The Protective Effect of White Ginseng against Biochemical and Pathological Changes Induced by Aflatoxins in Rats

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Abstract: The objective of the present study was to explore modification in toxico-pathological responses of rats toward aflatoxins (AFs) in the presence of white ginseng. The dietary supplementation with white ginseng (WG) at levels of of 0.0, 1 and 2 % (W/W) of the composition of daily rations, on the performance and toxicity of female Albino rats received aflatoxins-contaminated diets (1.011 mg/kg ration, of dry matter basis), were successively examined for six weeks, as attempt to prevent or minimize the negative probabilities due to ingesting aflatoxin(s) contaminated food. Thirty native apparently healthy female Albino rats with average weight of 100± 3.4 gm., were put under observation for two weeks, then they were divided into five equal groups of six rats each according to their live body weight for performing feeding trials. An exposure study extended for two different stages was conducted using female Albino rats. The 1st stage (pre-treatment) was suggested to compare the performance of animal groupings under the normal conditions before receiving any treatment, either level of contamination(s) or dosage(s) of additive, such stage extended for 2 weeks. The 2nd stage (treatment), the animals received different levels of aflatoxin(s) and the food additive (white ginseng), such stage extended for 4 weeks. Rats treated with AFs-contaminated diet alone showed depression, decrease in feed intake, body weight and loose feces. The activities of serum ALT, AST enzymes, which are reflecting liver function, were obviously affected during exposure to aflatoxins, but such levels came back to normal as the level of the WG in the ration increased. Serum urea and creatinine concentrations had also severed and such severe effects came back to moderate when receiving the proposed additive. Livers exhibited fatty change, necrosis and newly formed bile ducts. Lesions in kidney included tubular necrosis and pink homogeneous tubular casts. Rats fed white ginseng only had no significant differences compared to the negative control group (fed on a sole diet without any additives). A concurrent treatment of AFs with white ginseng indicated a potentiation of the animal performance reflected by decreased severity of clinical signs and inccreased body weight gains. The studied food additive minimized and reduced significantly the deterioratsion of such performance which obviously observed in animal grouping received AFs-contaminated diet. Female rats were responding to contaminated diets and to the food additive as well. Thus, our data strongly suggested that deleterious effects of AFs could be overcome or, at least, significantly were diminished by WG. Moreover, this plant by itself did not show any toxic effects.

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1. Introduction:

Both human and animal health has been dramatically affected in outbreaks of acute mycotoxicosis, but these tragic events may be only a part of the cost to society in terms of impaired health and productivity from the ingestion of sub-clinical levels of aflatoxins (WHO, 1981; Saad, 1993 and Moos, 2002). Aflatoxins B1, B2, G1 and G2 were occurred on different foodstuffs when exposed to certain strains of Aspergillus flavus and Aspergillus parasiticus (Wogan, 1977 and Casado et al., 2001). AFB_1 is the most abundant and toxic form of all naturally occurring aflatoxins. AFB_1 represents 75% of all aflatoxins found in contaminated food and feeds. Many reports stated the harmful effects of aflatoxins on wide variety of animals and human; depression of growth and production (Saad, 1993), Immunosuppression (yeong–Hsiang, 2001), liver disorders (Guerre, et al., 1996 and Anong and Suparats, 2006), abnormalities of enzyme picture (Patrick et al., 2002 and Tulayakul et al., 2005). However, such harmful effects seem to be difficult to overcome since aflatoxicosis gave no reaction response for any available treatment of drugs and / or antibiotics (Abdelhamid, 1990 and saad, 1993). The metabolisms of aflatoxins are due mainly to sex and specie differences besides the mitochondrial enzymatic reaction. AFB1 is first metabolized mainly by the cytochrome P-450 enzyme (CYP450) system found in the microsome. This metabolism will produce a variety of metabolites such as AFB_1-epoxide and hydroxylated metabolites (AFM1, AFP1, AFQ1, AFB 2α and aflatoxicol). (Tylayakul et al., 2005 and Anong 2006). The rate of metabolism, the repeativeness of exposure, the dosage and type of

mycotoxin (s) were reported as limiting factors affect the type of toxic action of mycotoxicosis (Saad, 1994; Jia-Sheng and John, 1999; Hussein and Jeffrey, 2001).

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).

Ginseng, a traditional medicinal herb in Asia, has become internationally popular in recent years. The traditional source of ginseng root has been Asian ginseng (Panax ginseng C.A. Meyer) but American ginseng (Panax quinquefolium L.), a plant native to North America, is now also cultivated and used in many countries. While the full pharmaceutical activity of ginseng is due to a rage of compounds, the triterpene saponins, known as ginsenosides, are widely considered to be the most important components contributing to the multiple medicinal properties of both Asian and American ginseng. The importance of the saponins in Asian ginseng has been known for about 100 years and, with the advent of modern chromatographic techniques, about 30 triterpene saponin glycosides, designated as neutral ginsenosides, have been identified (Sticher, 1998). The presence of neutral ginsenosides in American ginseng was first demonstrated by Ando et al., (1971) with later studies confirming that a range of neutral ginsenosides exists in this species similar to the previously studied Asian ginseng (Panax ginseng). The two major groups of ginsenosides are the Rb and Rg groups, which have 20 (S) protopanaxadiol and 20 (S) protopanaxatriol, respectively, as the sapogenines. Rb group includes the ginsenosides Rb1, Rb2, Rc and Rd, while Rg group includes the Re, Rf and Rg1 ones as the main compounds. Among all these ginsenosides, Rb1 and Rg1 are the most effective compounds (Tanaka and Kasai, 1984).

