Arabian Journal of Chemistry (2011) **4**, 1–10

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Therapeutic ability of some plant extracts on aflatoxin B1 induced renal and cardiac damage

Nadia Ameen Abdulmajeed

Department of Biochemistry, Girls College of Education, King Abdulaziz University, Jeddah, Saudi Arabia

Received 6 June 2010; accepted 7 June 2010
Available online 15 June 2010

KEYWORDS

Aflatoxicosis;
Antioxidants;
Energy metabolism;
Glycolytic enzymes;
Plant extracts

Abstract The aim of the study was to investigate the therapeutic effect of aqueous extracts of some traditional medicinal plants (including *Camellia sinensis* leaves, *Carum carvi* seeds, *Alpinia galangal* rhizomes, *Boswellia serrata* resins and *Cenchona officinalis* bark) compared to the anticancer drug, methotrexate (MTX) against aflatoxicosis induced by aflatoxin-B1 (AFB1) in both kidneys and hearts of rats. Administration of AFB1 induces oxidative stress in kidneys of AFB1-treated rats through elevating the level of malondialdehyde (MDA) and depleting the levels of tissue antioxidants, glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G-6-PDH) and vitamin C. Also the result revealed that aflatoxicosis interfere with the cellular energy supply of rat hearts through its inhibitory action on some markers of energy metabolism indicated by a decrease in glucose and glycogen contents of heart and a reduction in the activities of some glycolytic enzymes, phosphogluco-isomerase (PGI), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) compared to normal healthy animals.

Supplementation of the aqueous extracts of the different plant used, effectively ameliorated the deviation induced in both kidneys and hearts of animals in response to AFB1 administration. This effect was evident through reducing MDA level and up-regulating the inhibitory effect of AFB1 on the levels of antioxidants in kidneys as well as the energetic biomarkers in hearts. However, administration of MTX to AFB1-treated rats dramatically amplified the toxic effect of aflatoxicosis, indicated by marked increment in MDA level and decrease in the levels of antioxidants in kidneys of AFB1-MTX group in relation to AFB1 group. Also the same response was found in the bioenergetic markers of hearts. From the current investigation, it can be suggested that supplementation of

E-mail address: n-abdulmajed@hotmail.com

doi:10.1016/j.arabjc.2010.06.005

Production and hosting by Elsevier

the extracts of the different plants presented in this study was beneficial in modulating the alterations induced in kidney and heart under the toxic effects of AFB1.

1. Introduction

Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*. There are four naturally occurring aflatoxins, the most toxic being aflatoxin B1 (AFB1), and three structurally similar compounds namely aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Aflatoxins not only contaminate our food stuffs but are also found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals (Bennett and Klich, 2003; Fink-Gremmels, 1999).

Aflatoxins are well known to be potent mutagens, carcinogenic, teratogenic, immunosuppressive and also inhibit several metabolic systems, causing liver, kidney and heart damage. These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (Minto and Townsend, 1997; Wogan, 1999; Bintvihok, 2002; Wangikar et al., 2005; Salunkhe et al., 1987).

The mechanism of AFB1 toxic effect has been extensively studied. It has been shown that AFB1 is activated by hepatic cytochrome P450 enzyme system to produce a highly reactive intermediate, AFB1-8,9-epoxide, which subsequently binds to nucleophilic sites in DNA, and the major adduct 8,9-dihydro-8-(N7guanyl)-9-hydroxy-AFB1 (AFB1 N7-Gua) is formed. The formation of AFB1-DNA adducts is regarded as a critical step in the initiation of AFB1-induced carcinogenesis (Sharma and Farmer, 2004; Klein, 2002; Preston and Williams, 2005).

Although the mechanism underlying the toxicity of aflatoxins is not fully understood, several reports suggest that toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2) during the metabolic processing of AFB1 by cytochrome P450 in the liver. These species may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis (Towner et al., 2003; Sohn et al., 2003; Berg et al., 2004).

Natural substances that can prevent AFB1 toxicity would be helpful to human and animal health with minimal cost in foods and feed. Traditional medicinal plants were used by some authors for their antifungal, anti-aflatoxigenic and antioxidant activity (Joseph et al., 2005; Kumar et al., 2007).

Green tea (*Camellia sinensis*, family Theaceae) has been found to possess many health benefits including protection of oxidative DNA damage, lowering atherosclerotic index, and improving blood flow, liver function and oral health. It also has antimicrobial and antioxidant activities (Dufresne and Farnworth, 2001; McKay and Blumberg, 2002; Almajano et al., 2008; Panza et al., 2008).

The functional properties of tea are due to its polyphenols. Tea catechins are the principal tea polyphenols, which are mainly present in green tea and exhibit the most effective antioxidant activity compared to other tea polyphenols. Among tea catechins, epigallocatechin gallate (EGCG) is the most abundant and efficient catechin (Almajano et al., 2008; Panza et al., 2008).

Caraway (*Carum carvi* L., family, Umbelliferae) is another traditional medicinal plant. The aqueous extract of caraway is used as an aperitif, tranquilizer, carminative, diuretic. The seeds are used to treat ailments such as flatulence, colic pain and bronchitis (Bellakhdar et al., 1997; Lahlou et al., 2007; de Carvalho and da Fonseca, 2006).

Galangal (*Alpinia galangal*, family, Zingiberaceae) has traditionally been used as spice foods. This spice is, like other spices, rich in phenolic compounds such as flavonoids and phenolic acids which contribute to the overall its antioxidant activities (Juntachote and Berghofer, 2005). Galangal is used for medical purposes such as carminative, stomachic (Yang and Eilerman, 1999) antispasmodic, antiphlogistic and antimicrobial (Viljoen et al., 2003; Oonmetta-aree et al., 2006).

Frankincense (*Boswellia serrata*, family, Frankincense) is a gum resin secreted by trees of the genus *Boswellia* of Burseraceae. It has been used for therapeutic purposes as a remedy, especially in the traditional Ayurvedic medicine of India. It was reported that frankincense exerted anti-inflammatory, antidiabetic, antiarthritic, anticancer and immunomodulatory activities in animals (Al-Wadi and Gumaa, 1987; Reddy et al., 1986; Hostanska et al., 2002; Miklaeil et al., 2003).

The active principles, boswellic acids, prevent endotoxin-/galactosamine-induced hepatitis in mice (Safayhi et al., 1991). It also exhibits marked sedative and analgesic effects (Knaus and Wagner, 1996). *Boswellia* has also been observed to inhibit human leukocyte elastase (HLE), which may be involved in the pathogenesis of emphysema. It also stimulates mucus secretion and thus may play a role in cystic fibrosis, chronic bronchitis and acute respiratory distress syndrome (Safayhi et al., 1991, 1997; Knaus and Wagner, 1996; Rall et al., 1996).

Cinchona (*Cenchona officinalis*, family, Rubiaceae) is another medicinal plant used for treatment of some diseases. It exhibits antimicrobial activity against bacteria and fungi. The bark of cinchona contains the alkaloid quinine along with several other alkaloids effective against malaria and other parasitic infection, quinovic acid is effective as antitumor and quinidine is used in the treatment of heart diseases (Rojas et al., 2006; Mturi et al., 2002; Trepardoux, 2002; Raffauf et al., 1978; Prinz, 1990).

