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Antiaflatoxigenic Activities of Some Plant Aqueous Extracts Against Aflatoxin-B1 Induced Renal and Cardiac Damage

Azza M. Mohamed and Nadia S. Metwally
Department of Medicinal Chemistry, National Research Center, Cairo, Egypt

Abstract: The present investigation aims at assessing the antiaflatoxigenic effect of aqueous extracts of some traditional medicinal plants (namely, *Zingiber officinale* Roscoe rhizome, *Cinnamomum zeylanicum* bark, *Trigonella foenum graecum* seeds, *Camellia sinensis* leaves and *Salvia officinalis* leaves) compared to the anticancer drug, methotrexate (MTX) against aflatoxin-B1 (AFB1) induced renal and cardiac damage in rats. The results revealed that 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. The results also showed 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 above mentioned plants, 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 releasing the inhibitory effect of AFB1 on the levels of antioxidants in kidneys as well as on the energetic biomarkers in hearts. However, administration of MTX to AFB1-treated rats dramatically amplified the toxic effect of aflatoxicosis induced in both kidneys and hearts, 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 a marked decrease in the bioenergetic markers in hearts of AFB1- MTX treated animals versus AFB1-treated ones was documented. From the current investigation, it can be concluded that supplementation of the extracts of the different plants presented in this study was beneficial in modulating the alterations induced in kidneys and hearts of rats under the effect of AFB1.

Key words: Medicinal plants, aflatoxin-B1, oxidative stress, energy

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). Aflatoxins are not only contaminate our food stuffs, but also are found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals (Fink-Gremmels, 1999; Bennett and Klich, 2003).

AFB1 is well known to be potent mutagenic, carcinogenic, teratogenic, immunosuppressive and also inhibit several metabolic systems, causing liver, kidney and heart damage (Wogan, 1999; Bintvihok, 2002; Wangikar *et al*., 2005). These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (Salunkhe *et al*., 1987).

Corresponding Author: Azza M. Mohamed, Department of Medicinal Chemistry, National Research Center, Cairo, Egypt Fax: 202-33370931

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 (Sharma and Farmer, 2004). The formation of AFB1- DNA adducts is regarded as a critical step in the initiation of AFB1-induced carcinogenesis (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 (Towner *et al*., 2003). These species may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis (Berg *et al*., 2004).

The use of synthetic chemicals as antimicrobials has greatly contributed to management of such losses, but indiscriminate application of chemicals has led to a number of ecological and medical problems due to residual toxicity, carcinogenicity, teratogenicity, hormonal imbalance, spermatotoxicity, etc. (Pandey, 2003). Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. Their medicinal use has been gradually increasing in developed countries. So, natural substances that can prevent AFB1 to toxicity would be helpful to human and animal health with minimal cost in foods and feed. Traditional medicinal plants were applied by some researchers for their antifungal, antiaflatoxigenic and antioxidant activity (Joseph *et al*., 2005; Kumar *et al*., 2007).

Ginger rhizome (*Zingiber officinale*), commonly known as ginger, is utilized worldwide as a spice and a flavoring agent. It has traditionally been used as a treatment for rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Afzal *et al*., 2001). Ginger extracts have been reported to have antioxidant (Masuda *et al*., 2004; Ajith *et al*., 2007a), anticancer (Lee *et al*., 2008), anti-inflammatory (Lantza *et al*., 2007) and antithrombotic effects (Thomson *et al*., 2002).

Cinnamon (*Cinnamomum eylanicum*) is a widely used spice and has many applications in perfumery, flavoring and pharmaceutical industries. Essential oils of cinnamon have been reported to have antimicrobial and antioxidant potency (Rodr´iguez *et al*., 2007; Singh *et al*., 2007). It has beneficial effects in the treatment of diabetes as it has insulin potentiating actions (Anderson *et al*., 2004).

Tea 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 (Dufresne and Farnworth, 2001). It also posses antioxidant and antimicrobial activities (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 (*Camellia sinensis*) 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 (Tulayakul *et al*., 2007).

