synthase (Fas) and TNF in the liver of rats fed AFs-contaminated diet for 3 weeks. The results depicted are normalized to levels of β-actin gene. Data are mean of ratios of intensity for each gene divided by that for β-actin.

density (OD max) for each band of the target amplification product and the corresponding OD max of β-actin (Fig. 2). These results indicated that exposure to AFs resulted in a significant increased in mRNA expression of the Fas gene accompanied with increased in TNF gene expression in the hepatic tissues compared to the other experimental groups. Treatment with quercetin alone at the low and high doses induced insignificant changes in the expression of Fas and TNF genes compared to the control group. However, animals fed AFs and treated with quercetin showed a significant improvement in both Fas and TNF gene expression toward the control level. This improvement was more pronounced in the group received the high dose of quercetin (Fig. 2).

The biochemical results were confirmed by the histological, histochemical and electron microscopic examination of the liver tissue. The histological examination of the liver sections in the control rats (Fig. 3a) or those treated with quercetin at the low or high dose (Fig. 3b and c) showed normal structure of hepatocytes with no cellular infiltration around the central vein, portal area or in between the hepatocytes. The microscopic examination of liver sections from AFs-treated group showed vacuolation and mononuclear cellular infiltration in between the hepatocytes (Fig. 3d). The liver sections of the animals treated with AFs plus the low dose of quercetin showed prominent improve in hepatocytes (Fig. 3e) however, those treated with AFs plus the high dose of quercetin showed prominent improve in hepatocytes but the fibrous tissues are still present (Fig. 3f).

The histochemical examination of the liver section of the control rats (Fig. 4a), low dose of quercetin (Fig. 4b) and high dose of quercetin (Fig. 4c) stained with bromophenol blue stain for protein evaluation in the hepatocytes cytoplasm and nucleus membrane showed normal distribution of blue color. The liver section of rats treated with AFs showed a decrease in protein reaction in hepatocytes (Fig. 4d). However, the liver section of rats treated with AFs plus low dose (Fig. 4e) and high dose of quercetin (Fig. 4f) showed obvious improvements in protein reaction.

The electron microscopic examination of the liver sections of the control group (Fig. 5-1) showed normal hepatocytes with nucleus, mitochondria, cisternae of rough endoplasmic reticulum and numerous glycogen granules in the form of rosettes dispersed in the cytoplasm. The examination of liver of rat treated with quercetin at the low or high dose (Fig. 5-2) showed hepatocytes with normal kupffer cells. However, the liver of rats treated with AFs showed hepatocyte with abnormal vacuolated nucleus and loss of normal chromatin pattern (Fig. 5-3), cytoplasmic large and small fatty droplets, the endoplasmic profile and glycogen rosette were disappeared (Fig. 5-4). The liver of rat treated with AFs plus low dose of quercetin (Fig. 5-5) showed decrease in number of mitochondria and fragmented. The rough endoplasmic reticulum are ruptured and lost of its ribosomes and great increase in glycogen rosette. Moreover, the liver of rats treated with AFs plus high dose of quercetin (Fig. 5-6) showed the same picture of regeneration structure of organelles and the cytoplasm contains mitochondria and more or less aggregation of endoplasmic reticulum.

4. Discussion

Hepatocarcinogenesis is a multistage, multifactorial process, involving viral, chemical and several other factors, including aflatoxins, alcohol and tobacco consumption [62]. Nevertheless, special emphasis has been placed on aflatoxin, due to the frequency with which they occur as food contaminants, together with their potency as liver carcinogens in numerous animal species [1,3]. Previously, we have reported that aflatoxin administration resulted in excessive lipid peroxidation [25] with concomitant decrease in reduced glutathione (GSH) [3], increased protein oxidation and DNA damage [1] in rat liver. In the current study, the protective effects of quercetin against