Methotrexate (MTX) is widely used as a cytotoxic chemotherapeutic agent to treat various neoplastic diseases such as acute lymphoblastic leukemia, lymphoma, solid cancers and autoimmune diseases such as psoriasis and rheumatoid arthritis (Antunes et al., 2002; Brock and Jenning, 2004; Doan and Massarotti, 2005). However, the efficacy of this agent often is limited by severe side effects and toxic sequelae. Since the cytotoxic effect of MTX is not selective for the cancer cells, it also affects the normal tissues.

MTX administration has been reported to cause acute renal failure, hepatotoxicity characterised by necrosis and fibrosis, neurotoxicity and intestinal mucosa oxidative stress (Walker et al., 2000; Sener et al., 2006; Jahovic et al., 2003; Vezmar et al., 2003; Uzar et al., 2006; Miyazono et al., 2004; Cetiner et al., 2005).

Relatively little is known about metabolic alterations occurring in kidney and heart during aflatoxicosis. So the objective of this study is to evaluate the metabolic disorders induced pathology in kidneys and hearts of rats by the exposure to AFB1. Also, the therapeutic benefits of some medicinal plant aqueous extracts in comparison to the currently available anticancer drug, MTX, against these disorders were also investigated.

This can be achieved through measuring some markers of oxidative tissue damage in kidney such as thiobarbituric acid reactive substances (TBARS, index of lipid peroxidation), the antioxidant markers, glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G-6-PDH), vitamin C and some indices of energy metabolism in heart [as glycogen, glucose and the glycolytic enzymes, phosphogluco-isomerase (PGI), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH)].

2. Materials and methods

2.1. Chemicals

All chemicals used were of high analytical grade, product of Sigma (US), Merk (Germany) and BDH (England). AFB1 was obtained from Sigma chemical company (St. Louis, Missouri, USA).

2.2. Preparation of plant extracts

Twenty grams of dried plants were slowly boiled in 200 ml of distilled water and heated for 30 min. The extracts were then centrifuged, filtered, frozen at −20 °C and then lyophilized. The obtained dry extract was dissolved in water and used directly for the treatment of animals using a particular dose according to (Paget and Barnes, 1964).

2.3. Animals

Eighty male albino rats of Sprague–Dawley strain weighing 100–120 g were used for this study. Animals were housed in clean metabolic cages and maintained under standard conditions (23 ± 2 °C and 12 h light/dark cycles). They were given standard pellet diet and water *ad libitum* and kept for two weeks to acclimatize to the environmental conditions.

2.3.1. Experimental design

The animals were divided into 8 groups:

- Group1: normal control rats.
- Group2: aflatoxin-treated group (1 mg/kg body weight of rats).
- Group 3: aflatoxin-green tea leaves (*Camellia sinensis*) treated group (200 mg/kg body weight of rats).
- Group 4: aflatoxin-caraway seeds (*Carum carvi*) treated group (2 mg/kg body weight of rats).
- Group 5: aflatoxin-galangal rhizomes (*Alpinia galanga*) treated group (1 mg/kg body weight of rats).
- Group 6: aflatoxin-cinchona bark (*Cinchona officinalis*) treated group (1 g/kg body weight of rats).
- Group 7: aflatoxin-frankincense resins (*Boswellia serrata*) treated group (1.25 g/kg body weight of rats).
- Group 8: aflatoxin-methotrexate treated group (0.5 mg/kg body weight of rats).

Aflatoxin B1 was administered intraperitoneally at a single dose (1 mg/kg) to the rats of different experimental groups except normal control group which injected intraperitoneally with normal saline, 0.9% NaCl. The extracts of different plants were administered orally one week after AFB1 injection daily for three weeks. Methotrexate was administered intramuscular after one week of aflatoxin injection and this was repeated 24 h later (Preetha et al., 2006; Rofe et al., 1994).

At the end of the experiment (4 weeks), animals in all groups were sacrificed. The kidney and the heart from different animal groups were immediately removed, weighed and homogenized in ice cold bi-distilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 min at 10,000 rpm at 4 °C and the supernatants were used for different biochemical analysis except glycogen which was extracted by using 30% potassium hydroxide for its biochemical determination.

2.4. Biochemical analysis

All the following parameters were measured spectrophotometrically.

2.4.1. Lipid peroxidation

Lipid peroxidation was assayed by measuring the formed malondialdehyde (MDA) (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method. MDA concentration was calculated using extinction coefficient value (ε) of 1.56×10^5/M/cm (Buege and Aust, 1978). Results are expressed as μmol MDA formed/g tissue.

2.4.2. Vitamin C

Vitamin C was estimated by the method of Jagota and Dani (1982) using Folin–Ciocalteu reagent. The colour developed was read at 760 nm. The vitamin C content is expressed as μg/g tissue.

2.4.3. Glucose and glycogen estimations

Glucose level was estimated using Diamond Diagnostic Kits (Trinder, 1962). Glycogen content was estimated by the method of Nicholas et al. (1956) using 30% KOH and anthrone reagent.

2.4.4. Glucose-6-phosphate dehydrogenase assay (G-6-PDH)

The enzyme activity was assayed in a reaction mixture contained triethanolamine buffer (86 mM, pH 7.6), $MgCl_2$ (6.9 mM), glucose-6-phosphate (1 mM), NADP (0.39 mM). The reduction of NADP was followed at 340 nm and one unit of activity is defined as the reduction of 1 nmol $NADP^+$/min/mg protein (Bergmeyer, 1974).

2.4.5. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

GAPDH activity in the oxidative phosphorylation was determined by monitoring NADH generation at 340 nm. The reaction mixture of 1 ml contained 50 mM Tris buffer, pH 8.5, 10 mM sodium arsenate, 1 mM NAD+ and 2 mM D-G3P (Serrano et al., 1991).

2.4.6. Glucose isomerase (GI)

The enzyme activity was measured in a reaction medium containing Tris-HCl buffer (0.2 M, pH 7.4), fructose-6-phosphate (5 mM), $MgCl_2$ (10 mM), NADP (0.2 mM). The increase in extinction at 340 nm due to NADPH production was recorded (Wu and Racker, 1959).

2.4.7. Lactate dehydrogenase assay (LDH)

The enzyme activity was evaluated in a reaction medium of Tris buffer (50 Mm, pH 7.5), sodium pyruvate (0.6 mM), NADH (0.18 mM). The rate of NADH consumption is determined at 340 nm and is directly proportional to the LDH activity in the sample (Bergmeyer, 1975).

2.5. Statistical analysis

The results are expressed as mean ± standard deviation (SD). Differences between groups were assessed by one-way analysis of variance using the SPSS software package for Windows. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD).

