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Panax ginseng C.A. Meyer Extract Protects Rat Erythrocyte from the Oxidative Damage Induced by the Synergistic Effects of Subchronic Treatment with Aflatoxin B_1 and Fumonisin

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Authors' contributions

This work was carried out in collaboration between all authors. Author KGAW carried out the experimental work, managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. Author FAM shared in the experimental work performed the statistical analysis and wrote the first draft of the manuscript. Author MAAW wrote the protocol, managed the project, managed the analyses of the study and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The current study aimed to evaluate the protective role of ginseng extract (GE) against the oxidative damage of erythrocytes resulted from the synergistic effects of subchronic (84 days) treatment with aflatoxin B_1 (AFB_1) and fumonisin B (FB) in male and female rats.
Study Design: Animals were divided into six groups of ten rats (5 males and 5 females) included: the control group; GE alone-treated group (150 mg/kg b.w); the group treated orally with AFB_1 (17 µg/kg b.w) during the first 2 weeks and fed FB_1-contaminated diet (100 mg/kg diet) during the 6th to 8th weeks; the group treated with GE during the

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mycotoxin protocol and continued till week 10; the group treated with GE 2 weeks before AFB_1 administration and continued till the end of FB_1 treatment and the group treated with GE for 4 weeks after the toxin protocol stopped.
Methodology: Blood samples were collected at the end of treatment for hematological and biochemical determinations.
Results: The results showed that the mycotoxins ingestion caused insignificant decrease in total hemoglobin (Hb) content, whereas they caused a significant increase in contents of Met-Hb, Carboxy-Hb and Sulf-Hb fractions accompanied by a significant decrease in Oxy-Hb faction, compared to the control group. Erythrocyte Met-HbR, SOD and GR activities were significantly decreased after mycotoxins treatment. In addition, mycotoxins ingestion induced a significant decrease in serum levels of iron and TIBC but did not significantly affect serum ferritin level. These effects were pronounced in male rats than in females. Treatment with GE ameliorated the erythrocytes damage induced by mycotoxins as indicated by the modulations in most of the investigated markers.
Conclusion: Treatment with GE has beneficial effect in counteracting erythrocyte membrane damage and hemoglobin changes induced by aflatoxin B_1 and fumonisin B_1. This effect may be probably through its potent antioxidative activity.

Keywords: Aflatoxin; fumonisin; mycotoxins; oxidative stress; erythrocytes; ginseng.

1. INTRODUCTION

Mycotoxins are fungal secondary metabolites commonly present in food. Ingestion of mycotoxin-contaminated products can lead to serious health problems, including immune suppression and even carcinogenesis [1,2]. Fumonisin and aflatoxin are produced by certain fungal strains belonging to the Fusarium and Aspergillus families, respectively [3]. Fumonisin B_1 (FB_1) and aflatoxin B_1 (AFB_1) are the most important toxins of each group, due to their prevalence as cereal contaminants and their toxicological potency. Humans and animals being constantly exposed to low levels of these mycotoxins, either individually or in combination [3].

Aflatoxins are hepatotoxic, immunosuppressive, carcinogenic, teratogenic and mutagenic [4-7], Several diseases are associated with the human consumption of these toxins, including toxic hepatitis and even primary hepatocellular carcinomas [3,8]. Fumonisins B have been shown to be the causative agent of equine leukoencephalomalacia and of porcine pulmonary edema syndrome [9]. Long-term studies indicated that fumonisin B_1 (FB_1) is hepatocarcinogenic [10,11] and nephrocarcinogenic in rats [12]. In addition, it has immunotoxic properties [13].

Li et al. [14] observed that co-contamination of maize with AFB_1 and FB_1 in a high-incidence area of human primary hepatocellular carcinoma in China, which led to the probable participation of both toxins in the genesis of this pathology being suggested. The physiological immuno surveillance, a key function to prevent tumor development, could be suppressed as consequence of the immunotoxic action of aflatoxins and fumonisins individually or as mixtures, as was previously stated in an experimental mycotoxicoses [15].

The possible genotoxicity of fumonisins has been evaluated in recent years, and it was proposed that this toxin could produce genetic damage by means of an indirect mechanism involving the cellular oxidative stress. Hassan et al. [16] observed that FB_1 caused DNA strand breaks in isolated rat liver nuclei, and concluded that such lesions may be caused by

increased lipid peroxidation. Taken together, the reactive oxygen species and lipid peroxidation have been considered to be main mechanisms in the toxicity of AFB_1 [17] and FB [6].

The erythrocytes play a very important physiological role in organism metabolism [18]. The main function of erythrocytes is the transport of oxygen (O_2) and mediation of carbon dioxide (CO_2) production [19,20]. As the red blood cell emerges from the bone marrow, it loses its nucleus, ribosomes, and mitochondria and therefore all capacity for cell division, protein synthesis, and mitochondrial-based oxidative reactions [20,21]. The behavior of erythrocytes deserves special attention. The erythrocyte membrane contains abundant polyunsaturated fatty acids, which are very susceptible to free-radical induced lipid peroxidation [22]. Erythrocytes have a powerful antioxidant protection system. However, at a high concentration of free radicals or insufficiency of the primary antioxidant protection, the oxidative damage of the erythrocyte membrane components leads to loss of the ability of erythrocytes to transfer O_2 and CO_2, and brings about cell hemolysis [23].

So far, antioxidants have attracted much interest with respect to their protective effect against damage by free radical that may be the cause for many diseases including cancer [17]. Panax ginseng (family Araliaceae) is one of the most widely used herbal medicines and is reported to have a wide range of therapeutic and pharmacological applications [18]. The major active components in P. ginseng are ginsenosides, containing a series of derivatives of the triterpene dammarane being attached by some sugar moieties. Ginsenosides can be divided into two groups according to the sugar moiety attaching position [22]. Ginsenoside has anti-oxidative abilities apart from its immunomodulatory, antihyperlipidemic and neuroprotective activities. Pretreatment with ginseng significantly attenuates H_2O_2 induced free radical production and protect against cell death [24]. The present study was designed to estimate the synergistic effect of subchronic administration of aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) on rat erythrocyte and to evaluate the protective role of Korean Panax ginseng extract against erythrocyte membrane damage and hemoglobin changes induced by these mycotoxins.

2. MATERIALS AND METHODS

2.1 Chemicals

Aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in the chemical methods were of the highest grade of commercially available materials.

2.2 Plant Materials

Korean Red ginseng was kindly provided by the Korean Society of ginseng, Seoul, Korea.

2.3 Preparation of *Korean ginseng* Extract

Korean red ginseng was extracted with 10 volumes of distilled water at 85°C for 8 h five times. The aqueous extract was combined and concentrated under reduced pressure to give darkish brown syrup, ginseng extract (GE). The moisture content of GE was 37.21%.

2.4 Determination of GE content

The ginsenosides content in the GE was determined by high performance liquid chromatography (HPLC) according to the method described by Ko et al. [25] as follows: an aliquot of GE dissolved in distilled water was passed through Sep-Pak C18 cartridge, and the cartridge was washed with distilled water. Subsequently, ginsenosides were eluted with 90% methanol and then analyzed by HPLC.