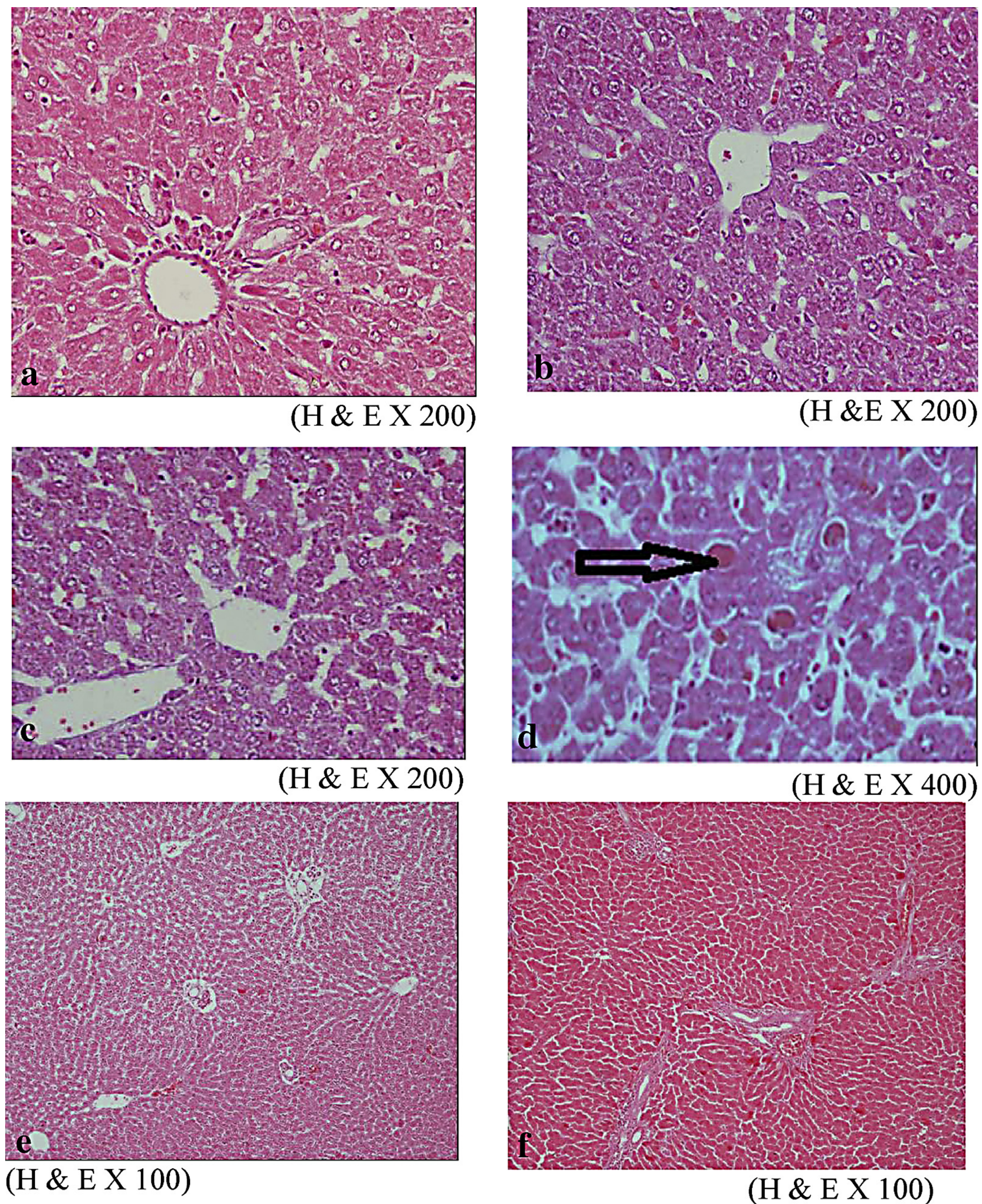

Fig. 3. Photomicrographs of liver sections from (a) control rats showing the normal structure of hepatocytes with no cellular infiltration around the central vein, portal area or in between the hepatocytes, (b) rats treated with low dose of quercetin, (c) rats treated with high dose of quercetin showing the normal structure of hepatocytes with no cellular infiltration around the central vein, portal area or in between the hepatocytes, (d) rats fed AFs-contaminant diet showing vacuolation and mononuclear cellular infiltration in between the hepatocytes (arrow), (e) rats fed AFs-contaminant diet plus low dose of quercetin and (f) rats received high dose of quercetin plus AFs showing almost a normal hepatocytes architecture and few fibrosis around blood vessels.

the toxicity resulted from the exposure to AFs in rat was evaluated. Test animals were given an extreme AFs challenge to ensure induction of severe responses. The selected doses of AFs and quercetin were literature based (Abdel-Wahhab et al. [3] and Choi et al. [19], respectively).

The current results indicated that quercetin alone had no significant effects on serum biochemical parameters of liver function in rats. Animals received AFs have shown a significant increase in ALT, AST and ALP. This increase in transaminases in AFs-treated animals is indicative of changes in the hepatic tissues [3]. These results clearly indicated that AFs has stressful effects on the hepatic tissue, consistent with those reported in the literature of mycotoxicosis. The significant increase in LDH, Cho, TriG, and TL in the group received AFs indicated severe hepatic parenchymal cells injury [5,8]. Whereas, the decrease in total protein indicated liver necrosis and/or kidney dysfunction [5,50,58].