3. Results

Table 1 reveals the abnormal levels of kidney oxidative stress biomarkers in rats that indicate the cellular damage caused by AFB1 treatment. The level of MDA in kidney tissue was markedly increased with concomitant significant decrease in the activities of tissue GR and G-6-PDH as well as in vitamin C level in AFBI-treated animals when compared with normal healthy ones. Administration of different plant extracts after AFB1 injection improve to large extent these parameters levels indicating the therapeutic role of these drugs.

This effect was pronounced in animals treated with green tea (G3), galangal (G5) and frankincense (G7) where these plants modulated most of these altered parameters nearly to their normal levels. However, supplementation of AFB1 treated rats with MTX dramatically amplified the toxic effect of AFB1 where the alterations in the studied parameters were more pronounced in AFB1-MTX treated animals than AFB1-treated ones.

The levels of some markers of energy metabolism in hearts of rats of different experimental groups are shown in Table 2. The result revealed marked decrease in glycogen and glucose contents in the hearts of AFB1 administered animals accompanied with a reduction in the glycolytic enzymes PGI, G3PDH and LDH. Administration of the extracts of plants under investigation, effectively up-regulate the decrease in these energetic biomarkers. But MTX treatment severely aggravated aflatoxicosis induced alteration in cardiac energy supply where the deviation in the studied energetic indices were more evidence in AFB1-MTX treated animals than AFB1-treated ones.

4. Discussion

This study was performed to evaluate the beneficial therapeutic ability of some traditional medicinal plant extracts against mycotoxicosis induced in rat kidneys and hearts by aflatoxin B1. Symptoms of mycotoxicosis are a result of interactions of mycotoxins with functional molecules and subcellular organelles in the animal cell.

Table 1 Levels of some oxidative stress markers in rat kidneys of different experimental groups.

Parameters	Normal G1	AF1B G2	AFB1-different plant extracts treated					MXT-treated G8	Anova *P*
			G3	G4	G5	G6	G7		
MDA	17.7 ± 1.1*	35 ± 2.27	18.1 ± 1.6*	19.38 ± 1.8*	15.17 ± 2.5*	18.14 ± 1.6*	19.4 ± 2.16*	45.6 ± 5.09**	< 0.0001
LSD	(2,8)	(1,3,4,5,6,7,8)	(2,8)	(2,8)	(2,8)	(2,8)	(2,8)	(1,2,3,4,5,6,7)	
GR	0.15 ± 0.02*	0.046 ± 0.011	0.13 ± 0.01*	0.13 ± 0.017*	0.14 ± 0.015*	0.12 ± 0.02*	0.13 ± 0.01*	0.034 ± 0.01	< 0.0001
LSD	(2,6,7,8)	(1,3,4,5,6,7,8)	(2,8)	(2,8)	(2,8)	(1,2,8)	(2,8)	(1,2,3,4,5,6,7)	
G-6-PDH	0.16 ± 0.02*	0.037 ± 0.006	0.14 ± 0.01*	0.12 ± 0.02*	0.14 ± 0.009*	0.11 ± 0.015*	0.15 ± 0.015*	0.024 ± 0.02**	< 0.0001
LSD	(2,4,6,8)	(1,3,4,5,6,7,8)	(2,7,8)	(1,2,7,8)	(2,7,8)	(1,2,7,8)	(2,3,4,5,6,8)	(1,2,3,4,5,6,7)	
Vitamin C	2.4 ± 0.2*	0.94 ± 0.06	2.29 ± 0.13*	1.70 ± 0.10*	2.32 ± 0.13*	1.68 ± 0.09*	1.910 ± 0.1*	0.60 ± 0.10**	< 0.0001
LSD	(2,4, 6,7,8)	(1,3,4,5,6,7,8)	(2,4,6,7,8)	(1,2,3,5,8)	(2,4,6,7,8)	(1,2,3,5,8)	(1,2,3,5,8)	(1,2,3,4,5,6,7)	

Data are means ± S.D. of five independent experiments. Numbers between brackets indicate significant correlation. Lipid peroxide is expressed in moles/gram tissue, enzymes are expressed in μmoles/min/mg protein, vitamin C is expressed in μg/gram tissue.

* $P < 0.0001$ compared to AFB1 group and MXT treated group.

** $P < 0.05$ compared to AFB1 treated group.

Table 2 Levels of some energy metabolism markers in rat hearts of normal and aflatoxin-B1 treated groups.

Parameters	Normal G1	AFB1 G2	AFB1-different plant extracts treated					MXT-treated G8	Anova *P*
			G3	G4	G5	G6	G7		
Glucose	5.59 ± 0.46*	2.21 ± 0.69	4.77 ± 0.93*	4.75 ± 0.35*	4.53 ± 0.93*	4.17 ± 0.14*	4.64 ± 0.53*	1.51 ± 0.06**	< 0.0001
LSD	(2,8)	(1,3,4,5,6,7,8)	(2,8)	(2,8)	(2,8)	(2,8)	(2,8)	(1,2,3,4,5,6,7)	
Glycogen	360.92 ± 23.9*	163.32 ± 12.5	353.27 ± 17.7*	350.41 ± 11.3*	346.67 ± 17.9*	305.94 ± 13.6*	315.59 ± 13.7*	136.1 ± 14.3**	< 0.0001
LSD	(2,6,7,8)	(1,3,4,5,6,7,8)	(2,6,7,8)	(2,6,7,8)	(2,6,7,8)	(1,2,3,4,5,8)	(1,2,3,4,5,8)	(1,2,3,4,5,6,7)	
GI	0.29 ± 0.02*	0.05 ± 0.01	0.276 ± 0.035*	0.25 ± 0.02*	0.23 ± 0.02*	0.196 ± 0.02*	0.197 ± 0.015*	0.03 ± 0.003**	< 0.0001
LSD	(2,5,6,7,8)	(1,3,4,5,6,7,8)	(2,6,7,8)	(2,6,7,8)	(1,2,6,7,8)	(1,2,3,4,5,8)	(1,2,3,4,5,8)	(1,2,3,4,5,6,7)	
GAPDH	0.263 ± 0.015*	0.063 ± 0.02	0.24 ± 0.02*	0.23 ± 0.03*	0.24 ± 0.02*	0.21 ± 0.01*	0.19 ± 0.03*	0.042 ± 0.01**	< 0.0001
LSD	(2,6,7,8)	(1,3,4,5,6,7,8)	(2,8)	(2,8)	(2,8)	(1,2,8)	(1,2,8)	(1,2,3,4,5,6,7)	
LDH	1.89 ± 0.146*	0..52 ± 0.11	1.76 ± 0.12*	1.67 ± 0.12*	1.57 ± 0.18*	1.43 ± 0.09*	1.64 ± 0.08*	0.42 ± 0.035**	< 0.0001
LSD	(2,6,8)	(1,3,4,5,6,7,8)	(2,6,8)	(2,6,8)	(2,8)	(1,2,3,4,7,8)	(2,6,8)	(1,2,3,4,5,6,7)	

Data are means ± S.D. of five independent experiments. Numbers between brackets indicate significant correlation. Glycogen and glucose are expressed in mg/gram tissue, enzymes are expressed in μmoles/min/mg protein.

* $P < 0.0001$ compared to AFB1 group and MXT treated group.