2.5 Fumonisin B_1 Production

Fumonisin B_1 (FB_1) was produced through the fermentation of corn by Fusarium verticillioides (F. moniliforme) obtained from plant pathology Institute, Agriculture Research Centre, Giza, Egypt. The fermented corn was autoclaved; ground to a powder and the fumonisin B_1 content was measured by HPLC according to Voss et al. [26]. The corn powder was incorporated into the basal diet to provide the desired level of 100 mg/kg diet. The diet containing FBs was analyzed and the presence of FB_1 was confirmed by HPLC then it was stored at ⁻20ºC for the duration of the experiment.

2.6 Animals and Treatments

Male and female Sprague-Dawley rats (150-160 g) were obtained from animal house colony of the National Research Centre, Dokki, Egypt. The animals were maintained on standard laboratory diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolizable energy 12.08 MJ) and were housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled at the Animal House Lab., National Research Centre, Dokki, Cairo, Egypt. After an acclimatization period of 1 week, the animals were divided into six groups of ten rats/group (5 male and 5 female) as shown in Table 1 as follow: Group 1, untreated control group; group 2, received a daily oral dose of GE (150 mg/kg b.w) [6] for 12 weeks; group 3, received an oral daily dose of AFB_1 (17 µg/kg b.w) in corn oil [6] during the first 2 weeks and fed on FB_1-contaminated diet (250 mg/kg diet) [26] from week 6 to 8; group 4, received a daily dose of GE for 10 weeks (from week 1 to week 10), treated with AFB_1 for the first 2 weeks and fed FB_1-contaminated diet for 3 weeks (from week 6 to 8); group 5, received a daily dose of GE for 10 weeks (from week 1 to week 10), treated with AFB_1 on weeks 3 and 4 then fed on FB_1-contaminated diet on weeks 8, 9 and 10; group 6, treated with AFB_1 in the first 2 weeks and fed FB_1-contaminated diet during weeks 6-8 then treated with GE on week's 9-12. The study was performed with the approval of the Animal Care and Ethics Committee of the National Research Centre, Cairo, Egypt.

2.7 Blood Sampling and Hemolysate Preparation

At the end of the experimental period, animals were fasted overnight and following diethyl ether anesthesia, blood samples were withdrawn from the retroorbital venous plexus. Each blood sample was divided into 2 portions: the smaller portion was taken on heparin and the whole blood was used for the determination of hemoglobin and its derivatives while the other portion was centrifuged at 3000 r.p.m for 15 minutes at 4°C where the clear serum was obtained.

Table 1. Experimental groups and their respective treatments

Weeks / Groups	1	2	3	4	5	6	7	8	9	10	11	12
Group1 (control)	—	—	—	—	—	—	—	—	—	—	—	—
Group 2	GE	GE	GE	GE	GE	GE	GE	GE	GE	GE	GE	GE
Group3	AFB_1	AFB_1	—	—	—	FB	FB	FB	—	—	—	—
Group 4	GE+AFB_1	GE+ AFB_1	GE	GE	GE	FB + GE	FB + GE	FB + GE	GE	GE	—	—
Group 5	GE	GE	GE+ AFB_1	GE+ AFB_1	GE	GE	GE	FB+ GE	FB+ GE	FB+ GE	—	—
Group 6	AFB_1	AFB_1	—	—	—	FB	FB	FB	GE	GE	GE	GE

AFB_1: aflatoxin B_1 (17 µg/kg b.w); FB: fumonisin (250 mg/kg diet); GE: Ginseng extract (150 mg/kg b.w)

Hemolysate was prepared according to the method of Silva et al. [27]. Briefly, whole blood was centrifuged at 3000 r.p.m for 15 minutes, then, the buffy coat was removed and the packed red cells were washed three times with physiological saline. The washed cells were lysed by suspending in hypotonic phosphate buffer and centrifuged at 7000 r.p.m for 30 minutes. The resulting pellet is the erythrocyte membrane and the supernatant represents the hemolysate. The supernatant obtained was further used for enzymatic assays.

2.8 Determination of Autoxidation Rate of Oxyhemoglobin

Autoxidation rate of oxyhemoglobin was determined according to the method described by Mansouri and Winterhalter [28].

2.9 Determination of Osmotic Fragility

The percentage of hemolysis was assessed according to the method described by Nicak and Mojzis [29] as hemolysis was determined using a series of eleven osmotic mixtures. Each mixture composed of different concentrations of NaCl, HgCl and glucose. The percentage of hemolysis was calculated according to the following formula: Hemolysis % = (A sample/A H_2O)*100.

2.10 Determination of Total Hemoglobin Concentration

Total hemoglobin (Hb) concentration in whole blood was measured colorimetrically using a kit produced by Biodiagnostics Co. (Cairo, Egypt).

2.11 Determination of Relative Concentration of Hemoglobin Derivatives

Methemoglobin (Met-Hb) level was determined in blood sample using the method described by Evelyn and Malloy [30]. Oxyhemoglobin (Oxy-Hb), Sulfhemoglobin (Sulf-Hb) and carboxyhemoglobin (Carboxy-Hb) levels in the blood samples were determined spectrophotometrically according to the method described by Van Kampen and Zulstra [31].

2.12 Iron, Total Iron Binding Capacity (TIBC) and Ferritin Determinations

Iron concentration and TIBC were determined in serum by spectrophotometric analysis using commercially available kits produced by Stanbio Laboratory (Boerne, TX, USA). The quantitative determination of serum ferritin was carried out by enzyme linked immunosorbant assay (ELISA) using a kit purchased from Monobind Co., Inc., USA.

2.13 Enzyme Assays

The activity of methemoglobin reductase (Met-HbR) was estimated in the hemolysate by assessing the spectrophotometrically rate of NADH-oxidation using the chemical method described by Hegesh et al. [32]. Superoxide dismutase (SOD) and glutathione reductase (GR) activities were determined in the hemolysate according the instruction manual of a kit purchased from Randox Laboratories Co., UK and Oxis Research^{TM} Co., Inc., USA, respectively.

2.14 Statistical Analysis

The obtained data were subjected to one way analysis of variance (ANOVA) using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. Tukey test was used to clarify the significance between the individual groups at probability level $P< 0.05$ [33].

3. RESULTS

The results of the HPLC analysis of GE revealed that ginsenoside Rg3 was identified as the main component (4.04 mg/g). The extract contains also Rb1: 3.72; Rg2: 3.16; Rc: 1.89; Rb2: 1.71; Rd: 1.32; Rf: 1.02; Re: 0.95; Rh1: 0.88; Rg1: 0.54; Rh2: 0.11 mg/g.

The effects of the mycotoxins as well as ginseng extract (GE) or the combination of both on the autoxidation rate of oxyhemoglobin of male (A) and female (B) rats are shown in Fig. 1. The data revealed that the tested mycotoxins performed a marked elevation in the autoxidation rate of oxyhemoglobin which was higher in male than female rats comparing with the matched sex in the control group. In addition, the animal groups treated with GE and mycotoxins, in different combinations, showed a marked improvement in the autoxidation rate regarding both sexes. The highest degree of improvement was found in the sixth group.