In previous works, many authors observed that Korean Panax ginseng has a protective role against many toxicants (Mannaa et al., 2006; Khalil et al., 2008; El-Kady et al., 2006). However, the components of ginseng that might bring about the decreased cancer risk remain unknown.

The aim of the present work is to investigate the effects of AFs on lethality, on some haematological and biochemical parameter changes and on histological damages in the absence or presence of ginseng in order to appreciate the potential protective effects of this medicinal plant against AFs- toxicities. The effects of this herb alone were also evaluated.

2. Materials and methods

2.1- Experimental design.

Depending on our previous results (Abdel-Fattah, 2002; Abdel-Fattah and Abdel-Salam, 2004 and Abu-Seif, *et al.*, 2009), concerning the antimicrobial effects of herbs and medicinal plants, this study was achieved. Thirty female Albino rats were used in this study. Animals were divided into five groups as shown in Table (1). All groups were exposed to the main two stages of the experiment period as follows; the first 2 weeks were the pre-treatment period, followed by 4 weeks of treatment. Both the 1st and 2nd groups were fed on sound rations, free from aflatoxins, but the 1st one had no additive to act as "negative control", while the 2nd group fed on the same sound ration plus the studied additive at concentration equivalent to 2 % (w/w). The other three groups ingested aflatoxin(s) contaminated diets containing 225 mg B1, 30 mg B2, 70 mg G1 and 10 mg G2 /kg . The 3rd group exposed to contaminated diets only without any addition, but the 4th and 5th groups fed on aflatoxin(s) contaminated diets, plus the proposed additive (WG) at 1 and 2 % w/w. Feed intake and body weight gain were recorded daily. At the end of the treatment period all animals were fasted for 12 h, then blood samples were collected from the retroorbital venous plexus under diethyl ether anesthesia. Sera were separated using cooling centrifugation and stored at - 20 oC until analysis. After the collection of blood samples, animals were killed and samples of the liver and kidney of each animal were dissected.

Quantitative measurement of serum biochemical parameters included ALT, AST, urea and creatinine, were determined using commercial kits.

2.2. Experimental animals

One-month old Female white Albino rats weighting 100–110 g (purchased from animal house of National Research Centre, Cairo, Egypt) were maintained on standard lab diet (protein: 160.4; fat: 36.3; fibre: 41 g/kg and metabolizable energy 12.08 MJ), and 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 2 weeks, the animals were divided into five groups (6 rats/group) and housed in filter-top polycarbonate cages. All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre, Dokki, Cairo, Egypt.

2.3-Organisms. *Aspergillus parasiticus* (*A.parasiticus*) NRRL 2999 was obtained as lyophilized preparation from the Mycotoxin lab. , National Research Center, Dokki, Giza, Egypt.

2.4- Plant material:

American white ginseng was purchased from an Egyptian local market (Harraz Co., Cairo, Egypt).

2.5- Aflatoxins standards and chemicals:

All standards of Aflatoxins (B1, B1, G1, G2, and B2 α) were purchased from sigma company, USA. All Chemicals and solvents used were of ACS grade. Thin layer TLC aluminum plates recoated with 0.25 mm silica gel 60 (Merk).

2.6- Preparation of aflatoxin(s) - artificially contaminated ingredient.

A balanced ration of growing rats with 14 % crude protein and 3100 kg calorie were purchased and artificially infected (in vitro) with a certain strain of *Aspergillus parasiticus* (NRRL-2999) which identified as an aflatoxin(s) producing strain. The inoculated substrates were incubated at 28 °c for 18 days (Shotwell et al., 1966). Qualitative and quantitative assay for the presence of aflatoxins in the contaminated substrate has been caried out using HPLC (Agilent Technologies, Waldbronn, Germany) as recommended by AOAC (1990). The AFs within the contaminated material consisted of 225 mg B1, 30 mg B2, 70 mg G1 and 10 mg G2 /kg. The contaminated material was incorporated into the basal diet in the ratio 15.55. % of the daily ration, to provide the desired level of 1.011 mg of total AFs or 0.7 mg AFB1/Kg diet.

2.7- Sampling

2.7.1- Blood samples:

Blood samples were taken weekly from the jugular vein prior to the morning feeding, at the following times: 0, 6, 13, 20 and 27 days of treatment period. Blood samples were placed on ice, allowed to clot and after centrifugation; serum was separated and frozen at -20 °C until it was analyzed.

2.7.2- Sampling of feces and urine

During the 3rd wk of the experiment, total feces and urine of animals were collected twice daily over a 7-days period. Urine was collected from an indwelling cyatic catheter, which was also placed 1-day before the toxin administration. Feces were collected in fecal bags. Following the collection period, total samples of urine and feces from each animal were homogenized, and aliquot samples were stored at -20°C until further analysis. Sampling and methods of analyses were adopted as recommended by (AOAC, 1990).