** $P < 0.05$ compared to AFB1 treated group.

Results clearly indicate increase in MDA (index of lipid peroxidation, LPO) in kidneys of aflatoxin-treated rats as compared to control, suggesting kidney oxidative damage. This is in agreement with findings reported previously for rat liver (Abdel-Wahhab and Aly, 2003; El-Gibaly et al., 2003; Liebert et al., 2006; Naaz et al., 2007). As well as liver and kidney in mice (Verma and Nair, 1999; Choudhary and Verma, 2005).

Induction of LPO by AFB1 is considered one of the main manifestations of oxidative damage initiated by reactive oxygen species (ROS) and it has been linked with altered membrane structure and enzyme inactivation. It is exhibited that aflatoxin B1, with the help of microsomal cytochrome p-450 mediated oxidation, is biotransformed into aflatoxin 8-9-epoxide, which is a reactive intermediate and highly toxic (Niki et al., 2005; Sharma and Farmer, 2004; Klein et al., 2002).

Cytochrome p-450 exhibits a key function in the biotransformation of xenobiotics, catalyze the reductive transformation of foreign compounds and displays an oxidase activity resulting in ROS formation (Towner et al., 2003; Sohn et al., 2003). These species trigger cell damage through binding to cell macromolecules as well as membrane, leading to membrane peroxidation which affect the ionic permeability of the membrane and eventually leading to the impairment of cell functioning and cytolysis (Berg et al., 2004).

This is supported by previous investigation stated that aflatoxin is a potent nephrotoxic compound leading to kidney damage and changes in renal function. Also, previous histopathological studies indicated that the exposure to aflatoxin B1 led to sever degenerative renal damage (Wangikar et al., 2005; Dafalla et al., 1986; Gabal and Azzam, 1998; Tassari et al., 2006).

Oxidative damage in the cell or tissue occurs when the concentration of ROS (superoxide radical, hydroxyl radical and hydrogen peroxide) generated exceeds the antioxidant capability of the cell (Sies and Stahl, 1995) or when the antioxidant capacity of the cell decreases. Levels of non-enzymatic antioxidants (vitamin C) and enzymatic antioxidants (GR and G-6-PDH) are the main determinants of the antioxidant defense mechanism of the cell.

The significant reduction in vitamin C level as well as in activities of glutathione reductase (GR) and G-6-PDH (glutathione metabolizing enzymes) in kidneys of AFB1 administered rat could be responsible for increased lipid peroxidation observed during aflatoxicosis. This result is agreed with previous studies which revealed significant reductions in enzymatic and non-enzymatic antioxidants in AFB1-fed rat (El-Gibaly et al., 2003; Choudhary and Verma, 2005).

Vitamin C, which includes ascorbic acid and its oxidation product – dehydroascorbic acid, has many biological activities in human body. It can be defined as an enzyme cofactor, a radical scavenger, and as a donor/acceptor in electron transport at the plasma membrane (Davey et al., 2000).

GR is the key enzyme in the conversion of oxidized glutathione (GSSG) back to the reduced form (GSH). GSH scavenge the electrophilic moieties produced by toxic chemicals and conjugate them to less toxic products (Anilakumar et al., 2004). It also has an important role in the maintenance of vitamin C normal level. During free radical scavenging action, ascorbic acid is transformed into L-dehydroascorbate (Breimer, 1990). GSH is required for the conversion of L-dehydroascorbate back to ascorbate (Preetha et al., 2006). The normal vitamin C level is

a therapeutic benefit as it able to reduce oxidative stress through reacting with superoxide radical and hydroxyl radicals (Sies and Stahl, 1995).

So reduction in GR activity may lead to the fall in the level of reduced glutathione and hence decreases the conversion of L-dehydroascorbate to ascorbate and this probably explain the lowered level of ascorbic acid in the aflatoxin-treated animals. Glucose-6-phosphate dehydrogenase (G-6-PDH), a rate-limiting enzyme of the pentose phosphate pathway, is required for NADPH generation which is needed for the maintenance of GSH in its reduced form. Inhibition of G-6-PDH activity may reduce the capacity of the tissue to protect itself from the oxidative stress because less amount of NADPH is produced (Zhang et al., 2000).

Supplementation of different plant extracts, potentially was effective in reducing lipid peroxidation, and enhancing the antioxidant status of kidney in AFB1-treated animals. It is indicated by amelioration of MDA to near normal level and significant improvement the antioxidant defense system (GR, G-6-PDH and vitamin C), suggesting their antioxidant potentials. Two mechanisms can be postulated to the therapeutic effect of the used plant extracts against AFB1 renotoxicity. The first one is that these extracts may exert their beneficial actions through their direct antimicrobial activities. The second mechanism is based on their antioxidant abilities (proved from our data), which may be responsible for protecting the cells against the oxidative stress, possibly by increasing the endogenous defensive capacity of the kidney to combat oxidative stress induced by AFB1.

This emphasizes the fact that herbal medicines often contain multiple active substances which have antimicrobial and antioxidant activities. Some authors reported that several phenolic compounds contribute to the overall antioxidant and antimicrobial activities of herbs against human pathogens The mechanisms of phenolic compounds for antioxidant activity are mainly due to their redox properties and chemical structures, which can act as reducing agents, free radical scavengers or quenchers of the formation of singlet oxygen (Zheng and Wang, 2001; Pizzaleet al., 2002; Puupponen-Pimia et al., 2005).

Green tea (G3) was found to posses antimicrobial activity. It also contains polyphenols which have recently been reported to be a potent antioxidant and beneficial in oxidative stress, and to inhibit the initiation of AFB1-induced carcinogenesis in rats and mice. Epigallocatechin gallate, the main ingredient of green tea extract, is a strong chemopreventing of toxic effects of AFB1. It has an important role in converting AFB1 to aflatoxicol which is less toxic, enhancing of glutathione-S-transferase (GST) activity and reducing the metabolic conversion AFB1 to the AFB1-DNA adduct (Almajano et al., 2008; Panza et al., 2008; Chen et al., 2004; Raza and John, 2005; Chou et al., 2000; Ahmed et al., 2002; Tulayakul et al., 2007).

Caraway (G4) is rich in monoterpenes and is known for its antimicrobial activity. In addition, previous studies show that caraway contains potential antioxidant substances such as the flavonoids. Apart from antioxidant properties, caraway also has an important role in reducing cytochrome P450 metabolic activity. Thus this may reduce the metabolic conversion of AFB1 to the highly reactive intermediate, AFB1-8,9-epoxide which has the major role in AFB1 toxicity and carcinogenicity (Preston and Williams, 2005; Iacobellis et al., 2005; Satyanarayana et al., 2004; Naderi-Kalali et al., 2005).

Galangal (G5) was found to have antimicrobial effect which is related to the presence of several components acted as antimicrobial agents. 1,8-Cineole, the main component of galangal in the essential oil, has been previously reported to have an antibacterial activity. It has a mechanism of action against bacterial cells involving disruption of cytoplasmic membrane and coagulation of cell contents. Also, several researchers have reported that galangal showed antioxidant activity in a model system (Viljoen et al., 2003; Oonmetta-aree et al., 2006; Gachkar et al., 2007; Cheah and Abu-Hasim, 2000; Javanmardi et al., 2003).