Fenugreek (*Trigonella foenum graecum*) seeds are commonly used as spice in Indian homes. Fenugreek is a traditional medicinal herb posses antidiabetic and antiulcer potential (Sharma, 1986; Pandian *et al*., 2002). It prevents hyperlipidemia, atherosclerosis (Sharma *et al*., 1996) and cancer (Sur *et al*., 2001) in experimental animals. The seeds are reported to be rich in polyphenolic flavonoids (100 mg g^{-1}) (Gupta and Nair, 1999) which has antioxidant activity and was able to scavenge O^{-}_{2} and H_2O_2 (Kaviarasan *et al*., 2008).

Sage (*Salvia officinalis* L.) is the most popular herbal remedy to treat common health complications such as cold and abdominal pain (Gali-Muhtasib *et al*., 2000). Generally Salvia species are reported to have a wide range of pharmacological effects, such as anti-bacterial, fungistatic, virustatic, astringent, eupeptic, anti-hydrotic and hypoglycemic effects (Cherevatyi *et al*., 1980;

Farag *et al*., 1986; Eidi *et al*., 2005). In addition, they are well known also for their anti-oxidative properties (Hohmann *et al*., 1999; Wang *et al*., 2003) and antitumor (Liu *et al*., 2000), anticholestatic (Oh *et al*., 2002) effects.

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 (Jahovic *et al*., 2003; Sener *et al*., 2006), neurotoxicity (Uzar *et al*., 2006) and intestinal mucosa oxidative stress (Miyazono *et al*., 2004).

Relatively little is known about metabolic alterations occurring in both kidney and heart during aflatoxicosis, so the objective of this study is to evaluate the metabolic disorders induced pathology in rat kidneys and hearts in response to the exposure to AFB1. Also the ameliorative beneficial ability of some medicinal plant aqueous extracts compared to the currently available anticancer drug, MTX, against these disorders was also investigated. This can be achieved through measuring some markers of oxidative tissue damage in kidney such as malondialdehyde (MDA, index of lipid peroxidation) and the antioxidant markers, glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G-6-PDH) and vitamin C as well as some indices of energy metabolism in heart such as glycogen, glucose and some glycolytic enzymes namely, phosphogluco-isomerase (PGI), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH).

MATERIALS AND METHODS

This study was conducted in Medicinal Chemistry Department, National Research Center, Cairo, Egypt during winter.

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).

Plants

All plants used were purchased from the local market.

Preparation of Plant Extracts

One hundred grams of dried plants, shown in Table 1, were mixed with 1000 mL of distilled water and the mixture was boiled at 100°C under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at -20°C and then lyophilized. The lyophilized products of different plant extracts obtained were dissolved in water before administration at the stated doses given in Table 1.

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

Table 1: Studied plants

Scientific name	Family	Popular name	Used parts	Dose administered (mg kg^{-1} b. wt.)	References
Zingiber officinale Roscoe	Zingiberaceae	Ginger	Rhizomes	500	Al-Qattan *et al.* (2008)
Cinnamomum zeylanicum	Laureceae	Cinnamon	Bark	80	Kannappan and Anuradha (2008)
Trigonella foenum græcum	Leguminosae	Fenugreek	Seeds	50	Puri *et al.* (2002)
Camellia sinensis	Theaceae	Green tea	Leaves	200	Ito *et al.* (1989)
Salvia officinalis	Labiatae	Sage	Leaves	200	Amin and Hamza (2005)

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.

Experimental Design

The animals were divided into 8 groups:

Group 1: Normal control rats
Group 2: Aflatoxin-treated group.
Group 3: Aflatoxin-ginger rhizome (*Zingiber officinale*) treated group
Group 4: Aflatoxin-cinnamon bark (*Cinnamomum zeylanicum*) treated group
Group 5: Aflatoxin- green tea leaves (*Camellia sinensis*) treated group
Group 6: Aflatoxin-fenugreek seeds (*Trigonella foenum graecum*) treated group
Group 7: Aflatoxin-sage leaves (*Salvia officinalis*) treated group
Group 8: Aflatoxin-methotrexate treated group

Aflatoxin B1 was administered intraperitoneally as a single dose (1 mg kg^{-1}) (Preetha *et al*., 2006) 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 at a dose of 0.5 mg kg^{-1} bodyweight and this was repeated 24 h later (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 bidistilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 min at 10000 g at 4°C and the supernatants were used for different biochemical analysis except glycogen.