2.8- Histological study:

At the end the treatment stage, all animals were slaughtered, liver and kidney were dissected out, organs were fixed in 10% neutral buffer formalin and processed for histopathological examination using routine paraffin embedding technique. Sections of 5 mm thickness stained with hematoxylin and eosin (H&E) were examined for morphological alterations and morphometric measurements. The slides were examined under 400 imagnification using an optical microscope (Carl Zeiss, Germany) as reported by Pearse (1979) and Sheehan and Harapcbak (1980).

2.9- Analytical methods:

2.9.1- Aflatoxins analysis:

Analysis of aflatoxins.

Aflatoxin(s) in feed and feces samples were extracted by B.F. method as described in AOAC (2000). The AFB1, its metabolites B2α and aflatoxicol were analyzed in urine, and serum samples according to method of Richarda and Lyona (1986).

Visualization and quantitation of aflatoxin B1, aflatoxicol and aflatoxin B2α.

AFB_1, aflatoxicol and AFB2α, were located by UV exposure. Extracts were dissolved in soul chloform and vortex, 20µl aliquot and 10µ of the standards were stopped on TLC plates and developed in dark room with chloroform: acetone (90:10). After drying the spots were examined with U.V at a wave length of 365 nm. For the quantitative determination of the compounds, the silica plates were developed in solvent system (chloroform: actone, 90:10) and scanned in a Vitatron LTD 100 densitometer equipped with a mercury lamp (excitation at 366 nm and emission at 460 mm). The recorded areas of the spots were compared with standards of the respective compounds.

2.9.2- Serum biochemical parameters determination:

Blood was collected from the jugular vein of rats anesthetized with ether vapors on the days, 0, 14 and 28 of treatment period. Serum separated from clotted blood was stored at -20 ^{o}C for estimation of different biochemical parameters which included, total aflatoxins (AOAC method, 1990), ALT (Randox UK, catalogue no. AL 484), AST (Randox UK, Catalogue no. TR 1697) creatinine (Randox UK, catalogue no. CR 523), and urea (Randox UK, catalogue no. CH 280).

2.10- Statistical analysis

The data was subjected to the analysis of variance test. Different group means were compared by Duncan's multiple range test using a computer statistical package ($p \leq 0.05$) (Duncan, 1955).

3. Results and Discussion:

(1) Effects of ingesting aflatoxins and the WG additive on the performance of treated animals:

The selected doses of aflatoxin were literature based (Gelderblom et al., 2002) however; the selected

dose of WG was based on previous work (Abdel-Wahhab and Ahmed, 2004, Saad and Abdel-Fattah, 2008). The levels of aflatoxin B_1 selected in the present study were derived from the reported LD50 of both substances. Aflatoxin B_1 LD_{50} through oral route for rats has been reported as 5 mg/kg b. wt. (Betina, 1984). To produce a clinical aflatoxicosis, 1/10 LD_{50} (1 mg/kg b. wt.) was selected for daily doses.

In the current study, we evaluated the protective effects of WG against AFB_1 which promoting hepatic tumours in rat. The effect of different treatments on body weight gain of rats is depicted in Table (1). During the pre-treatment stage the average of body weight and body weight gain of both five groups show almostly similar values and ranges.

Rats fed with AFs at level 1.011 mg/kg diet in the present study exhibited a significant decrease in feed intake and body weights, the negative and positive control groups showed almostly the same values obtained from groups 4 and 5 which fed on high level of contamination simultaneously with the additive (WG) at concentrations of 1 and 2%.. Reduced weight gains have been observed in rats following an exposure of AFs-contaminated diets (Saad and Abdel-Fattah, 2008).

Our results indicated that ingestion aflatoxin-contaminated diets resulted in a significant decrease in food intake and consequently the body weight gain was also reduced. Similar decrease in food consumption and body weight was reported in rats fed AFs-contaminated diet (Mayura et al., 1998, Abdel-Wahhab and Aly, 2003; El-Nekeety et al., 2007, Saad and Abdel-Fattah, 2008). The reduced feed intake may indicate protein catabolism, thereby contributing to the observed kidney injury and causing impaired glomerular filtration (Tessari et al., 2006). On the other hand, the decrease in body weight in the animals treated with the aflatoxins alone may be due to the effects of these aflatoxins on the balance between orexigenic and anorexigenic circuits that regulate the homeostatic loop of body weight regulation, leading to cachexia (Rastog et al., 2001). In this regards, Abdel-Wahhab et al. (2006) reported that rats treated with AFB_1 showed a significant decrease in leptin. Low leptin concentration is usually associated with the high levels of cortisol and IL-6 which together act to influence the feeding response, causing weight loss in patients with pancreatic cancer (Barber et al., 2004). This correlation may explain the recorded decrease in body weight in animals ingested AFB1 and FB. Since leptin and its receptor are the key players in the regulation of energy balance and body weight control (Yuan et al., 2004; Abdel-Wahhab et al., 2006). Moreover, food intake was improved when the combined treatments of toxin plus WG was applied. Whereas, significant differences were still found between the control or WG alone groups and the other treatment groups.