Frankincense (G6) oils was reported to exhibited antibacterial and antifungal activities. The water-soluble fraction of the frankincense show hepato-protective and reno-protective property. It possess antioxidant activity as it has the ability to inhibit induced nitric oxide (NO) and inflammatory mediators production under in vivo and in vitro condition (Umez, 2000; Pandey et al., 2005; Gayathri et al., 2007).

Cinchona (G7) (*Cinchona officinalis*) was also exhibited antimicrobial activity against bacteria and fungi which may related to the alkaloids (namely, quinine, quinidine, cinchonine and cinchonidine) and steroids found in this plant (Rojas et al., 2006).

Administration of the anticancer drug, MTX, to AFB1-treated rats (G8) dramatically amplified the toxic effect of AFB1 induced in kidney which was documented by elevated MDA level with concomitant decrease in antioxidant defense systems, vitamin C, GR and G-6-PDH compared with normal animals. The behavior implicates the presence of oxidative tissue damage. These deviations were more evidence in AFB1-MTX treated animals than in AFB1-treated group.

This result is agreed with previous authors who reported that administration of MTX induces lipid peroxidation accompanied by significant reductions in antioxidant defense mechanism in different tissues including kidney, which is considered an important cause of destruction and damage to cell membranes and has been suggested to be a contributing factor to the development of MTX-mediated tissue damage (Cetiner et al., 2005; Rajamani et al., 2006). The significant reduction in the glutathione metabolizing enzymes (GR and G-6-PDH) as well as vitamin C induced by MTX may be explained by previous studies demonstrated that the cytosolic NAD(P)-dependent dehydrogenases are inhibited by MTX, suggesting that the drug could decrease the availability of NADPH in cells which is used by glutathione reductase to maintain the cytosolic antioxidant glutathione in reduced state (Cetiner et al., 2005; Caetano et al., 1997). Consequently, this may lead to a reduction in the effectiveness of the antioxidant vitamin C (Babiak et al., 1998).

Concerning with the effect of AFB1 administration on some energy metabolism biomarkers in rat hearts, the results revealed that marked decrease in glycogen and glucose contents of animal hearts in response to aflatoxicosis accompanied by a decrease in the glycolytic enzymes, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), glucose isomerase (GI) and lactate dehydrogenase (LDH), suggesting that AFB1 may interfere with the cellular energy supply through its inhibitory action on glycogen synthesis, glucose uptake (a decrease in glucose transport from blood to myocardial cells) and glycolysis in heart. Similar result was obtained by Tessier et al. (2003) who reported that cardiac glycolysis and glycogen synthesis are impaired by a bacterial endotoxin in rats. Our

results are consisted with some authors who reported that AFB1 decreases tissue glycogen by inhibiting the activities of biosynthetic enzymes and stimulating glycogenolysis through inhibiting intracellular cyclic adenosine monophosphate (cAMP) hydrolysis, which responsible for phosphorylase activation, the rate-limiting enzyme for glycogen (Kiessling, 1986; Bonsi et al., 1999).

Also it was found that AFB1 reduces glucose transport and affects some enzymes engaged in glucose metabolism in the liver. The reduction in glucose uptake may be attributed to the decrease in the number of GLUT 1 and GLUT 4 transporters in the sarcolemma in response to aflatoxicosis. These proteins are normally stored in cytoplasmic membranes and can be recruited to the plasma membrane as needed (Kiessling, 1986).

The loss of cardiac anaerobic glycolysis under the effect of AFB1 toxicity may be ascribed to the low level of intracellular glucose, in addition to the decrease in LDH activity may affect the re-generation of NAD from NADH, the oxidizing equivalents necessary to GAPDH, which sustains the continuous operation of glycolysis and hence the rate of glycolysis will be reduced (Brooks et al., 2003).

On the other hand, these enzymes may be released from cardiac muscle fibers due to aflatoxicosis causing tissue damage. The later suggestion is confirmed by histopathological investigations proved that gross cardiac anomalies due to AFB1 indicated by dilation of the heart and fusion of auriculo-ventricular valve leading to obstruction of passage of the blood from auricles to ventricles, vaculations and degeneration of cardiac muscle fibers (Liebert et al., 2006).

Glycogen utilization through glycolysis is one of the metabolic pathways which directly participate in generation of sufficient adenosine triphosphate (ATP) to meet energy demands of the heart. The myocyte requires continuos production of ATP to survive because it cannot be stored. Alterations of such bioenergetic sources which have a crucial role in cellular protection may render the heart susceptible to metabolic stress and affect the myocardial contractile performance, leading heart failure. This is supported by some authors who reported that exposure to aflatoxin B1 toxicity caused heart defects (Carvajal and Sánchez, 2003; Mancini et al., 1999; Pasha et al., 2007).

Administration of different plant extracts, effectively modulate the lowered levels of glycogen and glucose as well as the activities of the key enzymes involved in glucose metabolism in hearts of rats in response to aflatoxicosis. Modulation of the heart energy metabolism by the used drugs could be beneficial to the amelioration of events leading to cardiopathy. No comparable data are so far available for the effect of these plants on the heart energy metabolism, however, the possible mechanisms which may explain this good effect of the tested different plant extracts are that they may have a crucial role in inducing the enzymes implicated in glycogen synthesis and have the ability to increase glucose uptake by modulating the alterations in the glucose transporter GLUT in the cardiac muscle affected by AFB1. Also, the amelioration of glycolytic enzyme activities may attributed to the availability of glucose, in addition these extracts may have a direct beneficial action in cardiac tissue repair due to their antioxidant abilities as previously mentioned, thus preventing enzymes release.

Administration of MTX to AFB1-treated animals aggravates the cardiotoxic effect of AFB1 via decreasing the energy metabolic markers in the animal hearts which was ensured by the reduction in the glucose and glycogen contents of the heart with concomitant decrease in the tested glycolytic enzymes. These alterations were more severe in AFB1-MTX treated rats than in AFB1-treated group. The decrease in the glucose by MTX may ascribed to its ability to decrease glucose transport into the cells as one of its mechanism of actions against tumor cells. This finding is supported by previous authors who declared that MTX inhibited glucose uptake by Ehrlich ascites cells (Fung et al., 1986). The decrease in glycogen may attributed to either the inhibitory action of MTX on glycogen synthesis or the reduction of intracellular cardiac glucose, the substrate for glycogen synthesis. The decrease in glycolytic enzymes may be resulted from either the decrease in cardiocyte glucose content or the release of these enzymes due to tissue damage. This is ensured by some authors reported that treatment of rat hepatocyte with MTX for 24 h resulted in increase in enzyme leakage supporting the conclusion that MTX was cytotoxic agent in rat hepatocyte (Walker et al., 2000). Our data collectively support the hypothesis that cellular oxidative stress is unpredictable side effects of MTX and may consider a critical step in MTX-mediated injury (Sener et al., 2006).