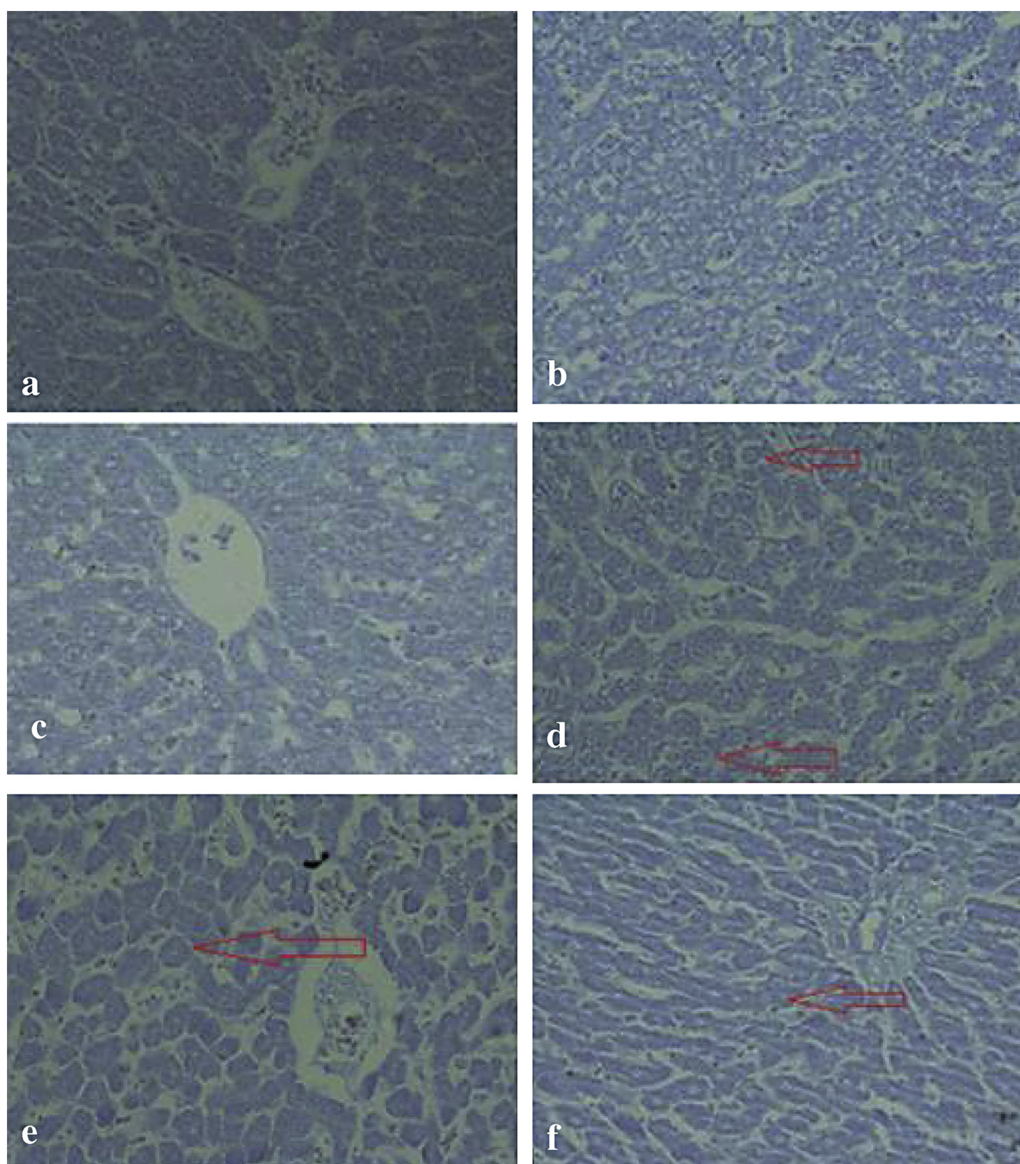

Fig. 4. Photomicrographs of liver sections stained with bromophenol blue stain for protein evaluation in the hepatocytes, cytoplasm and nucleus membrane from (a) control rats, (b) rats treated with low dose of quercetin, (c) rats treated with high dose of quercetin showing normal distribution of blue color, (d) rats Fed AFs-contaminated diet showing a decrease in protein reaction in hepatocytes, (e) rats fed AFs-contaminated diet plus low dose of quercetin and (f) rats fed AFs-contaminated diet high dose of quercetin showing obvious improvements in protein reaction (bromophenol blue stain 400×).

The present study has also revealed that animals fed AFs contaminated-diet have shown a significance increase in CEA level in serum, which is considered a specific biomarker for liver cancer and is synthesized mainly in the fetal stage; practically no production of this marker occurs in normal adult. Similar elevation in serum CEA was reported in rats treated with AFs [2]. Moreover, NO was found to be increased significantly in the animals treated with AFs. Although the role of NO in cell death is complex, the increased in NO level reported herein in the animals fed AFs-contaminated diet has suggested that this mycotoxin preferentially affect macrophage functions. On the other hand, the decrease in GPX and SOD in the liver of AFs-treated rats might indirectly lead to an increase in oxidative DNA damage [25]. Some studies on the mechanisms of mycotoxins-induced liver injury have demonstrated that glutathione and SOD play an important role in the detoxification of the reactive and toxic metabolites of this mycotoxin, and that the liver necrosis begins when the glutathione stores are almost exhausted [3].