Many authors stated that ingesting aflatoxin(s) contaminated food leads to negative effects on both animal and human performance (WHO, 1981, Park, 1983 and Saad, 1993; Abdel-Fattah, et al., 2006; Saad and Abdel-Fattah, 2008). It's worthy to mention that the group 2 which ingested the studied additive WG and sound diets showed non significant ($P<0.5$) effects compared with the negative control and the groups 4 and 5 which ingested the high level of contamination and the food additive at 1 and 2%. These findings are in agreement with those reported by Casado *et al.*, (2001) on rats and Biing-Hui *et al.*, (2002) working on swine.

Table 1. Means ± SE and comparison of body weight changes in all groups during experiment period (6 weeks).

Groups	**Means ± SE**					
	At zero time	After one week	After two weeks	After three weeks	After four weeks	After six weeks
Group 1	89.0 ± 1.52^{Aa}	117.3 ± 3.23^{Ab}	154.8 ± 2.53^{Bc}	166.4 ± 2.53^{Bc}	177.6 ± 2.15^{Bc}	191.3 ± 5.01^{Cd}
Group 2	84.0 ± 1.81^{Aa}	106.2 ± 3.68^{Ab}	136.4 ± 3.05^{Ac}	145.64 ± 2.87^{Ad}	159.1 ± 3.23^{Ae}	165.6 ± 2.67^{Be}
Group 3	84.0 ± 1.80^{Aa}	99.5 ± 3.30^{Ab}	132.6 ± 3.4^{Ac}	145.5 ± 4.28^{Ad}	154.2 ± 3.30^{Ad}	151.6 ± 3.58^{Ad}
Group 4	88.0 ± 2.06^{Aa}	105.6 ± 3.45^{Ab}	134.4 ± 2.84^{Ac}	149.0 ± 3.54^{Ad}	150.5 ± 4.47^{Ad}	143.3 ± 3.01^{Ad}
Group **5**	87.0 ± 65^{Aa}	105.4 ± 4.28^{Ab}	134.06 ± 2.06^{Ac}	140.11 ± 3.62^{Ac}	147.4 ± 4.14^{Ad}	148.4 ± 3.66^{Ad}
LSDp≤0.05	12.57					

N.B.: 1- The same capital litters in columns denotes no significant difference between treatments in the same period at ($p \leq 0.05$) and vise versa. But the differences in small litters in rows denote significant difference between periods in the same treatment at ($p \leq 0.05$) and vise versa. Day (0): Beginning of the experiment Day (42): End of the experiment

2- Some biochemical analysis of rat serum (liver and kidney function tests) as affected by dietary aflatoxin and / or WG treatment.

Data for selected serum constituents are presented, from the beginning and the end of the treatment period, in Table (2). There is no a consistent pattern shown in the metabolic indicators as influenced by the AFs and / or the WG food additive additive. The average(s) of the transaminases (ALT and AST) level and both urea

and creatinine concentrations showed the normal picture during the pre-treatment stage with no differences between groups (Table 2). During the treatment stage, the groups 1 and 2 showed constant level of both ALT and AST enzymes activities, while serum activities of ALT and AST of animals fed dietary AF only (group 3) had elevated ($p \leq 0.05$) compared with those fed sound rations with or without WG additive. Similarly, urea and creatinine concentrations were higher ($p \leq 0.05$) at the end of the treatment period for group which fed AFs-contaminated diet only. On the other hand, the level(s) of both ALT and AST activities were not significantly affected during treatment in the 4th and the 5th groups, leading to suggest the positive effect of studied additive on the liver function when the animals exposed to high level of aflatoxin(s) contamination.

Tracing the level(s) of transaminase ALT and AST in both animals of the 2nd group which received the additive only without any level of contamination, it could be easily noted that no changes were obtained during the two successive stages of the study. The obtained data were in accordance with those reported by Yeong-Hsiang *et al*. (2001), working on ducklings, Biing-Hui *et al*. (2002) on swine and Anong and Suparats (2006) on broilers.

High serum levels of AST and ALT are usually indicative of liver damage in animals (Lind et al. 1989) and humans (Gil et al. 1988; Hassal et al. 1990; Rati et al., 1991). It is of interest to mention that in animals fed diets contaminated with toxicants, the serum levels of these enzymes increased after liver damage because of increased membrane permeability or because of liver cell necrosis and cytosol leakage into the serum (Abdel-Wahhab, *et al.,* 2002 ; Saad and Abdel-Fattah, 2008 and Ozer *et al.*, 2008).

The intermediary metabolites produced during biotransformation of aflatoxins are held responsible for hepatotoxicity and the increase of serum activities of liver enzymes. They may cause cellular damage by covalent binding to cellular components such as enzymes, nucleic acids and proteins or by another mechanism. Damage of cellular components may play an important role in death of liver cells (Lind et al. 1989; and Teppema et al. 2002), hence, ALT and AST may be released to serum levels of these enzymes would increase.

Serum enzyme activities of AST and ALT are generally elevated in aflatoxicosis and are indicative for changes in the hepatic tissues and biliary system (Abdel-Wahhab et al., 2002), whereas increased levels of urea and creatinine may indicate protein catabolism and/or renal dysfunction (Abdel-Wahhab and Aly, 2003, 2005). Moreover, El-Nekeety et al. (2007) and Abdel-Wahhab et al. (2002) reported that rats fed FB-contaminated diet showed a significant increase in serum transaminases which indicated a necrosis in the liver tissue. These results clearly indicated that AFB_1 have stressful effects on the hepatic and renal tissues, consistent with those reported in the literature of mycotoxicosis (Sherif et al., 2009).