In conclusion, our results have shown that all aqueous extracts of the examined plants alleviate AFB1 induced kidney oxidative stress and alteration of cardiac energy metabolism and can be regarded as good therapeutic agents against aflatoxicosis.

References

Abdel-Wahhab, M.A., Aly, S.E., 2003. Antioxidants and radical scavenging properties of vegetable extracts in rats fed aflatoxin contaminated diet. J. Agric. Food Chem. 51, 2409.

Ahmed, I., John, A., Vijayasarathy, C., Robin, M.A., Raza, H., 2002. Differential modulation of growth and glutathione metabolism in cultured rat astrocytes by 4-hydroxynonenal and green tea polyphenol, epigallocatechin-3-gallate. Neurotoxicology 23, 289.

Almajano, M.P., Carbo, R., Jimenez, J.A., Gordon, M.H., 2008. Antioxidant and antimicrobial activities of tea infusions. Food Chem. 108, 55.

Al-Wadi, F.M., Gumaa, K.A., 1987. Studies on the activity of individual plants of an antidiabetic plant mixture. Acta Diabetol. Lat. 24, 37–41.

Anilakumar, K.R., Nagaraj, N.S., Santhanam, K., 2004. Protective effects of Amla on oxidative stress and toxicity in rats challenged with dimethylhydrazine. Nutr. Res. 24, 313.

Antunes, N.L., Souweidane, M.M., Li, E., Rosenblum, M.K., Steinherz, P.G., 2002. Methotrexate leukoencephalopathy presenting as Kluver–Bucy syndrome and uncinate seizures. Pediatr. Neurol. 26, 305.

Babiak, R.M., Campello, A.P., Carnieri, E.G., Oliveira, M.B., 1998. Methotrexate: pentose cycle and oxidative stress. Cell Biochem. Funct. 16, 283–293.

Bellakhdar, J., 1997. La Pharmacopee Marocaine Traditionnelle, Medecine Arabe Ancienne et Savoirs Populaires. Edition Ibis Press, p. 150.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497.

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

Bergmeyer, H.U., 1974. Method of Enzymatic Analysis. In: Verlag Chemic, Weinheim (Eds.), Academic Press, New York and London, pp. 644–649.

Bergmeyer, H.U., 1975. Determination of lactate dehydrogenase. J. Clin. Chem. Biochem. 13, 269.

Bintvihok, A., 2002. New insights to controlling mycotoxin danger in ducks. Feed Technol. 6 (1), 28.

Bonsi, P., Augusti-Tocco, G., Palmery, M., Giorgi, M., 1999. Aflatoxin B1 is an inhibitor of cyclic nucleotide phosphodiesterase activity. Gen. Pharmacol. 32, 615.

Breimer, L.H., 1990. Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: the role of DNA base damage. Mol. Carcinogen 3, 188.

Brock, S., Jenning, H.R., 2004. Fatal acute encephalomyelitis after a single dose of intrathecal methotrexate. Pharmacotherapy 24, 673.

Brooks, S.P.J., Cockell, K.A., Dawson, B.A., Ratnayake, W.M.N., Lampi, B.J., Belonje, B., Black, D.B., Plouffe, L.J., 2003. Carbohydrate metabolism in erythrocytes of copper deficient rats. J. Nutr. Biochem. 14, 648.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302.

Caetano, N.N., Campello, A.P., Carnieri, E.G.S., Kluppel, M.L.W., Oliveira, M.B.M., 1997. Effects of methotrexate (MTX) on NAD(P)+ dehydrogenases of HeLa cells: malic enzymes, 2-oxogluterate and isocitrate dehydrogenases. Cell Biochem. Funct. 15, 259.

Carvajal, K., Sánchez, R.M., 2003. Heart metabolic disturbances in cardiovascular diseases. Arch. Med. Res. 34, 89.

Cetiner, M., Sener, G., Sehirli, A.O., Eksioglu-Demiralp, E., Ercan, F., Sirvanci, S., Gedik, N., Akpulat, S., Tecimer, T., Yegen, B.C., 2005. Taurine protects against methotrexate-induced toxicity and inhibits leukocyte death. Toxicol. Appl. Pharmacol. 209, 39.

Cheah, P.B., Abu-Hasim, N.H., 2000. Natural antioxidant extract from galangal (*Alpinia galanga*) for minced beef. J. Sci. Food Agric. 80, 1565–1571.

Chen, J.H., Topoe, G.L., Liong, E.C., So, H.S.H., Leung, K.M., Tom, W.M., Fung, P.C.W., Nanji, A.A., 2004. Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. Am. J. Clin. Nutr. 80, 742.

Chou, F.P., Chu, Y.C., Hsu, J.D., Chiang, H.C., Wang, C.J., 2000. Specific induction of glutathione S-transferase GSTM2 subunit expression by epigallocatechin gallate in rat liver. Biochem. Pharmacol. 60, 643.

Choudhary, A., Verma, R.J., 2005. Ameliorative effects of black tea extract on aflatoxin-induced lipid peroxidation in the liver of mice. Food Chem. Toxicol. 43, 99.

Dafalla, R., Yagi, A.I., Adam, S.E.I., 1986. Experimental aflatoxicosis in Hybro-type chicks: sequential changes in growth and serum constituents and histopathological changes. Vet. Human Toxicol. 29, 222.

Davey, M.W., van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., 2000. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 80, 825.

de Carvalho, C.C.C.R., da Fonseca, M.M.R., 2006. Carvone: why and how should one bother to produce this terpene. Food Chem. 95, 413.

Doan, T., Massarotti, E., 2005. Rheumatoid arthritis: an overview of new and emerging therapies. J. Clin. Pharmacol. 45, 751.

Dufresne, C.J., Farnworth, E.R., 2001. A review of latest research findings on the health promotion properties of tea. J. Nutr. Biochem. 12, 404.

El-Gibaly, I., Meki, A.M., Abdel-Ghaffar, S.K., 2003. Novel B melatonin-loaded chitosan microcapsules: in vitro characterization and antiapoptosis efficacy for aflatoxin B1-induced apoptosis in rat liver. Int. J. Pharmacol. 260, 5.

Fink-Gremmels, J., 1999. Mycotoxins: their implications for human and animal health. Vet. Q 21, 115.

Fung, K.P., Ng, S.W., Lam, W.P., Choy, Y.M., 1986. Prevention by thymidine against toxicity and glucose uptake inhibition of methotrexate on cultured Ehrlich ascites tumour cells. Pharmacol. Res. Commun. 18, 759.

Gabal, M.A., Azzam, A.H., 1998. Interaction of aflatoxin in the feed and immunization against selected infectious disease in poultry. II. Effect on one-day-old layer chicks simultaneously vaccinated against Newcastle disease, infectious bronchitis and infectious bursal disease. Avian Pathol. 27, 290.

Gachkar, L., Yadegari, D., Rezaei, M.B., Taghizadeh, M., Astaneh, S.A., Rasooli, I., 2007. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem. 102, 898.