The effects of the mycotoxins alone, GE and mycotoxins together with GE in different treatments on the percentage of hemolysis of rats' erythrocytes are shown in Fig. 2. It is clearly noticed that erythrocytes of the mycotoxins treated-animals behave more degrees of hemolysis than those of either control or treated with GE; the highest degree was the mycotoxins alone treated rats; while those of the sixth group recorded a hemolysis behavior close to that of control.

Data presented in Table 2 shows the effects of the tested mycotoxins and GE on total hemoglobin and its derivatives. Mycotoxins ingestion induced insignificant decrease in total hemoglobin content, whereas Met-Hb, Carboxy-Hb and Sulf-Hb contents were found to be increased significantly accompanied by a significant decrease in Oxy-Hb, compared to the control group. Treatment with GE alone did not show any significant alteration in the levels of total hemoglobin or its derivatives compared to control group. The administration of GE to mycotoxins-intoxicated groups could hardly affect the levels of total hemoglobin, Oxy-Hb, Carboxy-Hb and Sulf-Hb whereas it significantly decreased Met-Hb formation compared to the group that was treated with both the mycotoxins alone.

Table 2. Effect of ginseng extract on total hemoglobin (Hb) content and its derivatives (expressed as % of total hemoglobin) in blood of mycotoxins- intoxicated rats

Groups	Total Hb (g/dl)	Oxy-Hb (%)	Met-Hb (%)	Carboxy-Hb (%)	Sulf-Hb (%)
Group 1	12.93 ± 0.13^{ab}	97.07 ± 1.23^{a}	1.53 ± 0.08^{c}	0.92 ± 0.11^{b}	0.48 ± 0.12^{d}
Group 2	13.74 ± 0.31^{a}	96.14 ± 2.09^{a}	2.31 ± 0.46^{c}	1.06 ± 0.08^{b}	0.49 ± 0.10^{d}
Group 3	11.81 ± 0.49^{b}	86.89 ± 3.02^{b}	9.10 ± 0.93^{a}	4.30 ± 1.02^{a}	1.27 ± 0.35^{c}
Group 4	12.71 ± 0.27^{b}	87.31 ± 1.86^{b}	7.51 ± 0.90^{ab}	3.73 ± 0.76^{ab}	1.68 ± 0.27^{ab}
Group 5	12.24 ± 0.70^{b}	88.49 ± 2.16^{b}	6.06 ± 0.93^{b}	3.63 ± 0.81^{ab}	1.82 ± 0.01^{bc}
Group 6	12.23 ± 0.32^{b}	88.13 ± 2.67^{b}	5.89 ± 0.39^{b}	2.74 ± 0.64^{ab}	1.45 ± 0.2^{a}

Values are mean ± SE for 10 rats per group.
Within each column, means with different letters are significantly different ($P \leq 0.05$) using one way (Tukey) ANOVA test.

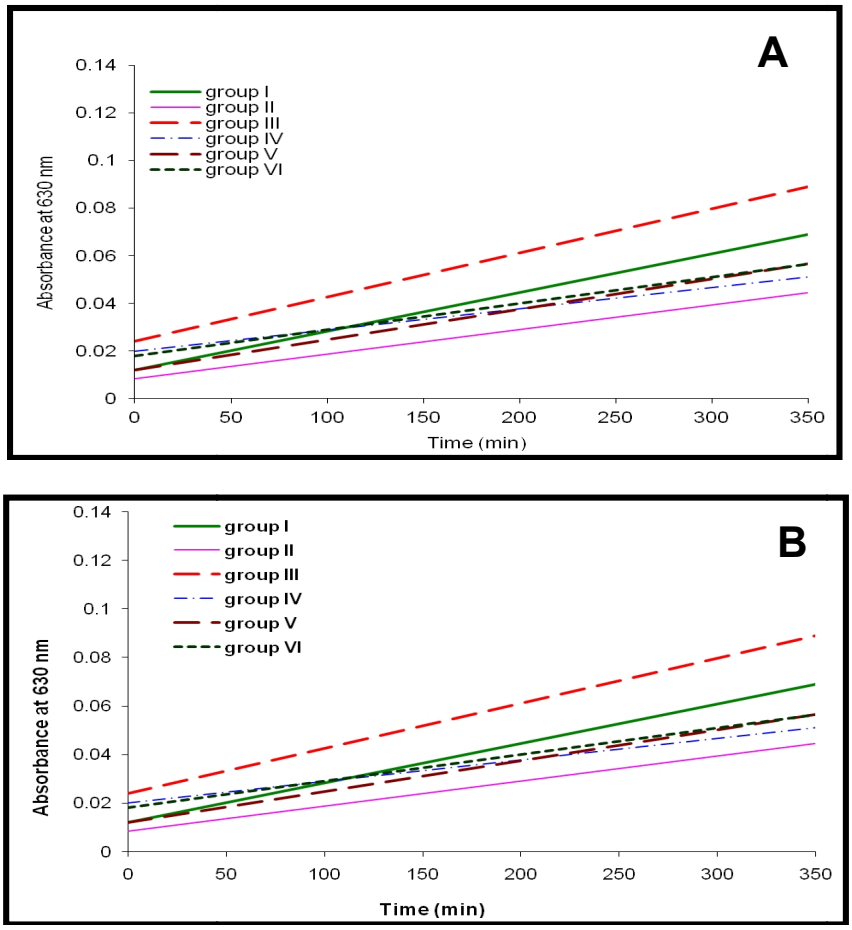

Fig. 1. Effects of AFB1 and FB1 alone or in combination with or without ginseng extract on the autoxidation rate of oxyhemoglobin of male (A) and female (B) albino rats.

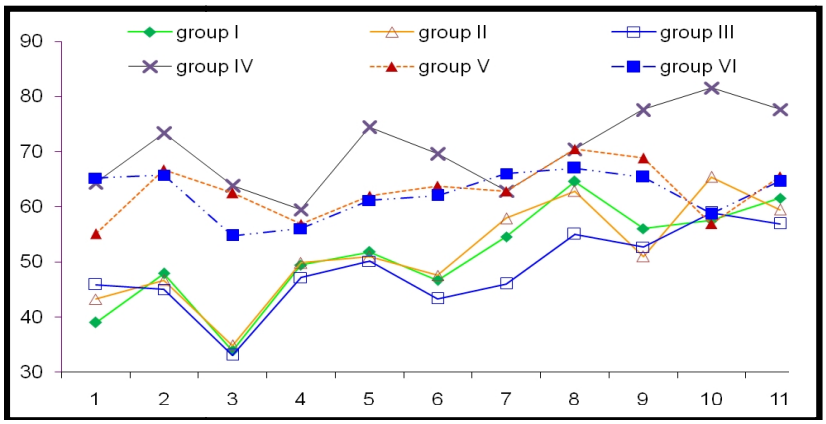

Fig. 2. Hemolysis behavior of RBCs of albino rats with different treatments of AFB_1 and FB_1 alone or in combination with GE as well as normal ones.

The effects of GE administration on iron status and total iron binding capacity (TIBC) in mycotoxins-intoxicated rats are presented in Fig 3. Mycotoxins ingestion resulted in a significant decrease in serum levels of iron and TIBC compared to the control group. At the same time, it did not significantly affect serum ferritin level (Fig. 4). The administration of GE during the mycotxins treatment in group 4 or before the mycotoxins treatment in group 5 offered little protection against mycotoxins-induced changes in iron and TIBC levels, whereas the administration of GE to rats after the mycotoxin treatment succeeded in ameliorating significantly the mycotoxins-induced changes in the mentioned parameters although their levels were still significantly different than the control group.