The results of our study (Table 2) are in agreement with the reports of other studies performed using antioxidants and hepatotoxic substances (Saad and Abdel-Fattah, 2008). Alterations in different serum biochemical parameters observed in the present study are in agreement with the previous reports (Durak et al. 1996; Huang et al. 1996; Naziroglu 1999; Manna et al., 2004; Abdel-Wahhab and Aly, 2003, 2005). Adding of WG during aflatoxins treatment succeeded to improve ALT, AST, activities and a significant improvement was also found in urea and creatinine concentrations.

Several studies on the mechanisms of aflatoxins induced liver injury have demonstrated that glutathione plays an important role in the detoxification of the reactive and toxic metabolites of these aflatoxins, and the liver necrosis begins when the glutathione stores are almost exhausted (Dilkin et al., 2003; Abdel-Wahhab and Aly, 2003, 2005). In the same regards, Kim et al., (1997) reported that ginseng has a potent protective action against CCL4-induced toxicity and it showed inhibitory effect on cytochrome P450-associated monoxygenase activities. Therefore, it is suggested that the protective effect of WG is attributed to its free radical scavenging activity (Abdel-Wahhab and Ahmed, 2004; Mannaa et al., 2006). Generally, these results indicated that WG have protective effects against liver injury induced by aflatoxins and it plays a role in increasing the antioxidant status as well as lowering the oxidative damage of nucleic acids in the body (Abdel-Wahhab and Ahmed, 2004; Mannaa et al., 2006). Furthermore, Yun et al. (1987) reported that prolonged administration of Korean red ginseng (KRG) extract resulted in substantial suppression of pulmonary tumorigenesis induced by such chemical carcinogens as aflatoxin B_1. It was reported that the non-saponin components of red ginseng (RG) suppressed the harmful effects of free oxygen radicals (O2, H2O2, and OH2), which exercise an important role in tissue degeneration (Kim et al., 1997). Moreover, Zhang et al., (1996) showed that hydroxyl radical formed by the Fenton reaction were completely inhibited by ginseng extract. This antioxidant effect of ginseng may be responsible for its wide pharmacological actions in clinical practice by a free radical reaction-inhibition mechanism.

Therefore, the protective effects of WG may be related to the antioxidant properties consequently decreased risk for most cancers including carcinomas of the esophagus, stomach, colon, pancreas, lung and liver (Matsuda et al., 1986; Jeong et al., 1997; Dayan and Paine, 2001). Recently, Li et al., (2008) postulated that Ginsenoside Rg1, cinnamic acid, and tanshinone IIA isolated from ginseng could serve as protective agents in cancer prevention and treatment.

Treatment of the intoxicated rats with WG resulted in significant improvement in kidney function as indicated by the marked decrease in serum urea and creatinine levels. These results were in conformity with those reported by Yokozawa et al., (1994) who demonstrated that WG and its active component, saponin, could significantly reduce the blood urea nitrogen and creatinine levels in the blood of nephrectomized rats. Other studies asserted the nephroprotective effect of Korean ginseng saponin against cisplatin-nephrotoxicity (Liu and Zhou 2000; Abdel-Wahhab and Ahmed, 2004). These authors suggested that Korean ginseng saponin reduced cisplatin-induced cytosolic free [Ca2+] ions overload and formation of DNA interstrand cross-link and DNA–protein cross-link.

Furthermore, Yokozawa and Liu (2000) demonstrated that ginsenoside could decrease the severity of renal injury induced by cisplatin. These authors suggested that decreased level of urea in serum in rats given WG reflected the protective action of ginsenoside against the renal dysfunction.

Our results may lead us to suggest that there is a significant liver and kidney dysfunction in the AFs-treated groups, 1.011mg of AFs/kg diet was sufficient to impair performance and cause liver and kidney damage in female Albino rats, adding of WG during the mycotxins treatment (groups 4 and 5) resulted in a significant improvement in ALT and AST activity as well as urea and creatinine concentrations. It was suggested that WG displays a pronounced hepatoprotective effect, assessed through the transaminases (ALT, AST) activities following hepatotoxicity in rats treated with AFs-contaminated diets.

Table 2. Means ± SE and comparison of enzymes levels and biochemical parameters in all groups during the AFs and WG treatments for 4 weeks.

Items / Groups	Means ± SE							
	*AST (IU·L^{-1})		ALT (IU·L^{-1})		Urea (mg·dl−1)		Creatinine (mg·dl−1)	
	Day (0)	Day (28)	Day (0)	Day (28)	Day (0)	Day (28)	Day (0)	Day (28)
	20							
Group 1	20.7±0.73^{Aa}	22.8±1.12^{Aa}	13.3±0.58^{Aa}	14.3±0.80^{Aa}	42.06±2.43^{A}	48.8±2.33^{Aa}	37.7±2.45^{Aa}	39.8±3.37^{Aa}
Group 2	21.01±1.08^{Aa}	27.07±0.69^{Aa}	12.9±0.48^{Aa}	17.02±1.05^{Aa}	44.07±2.83^{A}	45.6±2.85^{Aa}	38.7±2.94^{Aa}	43.6±3.01^{Aa}
Group 3	20.0±0.68^{Aa}	85.4±2.98^{Cb}	13.3±0.42^{Aa}	49.01±1.90^{Cb}	44.2±1.649^{A}	96.4±5.43^{Cb}	40.2±3.16^{Aa}	109.9±5.19^{Cb}
Group 4	20.03±1.07^{Aa}	76.27±2.85^{Cb}	13.3±0.29^{Aa}	32.4±1.136^{Bb}	43.3±2.28^{A}	77.00±3.16^{Bb}	37.12±2.22^{Aa}	91.1±4.21^{Bb}
Group **5**	19.6±0.76^{Aa}	40.8±1.46^{Bb}	13.1±0.36^{Aa}	35.5±1.21^{Bb}	46.96±2.08^{A}	66.5±3.03^{Bb}	40.9±1.49^{Aa}	80.30±3.31^{Bb}
LSD p≤0.05	10.03		7.5		9.67		11.53	