Treatment with quercetin resulted in a significant improvement in lipid profile in AFs-treated animals. Therefore, the antioxidant efficacy of quercetin may be due to its higher diffusion into the membranes [40] allowing it to scavenge oxyradicals at several sites through the lipid bilayer. It can be also resulting from its pentahydroxyflavone structure allowing it to chelate metal ions via the orthodihydroxy phenolic structure and by

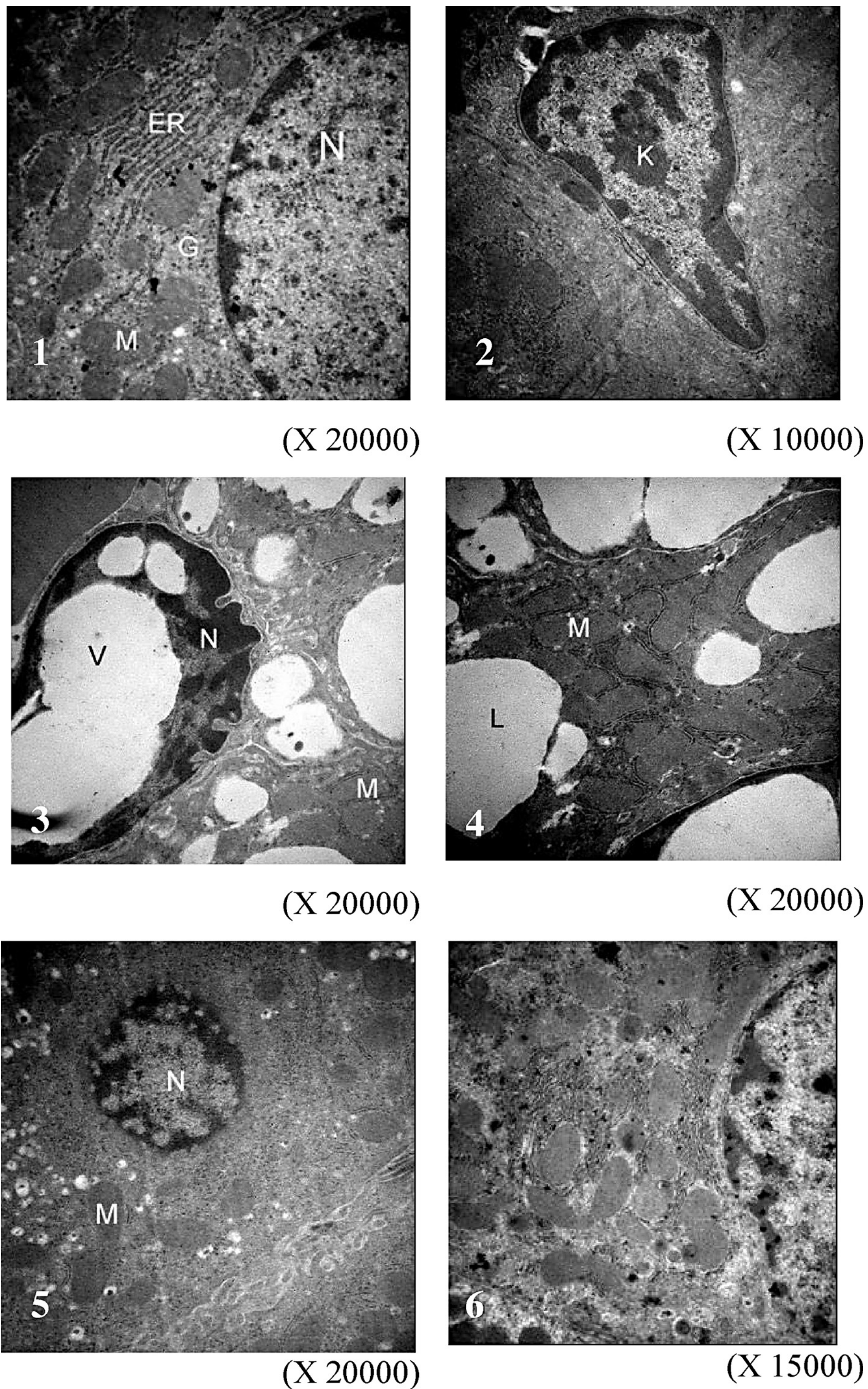

Fig. 5. TEM of an ultra thin section in a liver of: (1) control rats showing hepatocytes with nucleus (N), many mitochondria (M), cisternae of rough endoplasmic reticulum (ER) and numerous glycogen granules in the form of rosettes dispersed in the cytoplasm (G), (2) rats treated with quercetin at the low or high dose showing hepatocytes with normal kupffer cells, (3) rats treated with AFs showing hepatocyte with abnormal vacuolated nucleus (N) with loss of normal chromatin pattern, (4) rats treated with AFs showing cytoplasmic large and small fatty droplets. The endoplasmic profile and glycogen rosette are disappeared, and (5) rats treated with AFs plus low dose of quercetin showing nucleus, decreased in number of mitochondria and fragmented. The rough endoplasmic reticulum are ruptured and lost of its ribosomes and great increase in glycogen rosette and (6) rats treated with AFs plus high dose of quercetin showing same picture of regeneration structure of organelles. The cytoplasm contains mitochondria and more or less aggregation of endoplasmic reticulum.