The results revealed that mycotoxins ingestion significantly decreased erythrocyte Met-HbR (Fig.5), SOD (Fig. 6) and GR (Fig 7) activities compared to the control group. The intake of GE alone significantly increased the activities of these enzymes above the normal values of the controls. The administration of GE during or before the mycotoxins treatment induced insignificant increase in Met-HbR and SOD activities while GR showed significant increase compared to the mycotxins alone-treated group. Meanwhile, the administration of GE to rats pretreated with mycotoxin (group 6) significantly increased the activities of the three enzymes activity towards the normal values of the controls.

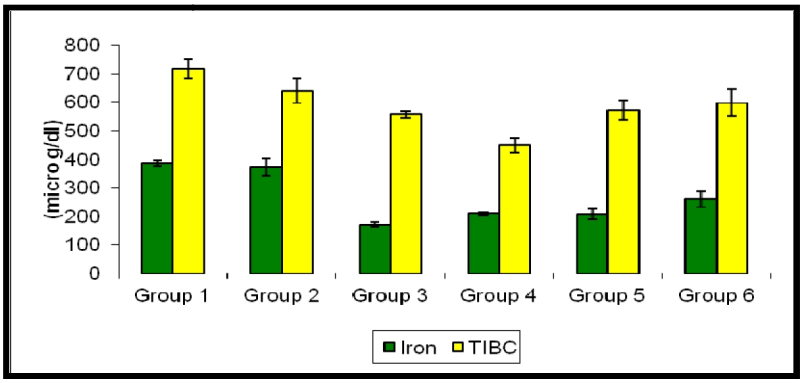

Fig. 3. Effect of ginseng extract on serum iron and total iron binding capacity (TIBC) in mycotoxins- intoxicated rats

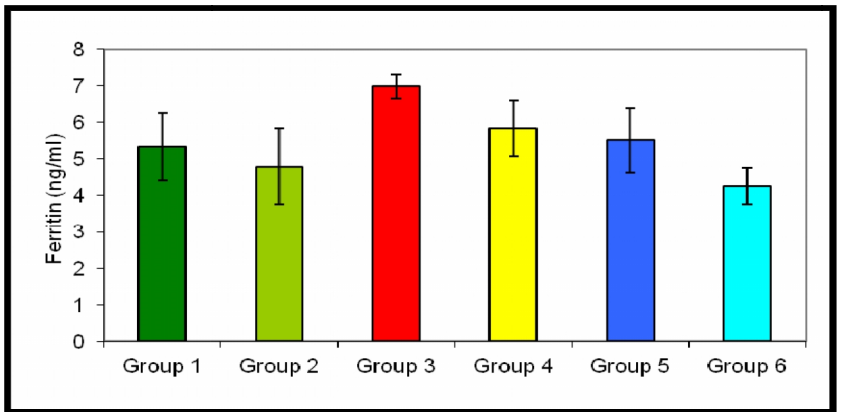

Fig. 4. Effect of ginseng extract on serum ferritin in mycotoxins- intoxicated rats

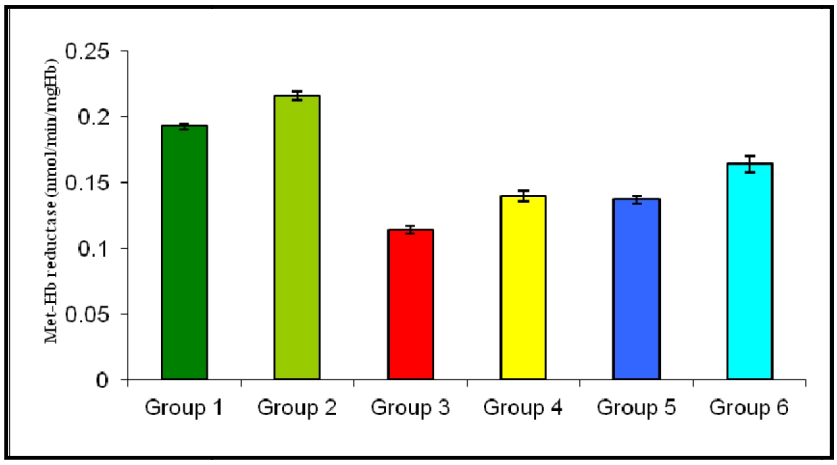

Fig. 5. Effect of ginseng extract on Met-Hb reductase in erythrocytes of mycotoxins-intoxicated rats

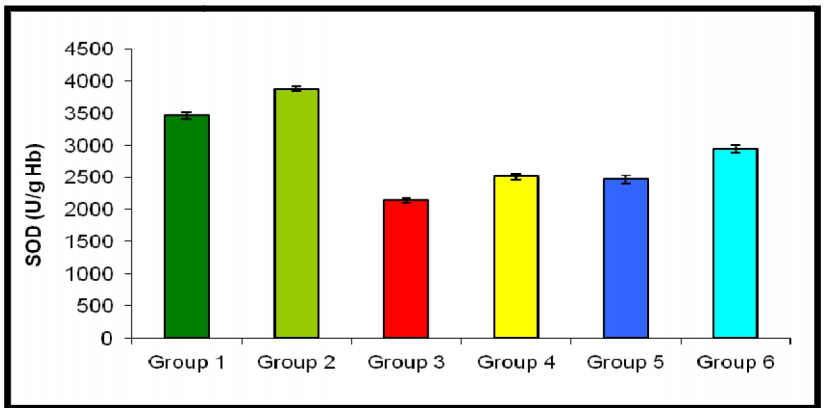

Fig. 6. Effect of ginseng extract on SOD in erythrocytes of mycotoxins-intoxicated rats

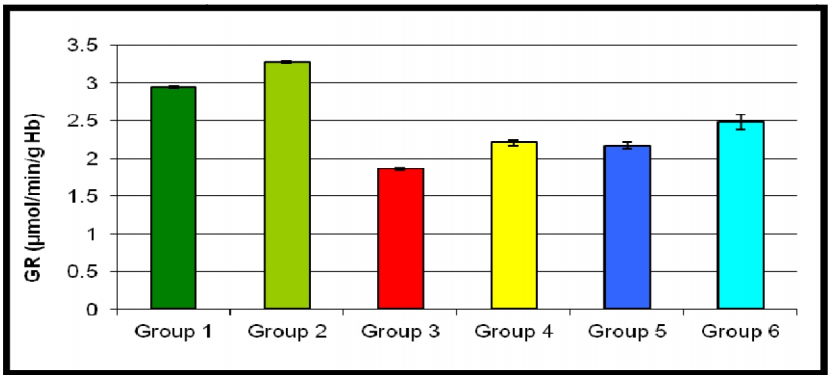

Fig. 7. Effect of ginseng extract on GR in erythrocytes of mycotoxins-intoxicated rats

4. DISCUSSION

Mycotoxins have been reported to have potential to generate free radicals in biological system [1,17,34-36]. Free radicals "atoms or molecules that contain one or more unpaired electrons" have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction [37] and the question of whether oxidative stress is a major cause of erythrocyte injury remains equivocal.