N.B.: 1- The same capital litters in columns denotes no significant difference between treatments in the same period at ($p \leq 0.05$) and vise versa. But the differences in small litters in rows denote significant difference between periods in the same treatment and the same parameter, at ($p \leq 0.05$) and vise versa.

2- [a] AST = aspartate aminotransferase; and ALT = alanine aminotrasferase..

Day (0): Beginning of the experiment

Day (28): End of the experiment

(3) The histopathological changes in different studied groups:

The biochemical results reported in the current study were confirmed by the histopathological study of the liver and kidney.

Liver: The results obtained from studies with slices prepared from the livers of AFs-treated rats indicate that there is some disturbance in protein synthesis throughout the course of the poisoning. One may speculate whether such an inhibition could play a role in the cellular necrosis.

Our histopathological study clearly illustrated the presence of diffuse coagulative necrosis and vacuolar degeneration in the hepatic tissue that induced by AFB1-intoxicated rats (group 3, Fig. 1). Such histopathological changes could be localized and reduced by co-administration of white ginseng parallel with AFB1 treatments in groups 4 and 5 in, which animals treated with WG at levels 1 and 2%, respectively. Theses two groups showed microscopic lesions slight to those of group 3 included local areas of either coagulative necrosis or vacuolar degeneration; especially with group 5 which showed slight degenerative changes.

The present results revealed that WG had no harmful effects on liver tissues. However, the liver of the animals in the aflatoxins-treated groups showed severe histopathological changes may be typical to those reported in the literature. In this concern, Gelderblom et al. (2002), Abdel-Wahhab et al. (2002) and El-Nekeety et al. (2007) stated that treatment with FB_1 resulted in hepatotoxicity, apoptosis and inhibitory effect on cell proliferation, interferes with normal growth related processes and hence the disruption of normal liver homeostasis. Moreover, AFB_1 treatment induced a severe cytotoxicity and inhibition of hepatocytes cell proliferation (Neal and Cabral, 1980; Mayura et al., 1998; Abdel- Wahhab et al., 1998, 2002, 2007).

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Kidneys: Kidneys of the groups given WG and AFs together also showed changes not similar to those observed in groups given AFs alone (Fig. 2). In group 3, there were severe and diffuse coagulative necrosis in the renal tubules and clowdy swelling of renal tubular cells with occlusions of their lumens with local oedematous changes in the kidneys of AFs-intoxicated rats for 4 weeks (group 3, Fig. 2) and such changes could be reduced (localized) with the co administration of white ginseng parallel with AFs-intoxications (groups, 4 and 5) as local degenerative and hemorrhagic changes with hyper cellularity of renal glomeruli (group 4) with addition to local necrotic areas (group 5) as shown in Fig.(2). A similar parallel study in rabbits which concluded that aflatoxins can induce adverse effects on fertility parameters, liver and kidney functions, while ginseng could reduced such side effects of aflatoxicosis (Emam and Hussein, 2008). Histopathological lesions induced by AFs in liver and kidneys of rats in the present study were may be similar to those reported by other workers (Hussain et al., 2009; Saad and Abdel-Fattah, 2008; Abdel-Wahab et al., 2007).

Our results suggested that a potentiation of the toxic response was evident by increased severity of clinical signs, increased activities of some liver enzymes, decreased body weights of rats administered AFs. Alterations in serum biochemical and pathological parameters employed in the present study could detect some modification in toxic responses of rats administrated the two agents (AFs plus WG) concurrently suggesting that these parameters were compromised by concurrent exposure to AFs and WG. Since both the levels of AFs in the present study produced toxic changes as evident by all the parameters used, it is possible that the modulation of the toxic response by AFs might have been masked by the alterations induced by WG dose levels.