Gayathri, B., Manjula, N., Vinaykumar, K.S., Lakshmi, B.S., Balakrishnan, A., 2007. Pure compound from *Boswellia serrata* extract exhibits anti-inflammatory property in human PBMCs and mouse macrophages through inhibition of TNF alpha, IL-1beta, NO and MAP kinases. Int. Immunopharmacol. 7, 473–482.

Hostanska, K., Daum, G., Saller, R., 2002. Cytostatic and apoptosis – inducing activity of boswellic acids towards malignant cells lines in vitro. Anticancer Res. 22, 2853.

Iacobellis, N.S., Lo Cantore, P., Capasso, F., Senatore, F., 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J. Agric. Food Chem. 12, 53–57.

Jagota, S.K., Dani, H.M., 1982. A new colorimetric technique for the estimation of vitamin C using folin phenol reagent. Anal. Biochem. 127, 178.

Jahovic, N., Cevik, H., Sehirli, A.O., Yegen, B.C., Sener, G., 2003. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. J. Pineal Res. 34, 282.

Javanmardi, J., Stushnoff, C., Locke, E., Vivanco, J.M., 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chem. 83, 547.

Joseph, G.S., Jayaprakasha, G.K., Selvi, A.T., Jena, B.S., Sakariah, K.K., 2005. Antiaflatoxigenic and antioxidant activities of Garcinia extracts. Int. J. Food Microbiol. 101, 153–160.

Juntachote, T., Berghofer, E., 2005. Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal. Food Chem. 92, 193.

Kiessling, K.H., 1986. Biochemical mechanism of action of mycotoxins. Pure Appl. Chem. 58, 327.

Klein, P.J., Van Vleet, T.R., Hall, J.O., Coulombe Jr., R.A., 2002. Biochemical factors underlying the age-related sensitivity of turkeys to aflatoxin B(1). Comp. Biochem. Physiol. C 132, 193.

Knaus, U., Wagner, H., 1996. Effects of boswellic acid of *Boswellia serrata* and other triterpenic acids on the complement system. Phytomedicine 3, 77.

Kumar, R., Mishra, A.K., Dubey, N.K., Tripathi, Y.B., 2007. Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int. J. Food Microbiol. 115, 159.

Lahlou, S., Tahraoui, A., Israili, Z., Lyoussi, B., 2007. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. J. Ethnopharmacol. 110, 458.

Liebert, J.J., Matawsk, I., Bylka, W., Murias, M., 2006. Protective effect of *Aquilegia vulgaris* L. on aflatoxin B1-induced hepatic damage in rats. Environ. Toxicol. Pharmacol. 22, 58.

Mancini, D., Benaminovitz, A., Cordisco, M.E., Karmally, R.N.W., Weinberg, R.D.A., 1999. Slowed glycogen utilization enhances exercise endurance in patients with heart failure. J. Am. Coll. Cardiol. 34, 1807–1812.

McKay, D.L., Blumberg, J.B., 2002. The role of tea in human health: an update. J. Am. Coll. Nutr. 21 (1), 1.

Miklaeil, B.R., Maatooq, G.T., Badria, F.A., Amer, M.M.A., 2003. Chemistry and immunomodulatory activity of frankincense oil. Zeitschrift fur Naturforschung Biosciences 58, 230.

Minto, R.E., Townsend, C.A., 1997. Enzymology and molecular biology of aflatoxin biosynthesis. Chem. Rev. 97, 2537.

Miyazono, Y., Gao, F., Horie, T., 2004. Oxidative stress contributes to methotrexate-induced small intestinal toxicity in rats. Scand. J. Gastroenterol. 39, 1119.

Mturi, N., Musumba, C.O., Wamola, B.M., Oguto, B.R., Newton, C.R., 2002. Cerebral malaria: optimising management. CNS Drugs 17, 153–165.

Naaz, F., Javed, S., Abdin, M.Z., 2007. Hepatoprotective effect of ethanolic extract of *Phyllanthus amarus* Schum. et Thonn. on aflatoxin B1-induced liver damage in mice. J. Ethnopharmacol. 113, 503.

Naderi-Kalali, B., Allamesh, A., Rasaee, M.J., Bash, H.J., Behechti, A., Doods, K., Kettrup, A., Schramm, K.W., 2005. Suppressive effects of caraway (*Carum carvi*) extracts on 2,3,7,8-tetrachloro-dibenzo-p-dioxin-dependent gene expression of cytochrome P4501A1 in the rat H4IIE cells. Toxicol. in Vitro 19, 373.

Nicholas, V., Carroll, R., Longley, W., Joseph, H.R., 1956. The determination of glycogen in liver and muscle by the use of anthrone reagent. J. Biol. Chem. 220, 583.

Niki, E., Yoshida, Y., Saito, Y., Noguchi, N., 2005. Lipid peroxidation: mechanisms, inhibition, and biological effects. Biochem. Biophys. Res. Commun. 338, 668.

Oonmetta-aree, J., Suzuki, T., Gasaluck, P., Eumkeb, G., 2006. Antimicrobial properties and action of galangal (*Alpinia galangal* Linn.) on *Staphylococcus aureus*. Lebensmittel-Wissenschaft und-Technologie 39, 1214.

Paget, G.E., Barnes, J.M., 1964. Evaluation of drug activities. In: Alaurence, D.R., Bacharach, A.L. (Eds.), . In: Pharmacometrics, vol. 1. Academic Press, New York, p. 161.

Pandey, R.S., Singh, B.K., Tripathi, Y.B., 2005. Extract of gum resins of *Boswellia serrata* L. Inhibits lipopolysaccharide induced nitric oxide production in rat macrophages along with hypolipidemic property. Indian J. Exp. Biol. 43, 509–516.

Panza, V.S.P., Wazlawik, E., Schütz, G.R., Comin, L., Hecht, K.C., da Silva, E.L., 2008. Consumption of green tea favorably affects oxidative stress markers in weight-trained men. Nutrition 24, 433.

Pasha, T.N., Farooq, M.U., Khattak, F.M., Jabbar, M.A., Khan1, A.D., 2007. Effectiveness of sodium bentonite and two commercial products as aflatoxin absorbents in diets for broiler chickens. Anim. Feed Sci. Technol. 132, 103.

Pizzale, L., Bortolomeazzi, R., Vichi, S., Uberegger, E., Conte, L., 2002. Antioxidant activity of Sage (*Salvia officinalis* and *S. fruticosa*) and oregano (*Origanum onites* and *Origanum indercedens*) extracts related to their phenolic compound content. J. Sci. Food Agric. 79, 277.

Preetha, S.P., Kanniappan, M., Selvakumar, E., Nagaraj, M., Varalakshmi, P., 2006. Lupeol ameliorates aflatoxin B1-induced peroxidative hepatic damage in rats. Comp. Biochem. Physiol. 143, 333.

Preston, R.J., Williams, G.M., 2005. DNA-reactive carcinogens: mode of action and human cancer hazard. Crit. Rev. Toxicol. 35, 673.

Prinz, A., 1990. Discovery of the cardiac effectiveness of cinchona bark and its alkaloids. Wien Klin Wochenschr 102, 721–723.