Biochemical Analysis

All the following parameters are measured spectrophotometrically:

Lipid Peroxidation Determination

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).

Vitamin C Estimation

Vitamin C was estimated by the method of Jagota and Dani (1982) using Folin-Ciocalteu reagent. The colour developed was read at 760 nm.

Glycogen Estimation

Glycogen content was estimated by the method of Nicholas *et al*. (1956) using 30% KOH and anthrone reagent.

Glucose Determination

Glucose level was estimated using Diamond Diagnostic Kits (Trinder, 1969).

Glutathione Reductase (GR) Assay

GR was measured by the modified method of Erden and Bor (1984). The reaction mixture contained the following in the final concentration: 4.1 mM Tris-HCl, pH 7.5, 15 mM $MgCl_2$, 5.7 mM EDTA, 60 mM KCl, 2.6 IU GSSG and 0.2 mM of NADPH in final reaction volume of 1 mL. The

reaction was started by the addition of tissue extract containing approximately 100 µg of protein. The decrease in absorbance was monitored at 340 nm.

Glucose-6-Phosphate Dehydrogenase (G-6-PDH) Assay

G-6-PDH 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 (Bergmeyer *et al*., 1974).

Phosphoglucoisomerase (PGI) Assay

PGI 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 due to NADPH production was recorded (Wu and Racker, 1959).

Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) Assay

GAPDH activity was determined by monitoring NADH generation at 340 nm (Serrano *et al*., 1991). The reaction mixture of 1 mL contained 50 mM Tricine-NaOH buffer pH 8.5, 10 mM sodium arsenate, 1 mM NAD+ and 2 mM glyceraldehyde 3-phosphate.

Lactate Dehydrogenase (LDH) Assay

LDH activity was evaluated in a reaction mixture containing 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).

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).

RESULTS

Table 2 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 vitamin C level as well as in the activities of tissue GR and G-6-PDH in AFBI-treated animals when compared with normal healthy ones. Administration of different plant extracts after AFB1 injection (Gs 3-7) restore most of these parameters to near their normal levels. However, supplementation of AFB1 treated rats with MTX (G8) dramatically amplified the toxic effect of AFB1 where the alteration in the studied parameters was 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 3. The result revealed marked decrease in glycogen and glucose contents in hearts of AFB1 administered animals accompanied with a reduction in the glycolytic enzymes PGI, GAPDH and LDH. Administration of the extracts

Table 2: Levels of some oxidative stress markers in rat kidneys of different experimental groups

			AFB1-different plant extracts treated					
	Normal	AF1B						MXT-treated
Parameters	G1	G2	G3	G4	G5	G6	G7	G8
MDA	17.50±1.1	35.700±3.32[a]	18.40±2.30	15.60±2.20	15.90±2.10	16.00±1.50	16.50±1.20	48.600±5.13[ac]
Vitamin C	2.40±0.2	0.940±0.06[a]	2.30±0.13	2.40±0.12	2.13±0.20	2.30±0.14	1.90±0.17[b]	0.580±0.06[ad]
GR	0.15±0.02	0.046±0.011[a]	0.15±0.01	0.13±0.007	0.14±0.005	0.14±0.008	0.13±0.01	0.034±0.005[ac]
G-6-PDH	0.16±0.02	0.037±0.006[a]	0.15±0.013	0.14±0.02	0.16±0.009	0.14±0.006	0.15±0.015	0.024±0.007[ac]

Data are Mean±SD of 5 independent experiments. MDA is expressed in nmoles g^{-1} tissue, enzymes are expressed in µmoles/min/mg protein, vitamin C is expressed in µg g^{-1} tissue. GR and G-6-PDH are expressed in µmoles/min/mg protein [a]$p<0.0001$, [b]$p<0.01$ versus normal group (G1). [c]$p<0.05$, [d]$p<0.01$ versus AFB1-treated group (G2)

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 was more evident in AFB1-MTX treated animals than AFB1-treated ones. The percent change of the above tested parameters in different experimental AFB1 treatment groups (G2-G8) compared to normal group (G1) is shown in Fig. 1 and 2.