scavenging lipid alkoxyl and peroxyl radicals [15,47]. It was also suggested that quercetin acts as antioxidant by inhibiting oxidative enzymes such as xanthine oxidase, lipoxygenase and NADPH oxidase. Inhibition of these enzymes is also responsible for the attenuation of oxidative stress as they play key roles in the initial process of free radical-induced cellular damage [20]. Further, it has been reported that quercetin metabolites can also inhibit peroxynitrite-mediated oxidation, similar to free quercetin [32]. Besides direct hydrogen-donating properties, more attention has been focused on the influence of quercetin on signaling pathways and its indirect interaction with the endogenous antioxidant defense system [17]. A previous study suggested that polyphenols interact with cellular defense systems such as phases I (mainly the CYP450 complex enzymes) and II (e.g., glutathione transferases and glucuronyl transferases) detoxifying enzymes [46]. In particular, quercetin has been shown to bind directly to aryl hydrocarbon receptor (AhR) as natural ligand, thereby eliciting an induction of xenobiotic responsive elements (XRE) in CYP450 family genes [41].

In the same concern, Myhrstad et al. [43] showed that flavonoids such as quercetin increase the expression of the rate limiting enzyme in the synthesis of GSH; c-glutamylcysteine synthetase. Quercetin, being the most potent of the tested flavonoids, has been found to increase the expression of c-glutamylcysteine synthetase with a concomitant increase in the intracellular glutathione concentrations [42]. In the present work, quercetin protected liver from damage might be a consequence of the stabilization in the redox state and maintenance of the antioxidant capacity offered. It could be also attributed to calcium channel blocking activity exerted by quercetin [7]. Calcium contents in liver cells are liable to be increased during the process of experimental liver damage, and calcium channel blocking drugs were found to inhibit the development of hepatic damage induced by different hepatotoxins [44,57].

Glutathione is a cysteine-containing tripeptide that plays a critical role in the protection of tissues by directly interacting with ROS or as a cofactor for enzymatic detoxification [33,52]. Superoxide dismutase (SOD) converts superoxide radicals to H_2O_2, which induces hydroxyl radicals by Fenton and/or Haber–Weiss reactions if the agent is not removed by CAT and/or GSH [31]. Here, we showed that the levels of GPX and SOD were diminished in AFs-treated rats; whereas, this reduction was significantly prevented by co-administration with quercetin. On the other hand, lipid peroxidation is believed to be one of the main markers of ROS-mediated damage [4,24]. These reports with our present data lead to a possibility that quercetin may participate in the reduction of AFs toxicity in part by decreasing oxidative stress through enhancement of antioxidant defense systems. These results strongly suggest that quercetin metabolites rather than free quercetin are closely associated with activation of antioxidant defense systems as well as inhibition of lipid peroxidation in AFs-treated animals.

The histological, histochemical and electron microscopy results reported in the current study revealed that quercetin had no harmful effects on liver tissues. However, the liver of the animals in the AFs-treated group showed severe histological and histochemical changes typical to those reported in the literature. It was documented that AFB_1 treatment induced a severe cytotoxicity and inhibition of hepatocytes cell proliferation [5,38]. In the current study, treatment with AFs resulted in vacuolation and mononuclear cellular infiltration in between the hepatocytes and decrease in protein reaction in hepatocytes with abnormal vacuolated nucleus and loss of the normal chromatin pattern. Moreover, the cytoplasmic showed large and small fatty droplets and the endoplasmic profile and glycogen rosette disappeared. Similar to the current observations, Mayura et al. [38] and Abdel-Wahhab et al. [3] observed extensive bile duct proliferation and periportal hepatocellular degeneration in the livers of rats treated with AFB_1. Treatment with quercetin at the two tested doses overcomes these histological and histochemical changes in a dose dependent manner. In general, the antioxidant activities of phenolics are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl [56]. A direct relationship has been found between the phenolic content and antioxidant capacity of plants [21,51,55].

5. Conclusion

It could be concluded that treatment with AFs resulted in a significant cytotoxic, biochemical, histological and histochemical changes typical to those reported in the literature. Quercetin succeeded to induce a hepatoprotective effect against AFs-induced cytotoxicity and oxidative stress damage in liver tissue in a dose dependent manner. Quercetin itself was safe at the tested doses and it may be a candidate for the prevention as well as treatment of liver diseases.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgment

This work was supported by National Research Center, Dokki, Giza, Egypt. Project No. S90402.

References

[1] S.H. Abdel-Aziem, A.M. Hassan, M.A. Abdel-Wahhab, Dietary supplementation with whey protein and ginseng extract counteracts the oxidative stress and DNA damage in rats fed aflatoxins-contaminated diet, Mutat. Res. 723 (2011) 65–71.

[2] M.A. Abdel-Wahhab, H.H. Ahmed, M.M. Hagazi, Prevention of aflatoxin B_1-initiated hepatotoxicity in rat by marine algae extracts, J. Appl. Toxicol. 26 (3) (2006) 229–238.