Aflatoxin B_1 is metabolized by the cellular cytochrome p450 enzyme system to form the reactive intermediate, aflatoxin B_1-8,9-epoxide, which in turn reacts with macromolecules such as lipid and DNA, leading to lipid peroxidation and cellular injury [38]. Oxidative damage has also been indicated in the aetiology of the toxic effects of fumonisins [22,39]. The results reported by Yin [40] provide the evidence that fumonisins apparently increase the rate of oxidation, promote free radical intermediate production and accelerate the chain reaction associated with lipid peroxidation leading to disruption of membrane structure.

The current results revealed that ingestion of AFB_1 and FB_1 mycotoxins caused a statistically significant increase in the autoxidation rate of oxyhemoglobin and also in hemolysis in female and male rats compared to their control values. Heme proteins like hemoglobin (Hb) and myoglobin (Mb) contain redox-active transition metal iron that makes them susceptible to causing oxidative damage. Although the structure of the globin chain allows heme to bind oxygen with minimal oxidation of ferrous to ferric iron, autoxidation is not entirely prevented; low concentrations of ferric heme are normally present in vivo (methemoglobin, metmyoglobin) [41,42]. Methemoglobin (MetHb) is formed when the ferrous porphyrin complex of Hb is oxidized into the ferric form. Unlike Hb, MetHb cannot bind oxygen and therefore is physiologically inert. Normally less than 2% of circulatory Hb exists in the form of MetHb [43] because the rate of Hb oxidation is exceeded several hundred fold by the capacity of red blood cells to reduce oxidized heme [44]. The oxidized heme can then react with the peroxides (H_20_2) formed during the autoxidation process itself or elsewhere in the protein's vicinity [41,42]. It has been suggested that H_20_2 and some free radicals are involved in the autoxidation reaction of Oxy-Hb [45]. These authors showed that $3M\text{-}0_2$ is released from 1 M-haemoglobin tetramer (4M-haem) during the autoxidation reaction of Oxy-Hb. However, the autoxidation mechanism of Oxy-Hb to methemoglobin was discussed in detail elsewhere.

Both the globin-bound radical and ferryl heme iron can cause tissue damage, for example, by initiating lipid peroxidation reactions [41,42]. The rate of Oxy-Hb oxidation is increased by a number of agents including 8-aminoquinolines, sulfonamides, and by exogenous and endogenous nitrites, nitrates and nitric oxide (NO) [46,47].

In the current study, AFB_1 and FB_1 did not significantly decrease the quantity of total Hb but they significantly affect its function. This appeared from the significant decrease in Oxy-Hb content and the significant increase in Met-Hb and Carboxy-Hb contents. These changes may, therefore are consequence of the increase in autoxidation rate of Oxy-Hb which caused by free radicals generated during mycotoxins metabolism. Besides a direct oxidant effect of the free radicals that generated during the mycotoxins metabolism, AFB_1 and FB_1 could increase Hb oxidation by other mechanisms. One such mechanism could be induction of NO production. AFB_1 [48] and FB_1 [49] were reported to induce the production of NO through macrophage activation. Moreover, NO is produced in endothelial cells and macrophages by a family of isoenzymes known as nitric oxide synthases (NOSs.) [50,51].

Inducible nitric oxide synthase (iNOS) is induced in macrophages during activation. Moreover, NO can act as a mediator of a variety of biological effects, among which are suppression of macrophage functions, tumor, cytotoxicity, vasodilatation and inhibition of platelet aggregation [49]. According to Sun et al. [52], under physiologic oxidative stress NO might provide protection to cells by S-nitrosylation of some critical protein thiols (-SH) on Hb to form nitrosated proteins, preventing them from further oxidative modification by reactive oxygen species (ROS). In addition, the presence of different Hb-NO reaction products may variously influence the whole blood oxygen affinity. Higher concentrations of met-Hb and SON-Hb shift the oxyhemoglobin dissociation curve leftward and higher Hb Fe^{2+} NO concentrations shift it rightward [53]. On the other hand, the reaction of NO with the oxygen-heme complex of oxyhemoglobin (oxyHb, Fe^{2+}) to form methemoglobin (metHb, Fe^{3+}) [54,55] has been considered to be a NO• characteristic [56]. Conversely, increased oxidative stress and the resultant dysregulation of NO are implicated in the pathogenesis of many diseases. Nitrosative stress occurs with an increase in reactive nitrogen species and ROS formed from oxidative stress. For example, the peroxynitrite ($OONO^-$), generated from NO and superoxide is a very strong cytotoxic oxidant, which can irreversibly damage cells by oxidation of free thiols, nitration of tyrosine residues and lipid peroxidation [52].

The excessive peroxidation process has been suggested to result in the release of soluble products that may affect erythrocyte membrane. Cell membranes integrity is broken leading to cell hemolysis and free hemoglobin (Hb) is released into the extracellular environment during hemolysis or extravasation of red blood cells and exerts inflammatory and toxic effects arising from oxidative processes involving the reactive heme group [57,58].

The redox cycling of the mycotoxins leads to the formation of free radicals such as reactive oxygen species (ROS) and superoxide anion (O^2) and H_20_2 [35]. It has been reported earlier that ROS, O^2 and H_20_2 can directly damage red blood cell (RBC) membranes and metabolic machinery, causing rapid intravascular hemolysis. These radicals also denature hemoglobin, which then precipitates and covalently binds to the interior RBCs membrane, thus forming Heinz bodies. This process distorts the cell membrane, resulting in increased RBCs fragility, hemolysis, and destruction of RBCs by the reticuloendothelial system [59].

As expected, mycotoxins ingestion at the tested doses significantly decreased both serum iron and the body iron store TIBC levels but insignificantly affected serum ferritin (the major iron - storage protein). Our data are in analogue with the previously mentioned report [60]. It is well documented that iron is the most abundant, important and essential transition metal in biochemical reactions and a heterogeneous group of proteins contain iron in a variety of molecular forms [20,61]. Iron not only binds oxygen reversibly but also participates in a number of vital oxidation-reduction reactions [62]. The mechanism by which the mycotoxins might induce iron deficiency as explained by Harvey et al. [60] is the inhibition of iron absorption or transport resulting in decreased serum iron level and consequently TIBC level.

Enzymes for preventing oxidative denaturation in erythrocytes are including superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase (GR)-dependent regeneration of glutathione (GSH) and NADH- Methemoglobin reductase (Met-HbR) [62,63]. Physiologically, erythrocytes are well protected against ROS by abundant Cu, Zn-SOD which scavenges free radicals thus preventing metHgb formation [64]. In the current study, we observed decreased SOD, GR and Met-HbR activities in mycotoxins-treated group. SOD scavenges O2•- and inhibits the formation of peroxynitrite, thereby suppressing injury and regulating the bioavailability of NO [65]; Whereas, GR plays essential role in reducing of oxidized glutathione [66]. Met-HbR, which requires the reduced form of nicotinamide adenine

dinucleotide (NADH) as a cofactor is primarily responsible for methemoglobin reduction in RBCs [59].