Puupponen-Pimia, R., Nohynek, L., Alakomi, H.L., Oksman-Caldentey, K.M., 2005. Bioactive berry compounds – novel tools against human pathogens: mini-review. Appl. Microbiol. Biotechnol. 67, 8–18.

Raffauf, R.F., Leoqewsne, P.W., Ghosh, P.C., 1978. Anti-tumor plants V. Constituents of *Cinchona pubescens*. Lioydia 41, 432–434.

Rajamani, R., Muthuvel, A., Senthilvelan, M., Sheeladevi, R., 2006. Oxidative stress induced by methotrexate alone and in the presence of methanol in discrete regions of the rodent brain, retina and optic nerve. Toxicol. Lett. 165, 265.

Rall, B., Ammon, H.P.T., Safayhi, H., 1996. Boswellic acids and protease activities. Phytomedicine 3, 75–76.

Raza, H., John, A., 2005. Green tea polyphenol epigallocatechin-3-gallate differentially modulates oxidative stress in PC 12 cell compartments. Toxicol. Appl. Pharmacol. 207, 212.

Reddy, G.K., Dhar, S.C., Singh, G.B., 1986. Urinary excretion of connective tissue metabolites under the influence of a new non-steroidal anti-inflammatory agent in adjuvant induced arthritis. Agents Actions 22, 99.

Rofe, A.M., Bourgeois, C.S., Washington, J.M., Philcoxm, J.C., Coyle, P., 1994. Metabolic consequences of methotrexate therapy in tumor-bearing rats. Immunol. Cell Biol. 72, 43.

Rojas, J.J., Ochoa, V.J., Ocampo, S.A., Muñoz, J.F., 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. BMC Complementary Altern. Med. 6, 2–7.

Safayhi, H., Mack, T., Ammon, H.P.T., 1991. Protection by boswellic acids against galactosamine/endotoxin-induced hepatitis in mice. Biochem. Pharmacol. 41, 1536.

Safayhi, H., Rall, B., Sailer, E.R., Ammon, H.P.T., 1997. Inhibition by boswellic acids of human leukocyte elastase. J. Pharmacol. Exp. Ther. 281, 460.

Salunkhe, D.K., Adsule, R.N., Padule, D.N. (Eds.), 1987. Occurrence of Aflatoxin. Aflatoxin in Foods and Feeds. p. 44.

Satyanarayana, S., Sushruta, K., Sarma, G.S., Srinivas, N., Subba Raju, G.V., 2004. Antioxidant activity of the aqueous extracts of spicy food additives – evaluation and comparison with ascorbic acid in in vitro systems. J. Herb. Pharmacother. 4, 1–10.

Sener, G., Demiralp, E.E., Cetiner, M., Ercan, F., Yegen, B., 2006. -glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. Eur. J. Pharmacol. 542, 170.

Serrano, A., Mateos, M.I., Losada, M., 1991. Differential regulation by trophic conditions of phosphorylating and non-phosphorylating NADP + -dependent glyceraldehyde-3-phosphate dehydrogenases in *Chlorella fusca*. Biochem. Biophys. Res. Commun. 181, 1077.

Sharma, R.A., Farmer, P.B., 2004. Biological relevance of adduct detection to the chemoprevention of cancer. Clin. Cancer Res. 10, 4901.

Sies, H., Stahl, W., 1995. Vitamin E and C, carotene and other carotenoids as antioxidants. Am. J. Clin. Nutr. 62, 1315S.

Sohn, D.H., Kim, Y.C., Oh, S.H., Park, E.J., Li, X., Lee, B.H., 2003. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. Phytomedicine 10, 165.

Tassari, E.N., Oliveira, C.A., Cardoso, A.L., Ledoux, D.R., Rottinghaus, G.E., 2006. Effects of aflatoxin B1 on body weight, antibody titres and histology of broiler chicks. Br. Poult. Sci. 47, 357.

Tessier, J.P., Thurner, B., Jungling, E., Luckhoff, A., Fischer, Y., 2003. Impairment of glucose metabolism in hearts from rats treated with endotoxin. Cardiovasc. Res. 60, 119.

Towner, R.A., Qian, S.Y., Kadiiska, M.B., Mason, R.P., 2003. In vivo identification of aflatoxin-induced free radicals in rat bile. Free Radic. Biol. Med. 35, 1330.

Trepardoux, F., 2002. Emerine and quinine, a therapy to reduce Belliniiu 1835. Rev. Hist. Pharm. 50, 401.

Trinder, P., 1962. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6, 24.

Tulayakul, P., Dong, K.S., Li, J.Y., Manabe, N., Kumagai, S., 2007. The effect of feeding piglets with the diet containing green tea extracts or coumarin on in vitro metabolism of aflatoxin B1 by their tissues. Toxicon 50, 339.

Umez, T., 2000. Behavioral effects of plant-derived essential oils in the Geller-type conflict test in mice. Jpn. J. Pharmacol. 83, 150.

Uzar, E., Sahin, O., Koyuncuoglu, R.H., Uz, E., Bas, O., Kilbas, S., Yilmaz, H.R., Yurekli, V.A., Kucuker, H., Ahmet Songur, A., 2006. The activity of denosine deaminase and the level of nitric oxide in spinal cord of methotrexate administered rats: protective effect of caffeic acid phenethyl ester. Toxicology 218, 125.

Verma, R.J., Nair, A., 1999. Vitamin E prevents aflatoxin-induced lipid peroxidation in the liver and kidney. Med. Sci. Res. 27, 223–226.

Vezmar, S., Becker, A., Bode, U., Jaehde, U., 2003. Biochemical and clinical aspects of methotrexate neurotoxicity. Chemotherapy 49, 92.

Viljoen, A., van Vuuren, S., Ernst, E., Klepser, M., Demirci, B., Bas-er, H., 2003. *Osmitopsis asteriscoides* (Asteraceae) – the

antimicrobial activity and essential oil composition of a Cape-Dutch remedy. J. Ethnopharmacol. 88, 137.

Walker, T.M., Rhodes, P.C., Westmoreland, C., 2000. The differential cytotoxicity of methotrexate in rat hepatocyte monolayer and spheroid cultures. Toxicol. in Vitro 14, 475.

Wangikar, P.B., Dwivedi, P., Sinha, N., Sharma, A.K., Telang, A.G., 2005. Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. Toxicology 215, 37.

Wogan, G.N., 1999. Aflatoxin as a human carcinogen. Hepatology 30, 573.

Wu, R., Racker, E., 1959. Regulatory mechanisms in carbohydrate metabolism. III. Limiting factors in glycolysis of ascites tumor cells. J. Biol. Chem. 234, 1029.

Yang, X., Eilerman, R.G., 1999. Pungent principle of *Alpinia galanga* (L.) Swartz and its applications. J. Agric. Food Chem. 47, 1657.

Zhang, Z., Apse, K., Pang, J., Stanton, R.C., 2000. High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. J. Biol. Chem. 275, 40042.

Zheng, W., Wang, S.Y., 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49, 5165.