[3] M.A. Abdel-Wahhab, N.S. Hassan, A.A. El-Kady, Y.A. Khadrawy, A.A. El-Nekeety, S.R. Mohamed, H.A. Sharaf, F.A. Mannaa, Red ginseng

extract protects against aflatoxin B_1 and fumonisins-induced hepatic pre-cancerous lesions in rats, Food Chem. Toxicol. 48 (2) (2010) 733–742.
[4] M.A. Abdel-Wahhab, A.A. Ibrahim, A.A. El-Nekeety, N.S. Hassan, A.A. Mohamed, *Panax ginseng* C.A. Meyer extract counteracts the oxidative stress in rats fed multi-mycotoxins-contaminated diet, Comun. Sci. 3 (3) (2012) 143–153.
[5] M.A. Abdel-Wahhab, E.A. Omara, M.M. Abdel-Galil, N.S. Hassan, S.A. Nada, A. Saeed, M.M. El-Sayed, *Zizyphus Spina-Christi* extract protects against aflatoxin B_1-inhibited hepatic carcinogenicity, Afr. J. Tradit. Complement Altern. Med. 4 (3) (2007) 248–256.
[6] M.A. Abdel-Wahhab, A. Saeed, A. Hufner, NMR and radical scavenging activities of Patuletin from *Urtica urens* L. against aflatoxin B_1, Pharm. Biol. 43 (6) (2005) 515–525.
[7] I. Baranm, C. Ganea, RyR3 *in situ* regulation by Ca^{2+} and quercetin and the RyR3-mediated Ca^{2+} release flux in intact Jurkat cells, Arch. Biochem. Biophys. 540 (1-2) (2013) 145–159.
[8] C.C. Barton, E.X. Barton, P.E. Ganey, S.L. Kunkel, R.A. Roth, Bacterial lipopolysaccharide enhances aflatoxin B_1 hepatotoxicity in rats by a mechanism that depends on tumor necrosis factor, Hepatology 33 (1) (2001) 66–73.
[9] S.C. Bischoff, Quercetin: potentials in the prevention and therapy of disease, Curr. Opin. Clin. Nutr. Metab. Care 11 (2008) 733–740.
[10] A.W. Boots, G.R. Haenen, A. Bast, Health effects of quercetin: from antioxidant to nutraceutical, Eur. J. Pharmacol. 585 (2008) 325–337.
[11] A. Bregman, Laboratory Investigation and Cell Biology, John Wiley and Sons, New York, 1983, pp. 51–60.
[12] M.E. Brun, S. Gasca, C. Girard, K. Bouton, B. De Massy, A. De Sario, Characterization and expression analysis during embryo development of the mouse ortholog of MLL3, Gene 371 (2006) 25–33.
[13] K. Burton, A study of the conditions and mechanisms of the diphenylamine reaction for the estimation of deoxyribonucleic acid, Biochem. J 62 (1956) 315–323.
[14] W.F. Busby, G.N. Wogon, in: C.E. Searle (Ed.), Chemical Carcinogenesis, 2nd ed., American Chemical Society, Washington, DC, 1985, pp. 945–1136.
[15] G. Cao, E. Sofic, R.L. Prior, Antioxidant and prooxidant behavior of flavonoids: structure–activity relationships, Free Radic. Biol. Med. 22 (1997) 749–760.
[16] CAST, Mycotoxins: risks in plant, animal, and human systems, in: J.L. Richard, G.A. Payne (Eds.), Council for Agricultural Science and Technology Task Force Report No. 139, Ames, IA, USA, 2003, ISBN: 1-887383-22-0.
[17] J.C. Chen, F.M. Ho, L.C. Pei-Dawn, C.P. Chen, K.C. Jeng, H.B. Hsu, S.T. Lee, T.W. Wen, W.W. Lin, Inhibition of iNOS gene expression by quercetin is mediated by the inhibition of IjB kinase, nuclear factor-kappa B and STAT1, and depends on heme oxygenase-1 induction in mouse BV-2 microglia, Eur. J. Pharmacol. 521 (2005) 9–20.
[18] X. Chen, Protective effects of quercetin on liver injury induced by ethanol, Pharmacogn. Mag. 6 (2010) 135–141.
[19] K.C. Choi, W.T. Chung, J.K. Kwon, J.Y. Yu, Y.S. Jang, S.M. Park, S.Y. Lee, J.C. Lee, Inhibitory effects of quercetin on aflatoxin B_1-induced hepatic damage in mice, Food Chem. Toxicol. 48 (2010) 2747–2753.
[20] A.J. Day, F.J. Canada, J.C. Diaz, P.A. Kroon, R. Mclauchlan, C.B. Faulds, G.W. Plumb, M.R.A. Morgan, G. Williamson, Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase, FEBS Lett. 468 (2000) 166–170.
[21] M. Del Carmen García-Rodríguez, T. Nicolás-Méndez, A.R. Montaño-Rodríguez, M.A. Altamirano-Lozano, Antigenotoxic effects of (−)-epigallocatechin-3-gallate (EGCG), quercetin, and rutin on chromium trioxide-induced micronuclei in the polychromatic erythrocytes of mouse peripheral blood, J. Toxicol. Environ. Health A 77 (6) (2014) 324–336.
[22] R. Drury, E. Wallington, R. Cancerson, Carleton's Histological Technique, 4th ed., Oxford University Press, London, 1976.
[23] D.L. Eaton, E.P. Gallagher, Mechanisms of aflatoxin carcinogenesis, Annu. Rev. Pharmacol. Toxicol. 34 (1994) 135–172.
[24] E.S. El-Denshary, M.A. Al-Gahazali, F.A. Mannaa, H.S. Salem, N.S. Hassan, M.A. Abdel-Wahhab, Dietary honey and ginseng protect against carbon tetrachloride-induced hepatonephrotoxicity in rats, Exp. Toxicol. Pathol. 64 (2012) 753–760.
[25] A.A. El-Nekeety, S.R. Mohamed, A.S. Hathout, N.S. Hassan, S.A. Aly, M.A. Abdel-Wahhab, Antioxidants properties of *Thymus vulgaris* oil against aflatoxin-induced oxidative stress on male rats, Toxicon 57 (2011) 984–991.