Table 3: Levels of some energy metabolism markers in rat hearts of normal and AFB1 treated groups

			AFB1-different plant extracts treated					
	Normal	AF1B						MXT-treated
Parameters	G1	G2	G3	G4	G5	G6	G7	G8
Glycogen	360.92±23.9	163.320±12.5[a]	318.300±17[b]	340.40±16	343.70±18.9	350.90±15	348.60±23	116.70±14.2[ad]
Glucose	5.59±0.46	2.210±0.69[a]	5.700±0.38	5.90±0.35	4.70±0.98	5.60±0.14	5.54±0.53	1.51±0.06[ad]
PGI	0.29±0.02	0.050±0.01[a]	0.276±0.035	0.26±0.02	0.28±0.03	0.25±0.02	0.21±0.02c	0.03±0.002[ad]
GAPDH	0.27±0.015	0.063±0.02[a]	0.250±0.02	0.24±0.03	0.25±0.01	0.23±0.007[c]	0.025±0.004[ad]	0.24±0.008[c]
LDH	1.69±0.15	0.520±0.11[a]	1.760±0.12	1.65±0.12	1.76±0.12	1.53±0.15	1.34±0.09[c]	0.22±0.022[ad]

Data are Mean±SD of 5 independent experiments. Glycogen and glucose are expressed in mg g^{-1} tissue. Enzymes are expressed in μmoles/min/mg protein. [a]p<0.0001, [b]p<0.01, [c]p<0.05 versus normal group (G1). [d]p<0.001 versus AFB1-treated group (G2)

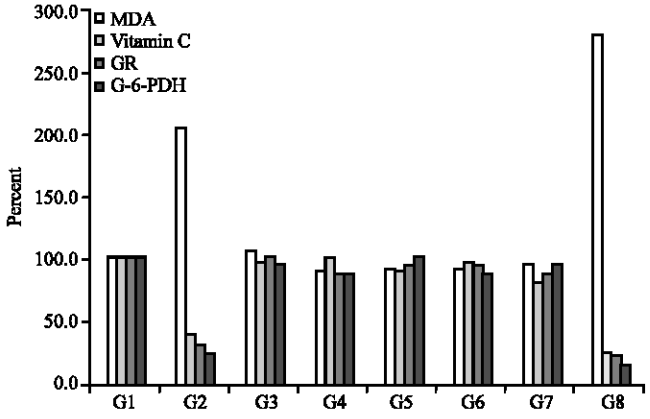

Fig. 1: Oxidative stress markers in rat kidneys of different experimental AFB1 treated- groups given as percent of control

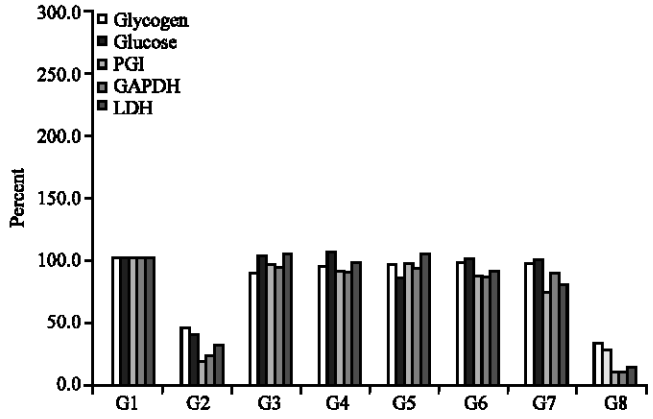

Fig. 2: Energy metabolism markers in rat hearts of different experimental AFB1 treated- groups given as percent of control

DISCUSSION

This study was performed to evaluate the beneficial antiaflatoxigenic ability of some traditional medicinal plant aqueous extracts against mycotoxicosis induced in rat kidneys and hearts by aflatoxin B1. 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 liver and kidney in rats and mice (Verma and Nair, 1999; Liebert *et al*., 2006; Naaz *et al*., 2007). 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 (Niki *et al*., 2005). 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 earlier investigation stated that AFB1 is a potent nephrotoxic compound leading to sever degenerative renal damage (Wangikar *et al*., 2005; Tessari *et al*., 2006).