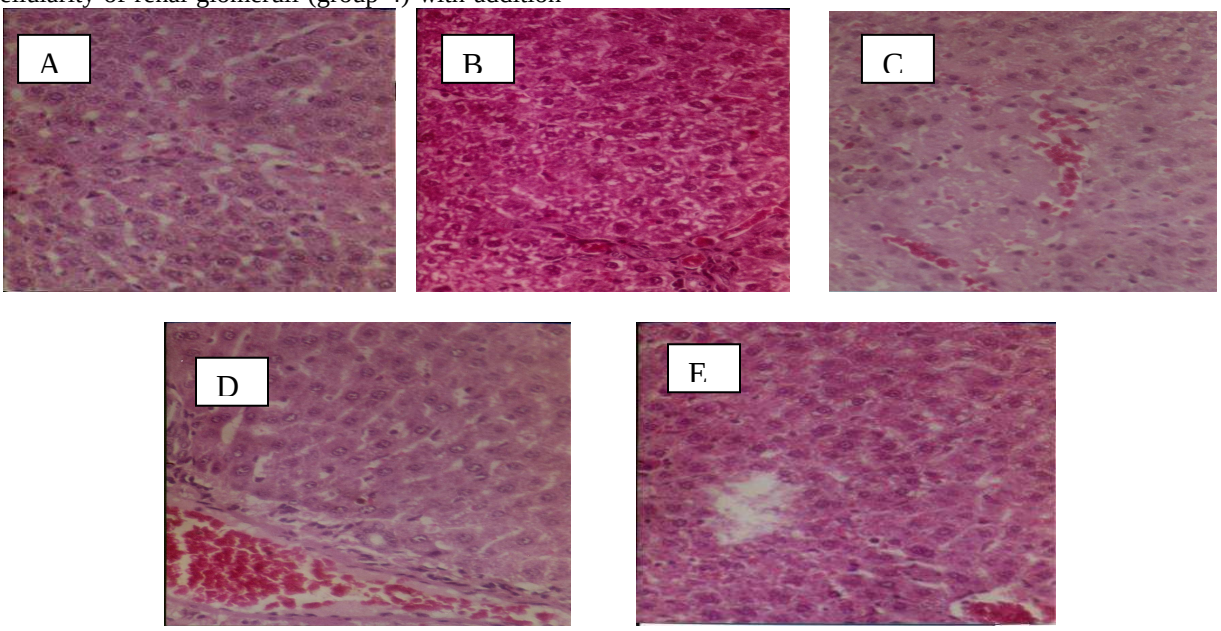

Fig. 1. Liver sections of (A) rat treated with WG alone (group 2) showing vacuolation and degenerative changes of some hepatocytes, (B) rat treated with AFs alone (group 3) showing vacuolation of hepatocytes, (C) rat (group 3) showing diffuse coagulative necrosis of the liver tissue and some haemorrhagic areas, (D) rat treated with WG at level of 1 % of daily ration (W/W) during AFs treatment (group 4) showing slight degenerative changes of some, centrilobular hepatocytic necrosis and congestion of the central vein; and (E) rat treated with WG at level of 2 % of daily ration (W/W)

during AFs treatment, showing slight degenerative changes of the hepatocytes with local necrotic area and congestion of some blood vessel (H&E) (X400).

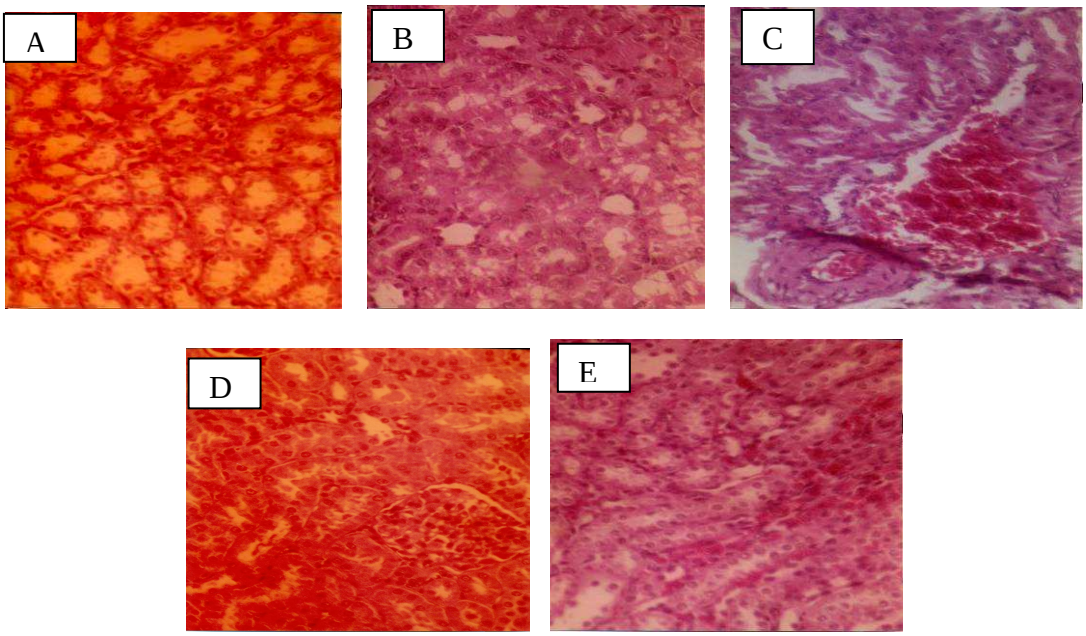

Fig. 2. Kidney sections of (A) rat treated with WG alone (group 2) showing slight degenerative changes of epithelial cells of renal tubules, (B) rat treated with AFs alone (group 3) showing severe necrotic changes of epithelia of the renal tubules and local haemorrhagic areas, (C) rat (group 3) showing severe and diffuse degenerative change of the renal tubular cells, severe hemorrhageareas and congested blood vessels, (D) rat treated with WG at level of 1 % of daily ration (W/W) during AFs treatment (group 4) showing degenerative changes of some renal tubules and hyper cellularity of renal glomeruli and (E) rat treated with WG at level of 2 % of daily ration (W/W) during AFs treatment, showing slight degenerative changes of the epithelial cells of renal tubules (H&E) (X400).