[26] J. Grimaud, Methods in Cellular Biology, European Course of Cellular Pathology, 1990, pp. 59.
[27] A.M. Hassan, S.H. Abdel-Aziem, M.A. Abdel-Wahhab, Modulation of DNA damage and alteration of gene expression during aflatoxicosis via dietary supplementation of *Spirulina* (Arthrospira) and whey protein concentrate, Ecotoxicol. Environ. Saf. 79 (2012) 294–300.
[28] J.E. Hustchins, W.M. Hagler Jr., Rapid liquid chromatographic determination of aflatoxins in heavily contaminated corn, J. Assoc. Off. Anal. Chem. 66 (1983) 1458–1465.
[29] M.K. Hwang, N.R. Song, N.J. Kang, K.W. Lee, H.J. Lee, Activation of phosphatidylinositol 3-kinase is required for tumor necrosis factor-a-induced upregulation of matrix metalloproteinase-9: its direct inhibition by quercetin, Int. J. Biochem. Cell Biol. 41 (2009) 1592–1600.
[30] IARC, Evaluation of carcinogen risks to humans. Some naturally occurring substances; foods, items and constituents, heterocyclic aromatic amines and mycotoxins IRAC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 56, IRAC, Lyon, France, 1993, pp. 243–395.
[31] F. Johnson, C. Giulivi, Superoxide dismutases and their impact upon human health, Mol. Aspects Med. 26 (2005) 340–352.
[32] L.O. Klotz, H. Sies, Defenses against peroxynitrite: selenocompounds and flavonoids, Toxicol. Lett. 140 (2003) 125–132.
[33] P. Larsson, L. Busk, H. Tjklve, Hepatic and extrahepatic bioactivation and GSH conjugation of aflatoxin B_1 in sheep, Carcinogenesis 15 (1994) 947–955.
[34] C.C. Lin, Y.F. Hsu, T.C. Lin, F.L. Hsu, H.Y. Hsu, Antioxidant and hepatoprotective activity of *punicalagin* and *punicalin* on carbon tetrachloride-induced liver damage in rats, J. Pharm. Pharmacol. 50 (1998) 789–794.
[35] S. Liu, W. Hou, P. Yao, B. Zhang, S. Sun, A.K. Nussler, L. Liu, Quercetin protects against ethanol-induced oxidative damage in rat primary hepatocytes, Toxicol. In Vitro 24 (2010) 516–522.
[36] J. Lu, Y. Zheng, L. Lou, D. Wu, D. Sun, Y. Feng, Quercetin reverses D-galactose-induced neurotoxicity in mouse brain, Behav. Brain Res. 171 (2006) 251–260.
[37] C. Manach, O. Texier, C. Morand, V. Crespy, F. Regerat, C. Demigne, C. Remesy, Comparison of the bioavailability of quercetin and catechin in rats, Free Radic. Biol. Med. 27 (1999) 1259–1266.
[38] K. Mayura, M.A. Abdel-Wahhab, K.S. McKenzie, A.B. Sarr, J.F. Edwards, K. Naguib, T.D. Phillips, Prevention of maternal and developmental toxicity in rats via dietary inclusion of common aflatoxin sorbents: potential for hidden risks, Toxicol. Sci. 41 (1998) 175–182.
[39] M.F. Molina, I. Sanchez-Reus, I. Iglesias, J. Benedi, Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver, Biol. Pharm. Bull. 26 (2003) 1398–1402.
[40] M.Y. Moridani, J. Pourahmad, H. Bui, A. Siraki, P.J. O'Brien, Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers, Free Radic. Biol. Med. 34 (2003) 243–253.
[41] J.O. Moskaug, H. Carlsen, M. Myhrstad, R. Blomhoff, Molecular imaging of the biological effects of quercetin and quercetin-rich foods, Mech. Ageing Dev. 125 (2004) 315–324.
[42] J.O. Moskaug, H. Carlsen, M. Myhrstad, R. Blomhoff, Polyphenols and glutathione synthesis regulation, Am. J. Clin. Nutr. 81 (2005) 277S–283S.
[43] M.C. Myhrstad, H. Carlsen, O. Nordstrom, R. Blomhoff, J.J. Moskaug, Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter, Free Radic. Biol. Med. 32 (2002) 386–393.
[44] T. Ohyama, K. Sato, K. Kishimoto, Y. Yamazaki, N. Horiguchi, T. Ichikawa, S. Kakizaki, H. Takagi, T. Izumi, M. Mori, Azelnidipine is a calcium blocker that attenuates liver fibrosis and may increase antioxidant defence, Br. J. Pharmacol. 165 (4b) (2012) 1173–1187.
[45] C.E. Perandones, A.V. Illera, D. Peckham, L.L. Stunzl, R.F. Ashman, Regulation of apoptosis *in vitro* in mature murine spleen T-cell, J. Immunol. 151 (1993) 3521–3529.
[46] J.L. Raucy, Regulation of CYP3A4 expression in human hepatocytes by pharmaceuticals and natural products, Drug Metab. Dispos. 31 (2003) 533–539.
[47] R. Ravichandran, M. Rajendran, D. Devapiriam, Antioxidant study of quercetin and their metal complex and determination of stability constant by spectrophotometry method, Food Chem. 146 (2014) 472–478.
[48] S. Rawal, J.E. Kim, R. Coulombe, AFB_1 in poultry: toxicology, metabolism and prevention, Res. Vet. Sci. 89 (2010) 325–331.
[49] SAS, SAS Institute, Inc., SAS User's Guide: Statistics, SAS Institute, Cary, NC, USA, 1982.
[50] S. Shyamal, P.G. Latha, S.R. Suja, V.J. Shine, G.I. Anuja, S. Sini, S. Pradeep, P. Shikha, S. Rajasekharan, Hepatoprotective effect of three