Oxidative damage in the cell or tissue occurs when the concentration of ROS 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 the activities of glutathione metabolizing enzymes, GR and G-6-PDH, in kidneys of AFB1 administered rat could be responsible for increased lipid peroxidation observed during aflatoxicosis. This result is coped with earlier studies which revealed significant reductions in enzymatic and non-enzymatic antioxidants in AFB1 fed rat liver (Abdel-Wahhab and Aly, 2003; Naaz *et al*., 2007). Vitamin C, which includes ascorbic acid and its oxidation product, dehydroascorbic acid, has many biological activities in human body. This 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). 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).

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 (Breimer, 1990; Preetha *et al*., 2006). 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. 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 were effective in reducing lipid peroxidation and enhancing the antioxidant status of kidney in AFB1-treated animals, indicated by amelioration of MDA to near normal level and significant improvement in the antioxidant defense system (vitamin C, GR and G-6-PDH) in relation to AFB1-treated animals, suggesting their antioxidant potentials. Two mechanisms can be postulated to this good effect of the used plant extracts against AFB1 renotoxicity. The first one is that these plant 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 researchers reported that several phenolic compounds were contributed 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; Pizzale *et al*., 2002).

Essential oil components from ginger were proved to exhibit antimicrobial efficacies against a variety of food-borne pathogens (Thongson *et al*., 2004). Also, ginger's high antioxidant value has confirmed in many *in vitro* and *in vivo* system (Masuda *et al*., 2004). It contains pungent phenolic substances collectively known as gingerols. [6]-Gingerol (1, [4'-hydroxy-3'- methyoxyphenyl-5-hydroxy]-3-decanone), one of the major pungent elements of ginger, has been found to exhibit antioxidant activity as determined by inhibition of phospholipids peroxidation induced by the $FeCl_3$-ascorbate system, scavenging a number of free radicals and inducing the antioxidant defense systems (Masuda *et al*., 2004; Ajith *et al*., 2007a). It had recently reported the nephroprotective activity of aqueous ethanol extract of ginger against cisplatin-induced acute renal toxicity in mice (Ajith *et al*., 2007b). Also it was found that ginger has the ability to attenuate the progression of diabetic structural nephropathy (Al-Qattan *et al*., 2008).

Also, it was reported that cinnamon has potential antifungal effect. It contains essential oils (namely benzaldehyde, α-phellandrene, linalool, linalyl acetate, cinnamaldehyde, eugenol, b-caryophyllene, benzoic acid, benzyl cinnamate) which are rich sources of terpenoids and phenolic compounds that appear to be responsible for much of their antimicrobial activity (Rodr´ıguez *et al*., 2007; Singh *et al*., 2007). Beside, It was proved that cinnamon increased the antioxidant potential and counteracted the formation of lipid peroxidation end products induced in animals in response to different stressors (Nardini *et al*., 1997 ; Lee *et al*., 2003a; Kannappan and Anuradha, 2008). It contains active compounds includes, cinnamic acid, 3, 4-dihydroxy cinnamic acid (caffeic acid), cinnamaldehyde, eugenol, phellandrene, epicatechin and camphene, are reported to possess antioxidant property (Lee *et al*., 2003a; Singh *et al*., 2007). These active antioxidant components act as an electron donor, which can react with free radicals such as hydroxyl and superoxide radicals to form more stable products and thereby, terminate the radical chain reaction (Dorman *et al*., 2000).

Green tea (G3) was found to posses antimicrobial activity (Almajano *et al*., 2008), 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 (Chen *et al*., 2004; Raza and John, 2005; Panza *et al*., 2008). Epigallocatechin gallate, the main ingredient of green tea extract, is a strong chemopreventing of toxic effects of AFB1 (Chou *et al*., 2000; Ahmed *et al*., 2002). 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 (Tulayakul *et al*., 2007).