4- Proportional urinary and fecal excretion of aflatoxib B_1, and its corresponding metabolites ($AFB_{2\alpha}$, aflatoxicol), for rats fed AFs-contaminated diets with or without WG.

Data in Table (3) represent the proportional excretions of AFB_1 and its metabolites ($AFB_{2\alpha}$ and aflatoxicol) via feces and urine. AFB_1 and $AFB_{2\alpha}$ concentrations in feces and urine varied according to dietary treatment. The cumulative excretion of $AFB_{2\alpha}$ is expressed in terms of AFB_1 equivalents. Results shown in Table (3) clearly indicate that treatment with WG in groups treated with dietary aflatoxin, had affected the route of AFs excretion and metabolism where major the most of the excreted AFB_1 was found as the metabolite $AFB_{2\alpha}$ in the urine (41.13 to 59.35%), whereas approximately 5.11 to 8.53% was found as $AFB_{2\alpha}$ in feces. In contrast, fecal and urinary excretion of $AFB_{2\alpha}$ in the group fed dietary aflatoxin only was very low (6.17 and 8.19%, respectively). No aflatoxicol amount was detected neither in feces nor urine samples tested.

These results indicate that the major excretory route was found to be the urine (accounting for 15.22% to 67.49% of the total AFs-excretion forms, whereas less than 10% of these forms were excreted in the feces. Treatment with WG improved the AFs excretion via feces and urine in identifiable forms, mainly $AFB_{2\alpha}$ and unchanged AFB_1 without any detectable amount of aflatoxicol. In this respect, our results were in contrast with those observed by Richarda and Lyona (1986) in pigs.

Fecal excretion of $AFB_{2\alpha}$ reduced significantly by the WG-treated groups, this might be explained by a more pronounced renal elimination, which in turn might result in lower biliary secretion of $AFB_{2\alpha}$ in these groups. Our results were in the same trend with those observed by (Bennett *et al.*, 1981), who found that the lower toxicities of AFB_1 and $AFB_{2\alpha}$ in mammals are mainly as a result of a faster rate of clearance via urine and feces compared with that of AFB_1. Our results indicated that AFB_1 metabolites are cleared at a much faster rate than AFB_1. Hence, the rate of AFB_1 biotransformation represents the main mechanism through which detoxification occurs.

It's worthy to report that data obtained from the analysed samples of excreta (faces + urine) of the different studied groups showed no traces of aflatoxicol. This finding might be due to the biotransformation of the ingested contaminants, aflatoxin B1, B2, G1 and G2 to other metabolites and /or the uncompetitiveness of Table (3).

Table (3) Proportional urinary (ng/ml) and fecal excretions (ng/gm) of aflatoxin B_1, and its corresponding metabolites ($AFB_{2\alpha}$, aflatoxicol), of female Albino rats fed aflatoxin (s)-contaminated diets with or without WG for 4 weeks.

Item		Groups										LSD (p≤0.05)
		Group 1		Group 2		Group 3		Group 4		Group 5		
		M+SD	%	M+SD	%	M+SD	%	M+SD	%	M+SD	%	
Feces	AFB1	0 ±0^{A}	0.0	0 ±0^{A}	0.0	165±9.78^{B}	0.49	210± 13.4^{C}	.0.63	297± 18.7^{D}	0.89	31.4
	AFB2α	0 ±0^{A}	0.0	0 ±0^{A}	0.0	1005±32057^{C}	6.17	1703±64.36^{B}	5.11	2843± 54.3^{D}	8.53	46.5
	Aflatoxicol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
Urine	AFB1	0 ±0^{A}	0.0	0 ±0^{A}	0.0	9380±84.5^{D}	28.14	3363 ± 113^{C}	10.09	2713± 56.5 ^{B}	8.14	85.2
	AFB2α	0 ±0^{A}	0.0	0 ±0^{A}	0.0	2730 ±22.15^{B}	8.19	13710±53.6^{C}	41.13	19783± 110 ^{D}	59.35	119.5
	Aflatoxicol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
Total recovery, %		0.0		0.0		42.99		56.96		76.91		-

N.B: 1- The same capital litters in columns denotes no significant difference between treatments in the same raw at ($p \leq 0.05$) and vice versa.

2- Excretion of $AFB_{2\alpha}$ expressed in terms of AFB_1 equivalents as calculated from the molecular weight of AFB_1 /molecular weight of $AFB_{2\alpha}$ (412/430) x mg of $AFB_{2\alpha}$.

In conclusion, we determined that aflatoxin could increase the liver enzyme levels and affect some hematological parameters. Increase in these parameters may occur due to peroxidation reactions, arising in aflatoxin biotransformation, and these reactions may inflict oxidative injury to cellular components. Administration of WG to rats received AFs-contaminated diet, resulted in a significant improvement in all biochemical parameters as well as improvement histopathological picture of the liver and kidney in different experimental groups. In the light of these results, WG was found to induce the potent protective action in rats may play a role in the prevention of hepatic cellular injury produced by aflatoxins.

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