herbal extracts on aflatoxin B_1-intoxicated rat liver, Singapore Med. J. 51 (2010) 326–331.
[51] K. Sikder, N. Das, S.B. Kesh, S. Dey, Quercetin and beta-sitosterol prevent high fat diet induced dyslipidemia and hepatotoxicity in Swiss albino mice, Indian J. Exp. Biol. 52 (1) (2014) 60–66.
[52] M.T. Siu, A.M. Shapiro, M.J. Wiley, P.G. Wells, A role for glutathione, independent of oxidative stress, in the developmental toxicity of methanol, Toxicol. Appl. Pharmacol. 273 (3) (2013) 508–515.
[53] S. Sreelatha, P.R. Padma, M. Umadevi, Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats, Food Chem. Toxicol. 47 (2009) 702–708.
[54] R.D. Stubblefield, O.L. Shotwell, C.W. Hesseltine, M.L. Smith, H.H. Hall, Production of aflatoxin on wheat and oats: measurement with a recording densitometer, Appl. Microbiol. 15 (1967) 186–190.
[55] K.M. Surapaneni, M. Jainu, Comparative effect of pioglitazone, quercetin and hydroxy citric acid on the status of lipid peroxidation and antioxidants in experimental non-alcoholic steatohepatitis, J. Physiol. Pharmacol. 65 (1) (2014) 67–74.
[56] D. Tátraaljai, E. Földes, B. Pukánszky, Efficient melt stabilization of polyethylene with quercetin, a flavonoid type natural antioxidant, Polym. Degrad. Stab. 102 (2014) 41–48.
[57] N. Thialbut, G. Peytavin, J.R. Glaude, Calcium channel blocking agents protect against acetaminophen-induced cytotoxicity in rat hepatocytes, J. Biol. Toxicol. 6 (1991) 237–238.
[58] C. Toskulkao, P. Lohakachonpan, T. Glinsukon, Time-course effects of ethanol pretreatment on hepatic necrosis and fat accumulation induced by aflatoxin B_1 in the rat, Toxicol. Lett. 55 (1999) 1–9.
[59] A. Vidhya, M. Indira, Protective effect of quercetin in the regression of ethanol-induced hepatotoxicity, Indian J. Pharm. Sci. 71 (2009) 527–532.
[60] R.A. Waller, D.B. Duncan, A Bayes rule for the symmetric multiple comparison problems, J. Am. Stat. Assoc. 64 (1969) 1484–1503.
[61] WHO, Forty-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, WHO, Geneva, 1998.
[62] J.H. Williams, T.D. Phillips, P.E. Jolly, J.K. Stiles, C.M. Jolly, D. Aggarwal, Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions, Am. J. Clin. Nutr. 80 (2004) 1106–1122.
[63] P. Yao, A. Nussler, L. Liu, L. Hao, F. Song, A. Schirmeier, N. Nussler, Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways, J. Hepatol. 47 (2007) 253–261.