In erythrocytes, the major antioxidant is GSH which protects important proteins such as spectrin, the oxidation of which can lead to increased membrane stiffness [67]. GSH not only supports antioxidant defense, but is also an important sulfhydryl buffer, maintaining -SH groups in Hgb and enzymes in the reduced state [68]. Several studies on the mechanisms of mycotoxins induced liver injury have demonstrated that GSH plays an important role in the detoxification of the reactive and toxic metabolites of these mycotoxins, and the liver necrosis begins when the glutathione stores are almost exhausted [69,70]. Previously, we reported that administration of AFB_1 and FB_1 to rats decreased protein synthesis and enhanced lipid peroxidation and MDA formation in liver tissue which is presumably results of free-radical-mediated toxicity [6,71]. The targets of oxidative damage are usually critical biomolecules such as protein, nucleic acids, and lipids [7,72]. The observed decrease in erythrocytes antioxidant status in AFB_1 and FB_1-treated rats may therefore be a manifestation of increased tissue oxidative stress caused by AFB_1 and FB_1 metabolism. We suggest that the inactivation of SOD, GR and Met-HbR, observed herein is the effect of action of the free radicals generated by mycotoxins metabolism which may damage the spatial structure of the antioxidant enzymes. In addition, MDA reactivity towards amino groups can result in inhibition of DNA and RNA protein synthesis in liver [72,73] and therefore affect enzymes synthesis.

The current data demonstrated that GE is considered as a potent antioxidant agent since it could produce marked decrease in Met-Hb formation that enhanced by the mycotoxins towards the normal value and as the cell hemolysis was also decreased. We observed also that the lowered activities of erythrocyte Met-HbR, SOD and GR caused by AFB_1 and FB_1 ingestion were effectively countered by GE. Additionally, GE could also ameliorate serum iron but not affect ferritin or TIBC. These positive alterations were detected significantly when GE given after the end of mycotoxins administration.

In previous works, we suggested that AFB_1 and FB_1 exert oxidative stress on hepatic tissue as indicated by increased hepatic MDA concomitant with significant decrease in hepatic total antioxidant capacity [6,71]. We have also evidenced that GE offer protective influence on these alterations. This in turn improves liver function and consequently increases its ability to synthesize the antioxidant enzymes and glutathione required for erythrocytes protection.

As indicated above, GE is composed of a number of ginsenosides (ginseng saponins). Ginsenosides appear to be responsible for most of the activities of ginseng including vasorelaxation, antioxidation, antiinflammation and anticancer. Among them, the most commonly studied ginsenosides are Rb1, Rg1, Rg3, Re and Rd [18]. The antioxidant activity of ginsenosides is further confirmed by several studies. Zhong and Jiang [74] examined cellular structures of free radical damage on myocardial cells induced by xanthine. They measured free radicals with an electron spin resonance technique and found that the ginsenosides Rb1, Rc, Re, Rg1, Rg2 and Rh1 counteracting the action of free radicals induced by xanthine. Additionally, Gillis [75] demonstrated the protective effects of ginsenosides on an injured rabbit pulmonary endothelium induced by a variant of reactive oxygen species. Furthermore, Wang et al. [76] demonstrated that the endothelial dysfunction induced by homocysteine and HIV protease inhibitors was effectively blocked by Rb1 and other ginsenosides and these results proved that Rb1 and other ginsenosides fully block reactive oxygen species production.

In an in vitro study, Li and Liu [77] determined the relationship between the structure of ginsenoside and its activity in AAPH-induced hemolysis of human erythrocytes. They found that the individual ginsenoside (20(S)-protopanaxadiol or 20(S)- protopanaxatriol) behaves as an antioxidant if a glucose is attached to the 20-position of the triterpene dammarane, such as Rc, Rb1, Rb3, Rg1, Rh1, Rg2, Re and R1, but as a prooxidant if there are no sugar moieties attached to the 20-position of the ginsenoside such as Rg3, Rh2 and Rd [22] concluded that Rb1and Rb3 are the most important antioxidative ingredients among all these ginsenosides.

Sex-related difference in metabolic activation and detoxification of AFB_1 and FB_1 can directly influence the metabolites formed in different tissues [78]. On reviewing the row data obtained, we observed that the mycotoxins toxicity was more pronounced in male rats than in females. For example, in mycotoxins-treated male, more inhibition in Met-HbR, SOD and GR was observed. Met-Hb formation and Oxy-Hb oxidation were significantly higher in male than in female. However, the lowered iron level and increased ferritin formation were found to be higher in female rats (data not shown). Male animals of many rodent strains were reported to have a significantly greater capacity for the liver metabolism and breakdown of chemicals (they have more cytochrome P450). This greater capacity for oxidative metabolism can cause the male animals of certain rodent strains to be more or less susceptible to toxicity from a chemical depending on whether oxidative metabolism represents a bioactivation or detoxification pathway for the chemical at the dose it is administered [79]. Previously, it was reported that AFB_1 exerts its biological effects after metabolic activation by cytochrome P-450-dependent monooxygenase to its reactive form i.e. AFB_1-epoxide which then interact with cellular macromolecules, particularly DNA [38,80] leading to lipid peroxidation and cellular injury. The above may explain variation in the response of male and female rats intoxicated by mycotoxin as well as those received GE.

5. CONCLUSION

The results described, together with our previous data [6] provide clear evidence that Panax ginseng extract administration has a beneficial effect in counteracting erythrocyte membrane damage and hemoglobin changes induced by AFB_1 and FB_1 ingestion probably partially through its potent antioxidative activity besides its indirect protective effect on hepatic tissue that may increase the ability of the liver to synthesize the antioxidant enzymes and glutathione required for erythrocytes protection.

CONSENT

Not applicable.