Fenugreek seeds are reported to be rich in polyphenolic flavonoids (namely vitexin, tricin, naringenin, quercetin, tricin-7-O-β-D-glucopyranoside, N, N'-dicarbazyl, glycerol monopalmitate, stearic acid, beta-sitosteryl glucopyranoside, ethylalpha-D-glucopyranoside, D-3-O-methyl-chiroinositol and sucrose) (Shang *et al*., 1998) which has antioxidant activity and was able to scavenge free radicals and enhance of endogenous antioxidant defenses GSH, vitamins C and E, thus prevent the process of lipid oxidation (Kaviarasan *et al*., 2008). Also, Dixit *et al*. (2005) reported the presence of gallic acid, o-coumaric acid, p-coumaric acid, rutin and caffeic acid in aqueous extract of fenugreek seeds which may also effectively scavenge the free radicals and thereby limit the progression of tissue damage.

Sage (*Salvia officinalis*) was reported to have antimicrobial potential effect (Cherevatyi *et al*., 1980), beside it contains several antioxidants such as water-soluble compounds; salvianolic acid A, salvianolic acid B and rosmarinic acid (Huang and Zhang, 1992), tanshinone IIA (Cao *et al*., 1996) and several phenolic glycosides, that prevent peroxidative damage, inhibit lipid peroxidations and free radicals generations *in vivo* and *in vitro* and induce endogenous antioxidant defense systems (Zupko *et al*., 2001; Amin and Hamza, 2005). Other Saliva species have been shown to inhibit acute liver injury induced by aflatoxin B1 (Liu *et al*., 2001) as well as protect against acute and chronic liver injury and fibrosis induced by CCl_4 (Lee *et al*., 2003b) in rats.

Administration of the anticancer drug, MTX, to AFB1-treated rats dramatically amplify 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 in AFB1- MTX treated animals than in AFB1-treated group, implicating the presence of oxidative tissue damage. This result is coped with earlier researchers 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 G6PDH) as well as vitamin C induced by MTX may be explained by earlier 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 (Caetano *et al*., 1997; Cetiner *et al*., 2005) and 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, GAPDH, PGI and 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 stated that cardiac glycolysis and glycogen synthesis are impaired by microbial endotoxin in rats. Present results are coped with some researchers 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 breakdown (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 (Kiessling, 1986). 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.

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 leading to degeneration of cardiac muscle fibers (Wangikar *et al*., 2005).

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 (Carvajal and Sánchez, 2003). The myocyte requires continuous 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 to heart failure (Mancini *et al*., 1999). This is supported by some researchers who reported that exposure to aflatoxin B1 toxicity caused heart defects (Pasha *et al*., 2006).

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. 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 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.

No comparable data are so far available for the effect of most of these plants on the heart energy metabolism, however earlier studies proved that trigonella seeds treatment could restore the altered expression of glycolytic enzymes in livers of diabetic animals and have the ability to increase glucose uptake through correcting the alterations in the distribution of GLUT4 in the skeletal muscle (Raju *et al*., 2001; Mohammad *et al*., 2006). Also, an aqueous extract of the cinnamon bark increased glucose metabolism roughly 20 fold *in vitro* in the epididymal fat cells (Anderson *et al*., 1978). Further, a methyl hydroxyl chalcone polymer derived from the cinnamon bark enhanced the glucose uptake, glycogen synthesis in 3T3-L1 adipocytes (Jarvill-Taylor *et al*., 2001). In addition, it was shown that the cinnamon bark extract (CBEt) modulates glucose, glucose-metabolizing enzymes in rats fed with a high-fructose diet (HFD) (Kannappan *et al*., 2006).

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 glycogen and glucose 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 glycogen may be 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 the glucose by MTX may be ascribed to its ability to decrease glucose transport into the cells as one of its mechanism of actions against tumor cells (Fung *et al*., 1986). 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 researchers reported that treatment of rat hepatocyte with MTX for 24 h resulted in an increase in enzyme leakage supporting the conclusion that MTX was cytotoxic agent in rat hepatocyte (Walker *et al*., 2000). Present 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 (Şener *et al*., 2006).

In conclusion, present 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 antiaflatoxigenic agents against aflatoxicosis.

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