ETHICAL APPROVAL

All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo, Egypt.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bondy GS, Pestka JJ. Immunomodulation by fungal toxins. J Toxicol Environ Health B Crit Rev. 2000;3:109-143.
2. Richard JL. Some major mycotoxins and their mycotoxicoses- an overview. Int J Food Microbiol. 2007;119:3-10.
3. Pitt JI. Toxigenic fungi and mycotoxins. Br Med Bull. 2000;56:184-192.
4. IPCS-WHO. Aflatoxins. In: WHO, ed. International program on chemical safety, Food Additives, Series 40. World Health Organization, Geneva. 1998;1-73.
5. Abdel-Wahhab MA, Nada SA, Amra HA. Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in the rats. J Appl Toxicol. 1999;19:199-204.
6. Abdel-Wahhab MA, Hassan NS, El-Kady AA, Khadrawy YA, El-Nekeety AA, Mohamed SR, Sharaf HA, Mannaa FA. Red ginseng extract protects against aflatoxin B_1 and fumonisins-induced hepatic pre-cancerous lesions in rats. Food Chem Toxicol. 2010;48:733-742.
7. Abdel-Aziem SH, Hassan AM, El-Denshary AS, Hamzawy MA, Mannaa FA, Abdel-Wahhab MA. Ameliorative effects of thyme and calendula extracts alone or in combination against aflatoxins-induced oxidative stress and genotoxicity in rat liver. Cytotechnol. 2013;10.1007/s10616-013-9598-7.
8. Mokhles M, Abdel-Wahhab MA, Tawfik M, Ezzat W, Gamil K, Mohamed MI. Detection of aflatoxin among hepatocellular carcinoma patients in Egypt. Pak J Biol Sci. 2007;10:1422-1429.
9. Kellerman TS, Mararas WIO, Thiel G, Gelderblom WCA, Cawood M, Coetzer JAW. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. Ondersterpoort J Vet Res. 1990;57:269-275.
10. Ueno Y, Iijima K, Wang SD, Suguira Y, Sekijima M, Tanaka T, Chen C, Yu SZ. Fumonisins as a possible contributing risk factor for primary liver cancer: a 3 year study of corn harvested in Haimen, China by HPLC and ELISA. Food Chem Toxicol. 1997;35:1143-1150.
11. El-Nekeety AA, El-Kholy W, Abbas NF, Ebaid A, Amra HA, Abdel-Wahhab MA. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. Toxicon. 2007;50:256-269.
12. Theumer MG, Lopez AG, Masih DT, Chulze SN, Rubinstein HR. Immunobiological effects of fumonisin B_1 in experimental subchronic mycotoxicoses in rats. Clin Diagn Lab Immunol. 2002;9:149-155.
13. Imam A, Abdel-Rahim F, Hassan NS, Shallan M, Abdel-Wahhab MA. Biochemical studies on the protective role of Aquilegia vulgaris L. during fumonisin toxicity. Egypt J Appl Sci. 2008;23:502-519.
14. Li FQ, Yoshizawa T, Kawamura O, Luo XY, Li YW. Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi. China. J Agric Food Chem. 2001;49:4122-4126.
15. Theumer MG, Lopez AG, Masih DT, Chulze SN, Rubinstein HR. Immunobiological effects of AFB_1 and AFB_1-FB_1 mixture in experimental subchronic mycotoxicoses in rats. Toxicology. 2003;186:159-170.

16. Hassan AM, Hassan NS, Mohamed SR, El-Nekeety AA, Abdel-Wahhab MA. Aquilegia vulgaris L extract countracts oxidative stress and cytotoxicity of fumonisin in rats. Toxicon. 2010;56:8-18.
17. Shon MY, Choi SD, Kahng GG, Nam SH, Sung NJ. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. Food Chem Toxicol. 2004;42:659-666.
18. Lü JM, Yao Q, Chen C. Ginseng compounds: An update on their molecular mechanisms and medical applications. Curr Vasc Pharmacol. 2009;7:293-302.
19. Misra HP, Fridovich K. The generation of superoxide radical during the autooxidation of hemoglobin. J Biol Chem. 1972;247:6960-6962.
20. Volpe EP. Blood and circulation. In: Dubuque WC, ed. Biology and Human Concerns. Brown, Wm. C. Publisher; 1993.
21. Bunn HF. Pathophysiology of the anemias. In: Wilson JD, Braunwald E, Isselbacher KJ, eds. Harrison's Principle of Internal Medicine. New York: McGraw-Hill Inc; 1998.
22. Liu ZQ, Luo XY, Sun YX, Chen YP, Wang ZC. Can ginsenosides protect human erythrocytes against free-radical-induced hemolysis?. Biochim Biophys Act. 2002;1572:58-66.
23. Lukyanenko LM, Kozlova NM, Slobozhanina EI. Activity of membrane-bound NADH-ethemoglobin reductase and physical state of lipids in erythrocyte membranes. Bioelectrochem. 2004;62:191-193.
24. Xie JT, Shao ZH, Vanden-Hoek TL, Chang WT, Li J, Mehendale S, Wang CZ, Hsu CW, Becker LB, Yin JJ, Yuan CS. Antioxidant effects of ginsenoside Re in cardiomyocytes. Eur J Pharmacol. 2006;532:201-207.
25. Ko SR, Choi KJ, Kim SC, Kim MW. Contents of crude saponin and ginsenosides in white ginsengs. Korean J Pharmacol. 1989;20:170-174.
26. Voss KA, Chamberlain WJ, Bacon CW, Norred WPA. Preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin B_1. Nat Toxins. 1993;1:222-228.
27. Silva CMM, Madeira VMC, Almeida LM, Custodio JBA. Hemolysis of human erythrocytes induced by tamoxifen is related to disruption of membrane structure. Biochim Biophys Act. 2000;1464:49-61.
28. Mansouri A, Winterhalter RH. Nonequivalence of chains in hemoglobin oxidation Biochem. 1973;12:4946-4949.
29. Nicak A, Mojzis J. Differences in hemolytic action of mercury ions on human and rat erythrocytes with relationship to the concentration of NaCl and glucose in vitro. Comp. Haematol Int. 1991;2:84-86.
30. Evelyn KA, Malloy HT. Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. J Biological Chem. 1938;126:655-60.
31. Van Kampen EJ, Julstra WG. Determination of hemoglobin and its derivatives. Adv Clin Chem. 1965;8:141-149.
32. Hegesh E, Calmanovici N, Avron M. New method for determining ferrihemoglobin reductase (NADH-methemoglobin reductase) in erythrocytes. J Lab Clin Med. 1968;72:339-344.
33. Steel RG, Torrie GH. Principles and procedures of statistics and biometrical approach. 2nd ed., McGraw-Hill Book Co., New York, Tronto and London; 1960.
34. Shen HM, Shi CY, Shen Y, Ong CN. Detection of elevated reactive oxygen species level in cultured rat hepatocytes treated with aflatoxin B_1. Free Radic Biol Med. 1996;2:139-146.
35. Galvano F, Piva A, Ritieni T, Galvano G. Dietary strategies to counteract the effects of mycotoxins: A review. J Food Protect. 2001;64:120-131.

36. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44-84.
37. Mansour SA, Mossa AH. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pest Biochem Physiol. 2009;93:34-39.
38. Stresser DM, Bailey GS, Williams DE. Indole-3-carbinol and B-naphthoflavone induction of aflatoxin B_1 metabolism and cytochrome P450 associated with bioactivation and detoxication of aflatoxin B_1 in the rat. Drug Metab Dispos. 1994;22:383-391.
39. Abel S, Gelderblom WCA. Oxidative damage and fumonisin B_1-induced toxicity in primary rat hepatocytes and liver in vivo. Toxicology. 1998;131:121-131.
40. Yin JJ, Smith MJ, Eppley RM, Page SW, Sphon JA. Effects of fumonisin B on lipid peroxidation in membranes. Biochimica et Biophysica Acta. 1988;1371:134-142.
41. Kanner J, Harel S. Desferrioxamine as an electron donor. Inhibition of membranal lipid peroxidation initiated by H_2O_2-activated metmyoglobin and other peroxidizing systems. Free Radic Res Commun. 1987;3:309-317.
42. Rogers MS, Patel RP, Reeder BJ, Sarti P, Wilson MT, Alayash AI. Pro-oxidant effects of cross-linked haemoglobins explored using liposomes and cytochrome c oxidase vesicle model membranes. Biochem J. 1995;310:827-833.
43. Jaffe ER, Hulquist DE. Cytochrome b5 reductase deficiency and enzymopenic hereditary methemoglobinemia. In: Stanbury JB, Wyngaarden JB, Fredrickson DS eds. The Metabolic and Molecular Basis of Inherited Diseases, 6th ed. McGraw Hill Information Services Company, New York; 1995.
44. Leukens JN. Methemoglobinemia and other disorders accompanied by cyanosis. In: Lee RG ed. Wintrobe's Clinical Hematology. McGraw Hill, Philadelphia; 1994.
45. Tomoda A, Onekama Y, Tsujit A. Changes in intermediate haemoglobins during autoxidation of haemoglobin. Biochem J. 1981;195:485-492.
46. Bodansky O. Methemoglobinemia and methemoglobin producing compounds. Pharmacol Rev. 1951;3(2):144-96.
47. Wennmalm A, Benthin G, Edlund A, Jungerster L, Kieler-Jensen N, Lundin S, Westfelt UN, Peterson AS, Waagstein F. Metabolism and excretion of nitric oxide in humans. Circ Res. 1993;73:1121-1127.
48. Abdel-Wahhab MA, Ahmed HH, Hagazi MM. Prevention of aflatoxin $_{B1}$-initiated hepatotoxicity in rat by marine algae extracts. J Appl Toxicol. 2006;26:229-238.
49. Dombrink-Kurtzman MA, Gomez-Flores R, Weber RJ. Activation of rat splenic macrophage and lymphocyte functions by fumonisin B_1. Immunopharmacol. 2000;49:401-409.
50. Hibbs JB, Taintor RR, Vavrin Z. Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science. 1987;235:473-476.
51. Olken NM, Marletta MA. NG-Methyl-L-arginine functions as an alternate substrate and mechanism-based inhibitor of nitric oxide synthase. Biochem. 1993;32:9677-9685.
52. Sun J, Steenbergen C, Murphy E. S-nitrosylation: NO-related redox signaling to protect against oxidative stress. Antioxid Redox Signal. 2006;8:1693-1705.
53. Stepuro TL, Zinchuk VV. Nitric oxide effect on hemoglobin-oxygen affinity. J Physiol Pharmacol. 2006;57:29-38.
54. Feelisch M, Stamler JS. The oxyhemoglobin assay. In: Feelisch M, Stamler JS, Feelisch M, Stamler JS eds. Methods in Nitric Oxide Research. John Wiley & Sons, New York; 1996.
55. Jia L, Bonaventura C, Bonaventura J, Stamler JS. S-Nitrosohaemoglobin: A dynamic activity of blood involved in vascular control. Nature. 1996 or 2006;380:221-226.

56. Tabrizi-Fard MA, Lee WN, Fung HL. Differential interactions of nitric oxide donors with rat oxyhemoglobin. Biochem Pharmacol. 1999;58:671-674.
57. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA. 2005;293:1653-1662.
58. Jeney V, Balla J, Yachie A, Varga Z, Vercellotti GM, Eaton JW, Balla G. Pro-oxidant and cytotoxic effects of circulating heme. Blood. 2002;100:879-887.
59. Rae HA. Onion toxicosis in a herd of beef cows. Can Vet J. 1999;40:55-57.
60. Harvey RB, Kubena LF, Elissalde MH, Corrier DE, Huff WE, Rottinghaus GE, Clement BA. Co-contamination of swine diets by aflatoxin and diacetoxyscirpenol. J Vet Diagn Invest. 1991;3:155-160.
61. Çimen BMY. Free radical metabolism in human erythrocytes. Clinica Chimica Acta. 2008;390:1-11.
62. Telen MJ, Kaufman RE. The mature erythrocyte. In: Greer, J.P., Foerster, J, eds. Wintrobe's Clinical Hematology. Philadelphia: Lippincott Williams & Wilkins; 1999.
63. Scott MD, Eaton JW, Kuypers FA, Chiu DT, Lubin BH. Enhancement of erythrocyte superoxide dismutase activity: effects on cellular oxidant defense. Blood. 1989;74:2542-2549.
64. Dumaswala UJ, Zhuo L, Jacobsen DW, Jain SK, Sukalski KA. Protein and lipid oxidation of banked human erythrocytes: role of glutathione. Free Radic Biol Med. 1999;27:1041-1049.
65. Gunduz K, Ozturk G, Sozmen EY. Erythrocyte superoxide dismutase, catalase activities and plasma nitrite and nitrate levels in patients with Behçet disease and recurrent aphthous stomatitis. Clin Exp Dermatol. 2004;29:176-179.
66. Bukowska B, Kowalska S. Phenol and catechol induce prehemolytic and hemolytic changes in human erythrocytes. Toxicol Lett. 2004;152:73-84.
67. Carroll J, Raththagala M, Subasinghe W, Baguzis S, D'amico O, Blak T, Root P, Spence D. An altered oxidant defense system in red blood cells affects their ability to release nitric oxide stimulating ATP. Mol Biosyst. 2006;2:305-311.
68. Baynes JW. Oxygen and life. In: Baynes JW, Domoniczak MH, eds. Medical Biochemistry. Philadelphia: Elsevier; 2005.
69. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther. 1973;187:211-217.
70. Abdel-Wahhab MA, Aly SE. Antioxidant property of Nagilia sativa (Black cumin) and Syzygium aromaticum (Clove) in rats during aflatoxicosis. J Appl Toxicol. 2005;25:218-223.
71. Abdel-Wahhab MA, Ibrahim AA, El-Nekeety AA, Hassan NS, Mohamed AA. Panax ginseng C.A. Meyer extract counteracts the oxidative stress in rats fed multi-mycotoxins-contaminated diet. Com Sci. 2012;3(3):143-153.
72. Hassan AM, Abdel-Aziem SH, Abdel-Wahhab MA. Modulation of DNA damage and alteration of gene expression during aflatoxicosis via dietary supplementation of Spirulina (Arthrospira) and whey protein concentrate. Ecotoxicol Environ Safety. 2012;79:294-300.
73. Sreenivas R, Prakasham RS, Krishna Prasad K, Rajesham S, Sarma PN, Venkateswar L. Xylitol production by Candida sp.: parameter optimization using Taguchi approach. Proc Biochem. 2004;39:951-956.
74. Zhong G, Jiang Y. Calcium channel blockage and anti-free-radical actions of ginsenosides. Chin Med J. 1997;110:28-29.
75. Gillis CN. Panax ginseng pharmacology: a nitric oxide link? Biochem Pharmacol. 1997;54:1-8.

76. Wang X, Chai H. Yao Q, Chen C. Molecular mechanisms of HIV protease inhibitor-induced endothelial dysfunction. J Acquir Immu Defic Syndr. 2007;44:493-499.
77. Li GX, Liu ZQ. The protective effects of ginsenosides on human erythrocytes against hemin induced hemolysis. Food Chem Toxicol. 2008;46:886-892.
78. Zain ME. Impact of mycotoxins on humans and animals. J Saudi Chem Soc. 2011;15:129-144.
79. Franklin MR, Yost GS. Biotransformation: a balance between bioactivation and detoxification, In: Williams PL, James RC, Roberts SM eds. Principles of Toxicology: Environmental and Industrial Applications. John Wiley & Sons, Inc; 2000.
80. Essigman JM, Croy RG, Bennet RA, Wogan GN. Metabolic activation of AFB_1; pattern of DNA adduct formation, removal and excretion in relation to carcinogenesis. Drug Metab Rev. 1982;13:581-602.

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