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INFLUENCE OF GARLIC AND PANAX GINSENG SUPPLEMENTATION ON MALE RABBITS EXPOSED TO AFLATOXIN B_1

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SUMMARY

This study was undertaken to evaluate the effectiveness of garlic and panax ginseng as dietary additions in alleviating the toxicity of aflatoxin B_1 (AFB_1) in male Chinchilla rabbits.

thirty rabbits (12-14 weeks age and mean body weight 1589 ± 5.079 gm). were classified into nearly 6 similar groups in their average body weights : first and second groups served as positive and negative controls, third and fourth groups received Aflatoxin B_1 (AFB_1) plus rather garlic or panax ginseng respectively. Fifth and sixth groups received only garlic and panax ginseng respectively. Rabbits were orally administered their respective doses of (AFB_1) every other day for 10 weeks.

The hemato-biochemical parameters and enzymatic activies were evaluated. Results showed that AFB_1 significantly ($P<0.05$) decreased hemoglobin (Hb) total erythrocytic count (TEC) and (PCV). Garlic and panax ginseng caused an increase in these parameters and counteracted the negative effect of AFB_1 in the treated groups. Additionally, serum concentrations of total protein, albumin and glucose were significantly ($P<0.05$) declined by treatment with aflatoxin B_1. Garlic and panax ginseng caused non significant increases in these parameters and lessen the harmful effect of AFB_1. On the other hand, aflatoxin treatment caused significant increases ($P<0.05$) in the activities of serum asparatate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the treated groups also caused significant increases ($p<0.05$) in cholesterol and total bilirulin. Garlic and panax ginseng caused significant decreases in these parameters and alleviated the harmful effects of AFB_1.

Whereas total leukocyte count (TLC), urea and creatinine were not significantly affected by aflatoxin treatment. Generally, it is interesting feature that the dietary additions of macerate garlic or panax ginseng alone in G5 and G6 respectively had no negative effects on most of the previous parameters. Also the presence of garlic or panax ginseng could diminished the adverse effects of AFB_1 on most of hematological and biochemical values and enzymatic activities in rabbits.

INTRODUTION

Aflatoxin (AF) are produced as a by–product of the mould Aspergillus flavus and Aspergillus parasiticus and are considered as the most potent naturally occurring carcinogens known.

When live stock eat aflatoxin – contaminated feed it cause many health and performance problem (Patterson, 1976). Aflatoxin B_1 (AFB_1) is a potent hepatotxic and hepato carcinogenic mycotoxin, the acute toxic effects of AFB_1 include hemorrhaging and death. The subacute or chronic effects of exposure can affects growth, feed efficiency and general well – being Coffey et al. (1999). Lipid peroxidation and oxidative DNA damage are the principal manifestations of AFB_1 induced toxicity, which could be mitigated by antioxidant Souza et al., (1999). Aflatoxin B_1 itself is inactive but is metabolized by microsomes to a reactive compound that inhibit RNA polymerase activity Garnetr (1973). Clinical signs of aflatoxicosis in animals include gastrointestinal dysfunction , reduced reproductivity , reduced feed utilization and efficiency, anemia and jaundice (Tung et al, 1975).

Garlic (Allium Sativum L.) and Panax ginseng are intriguing herbs with a long history of medicinal use for a variety of diseases including detoxification (Dausch and Nixon, 1990) Mesbah and Abou –EL- Ela reported that garlic acts as hypoglycemic and hypolepidmic and antioxidant and detoxified of heavy metals and an immune system modulator. Garlic function in detoxification pathway may be related to its ability to affect sulfur metabolism.

Garlic have a stimulating effect on certain enzymes that are known to be involved in removing toxic substances Dausch and Nixon (1990), Horic et al. 1989). Horic et al. (1984) have suggested its possible role of protecting the membranes from lipid peroxidation. Root of panax ginseng is a herbal drug widely used as an untradional medicine for treatment of cardio – vascular diseases (Lin and Gandolfi, 1997) For thousands of years, it had been used as an aseptic, stomachic and erythropiotic agent. It contains several types of polyacetic alcohols (Nakaqgawa et al., 1985) and these alcohols suppressed (in vitro) the growth of cultured tumercells. (Matsunaga et al., 1994). Panaxytrol (alcoholic extract of panax ginseng) also suppreses the biosyntheois of B16 melanoma transplant-

ed into mice and shows a simultive effect on the anti – tumor activity of mitomycin in cultursd tumor cells Matsunaga et al (1994) the objectives of the present study were

1) to study the effects of treatment by aflatoxin B_1 (AFB_1) on some blood criteria and enzymatic activities of male rabbits.

2) to evaluate the effects of garlic or panax ginseng in alleviating the harmful effects of AFB1 on the above parameters.

MATERIALS AND METHODS

The present study was carried out at a special unit related to a private clinic. Thirty male Chinchilla rabbits aged 12-14 wks and weighed (1589 ± 4.07 g) were used in this study. The animals were individually housed in cages and weighed weekly throughout the experimental period of 10 weeks. Feed and water were provided ad-libitum. The animals were fed on ration pellets consisting of 30% berseem hay (Trifolium alexandrinum), 25% yellow maize, 26.2% wheat bran, 14% soy-bean meal, 3% molasses, 1% calcium chloride, 0.4% sodium chloride, 0.3% mixture of minerals and vitamins and 0.1% methionine. Chemical analysis indicated that it contained 17.5% crude protein, 14.0% crude fiber and 2.7% fat. The animals were randomly divided into 6 groups of 5 animals each and were assigned as follows:

Group 1: Fed the basal diet only and served as positive control (G1).

Group 2: was orally treated with AFB_1 (25 ug/kg BW. every other day and served as negative control (G2).This dose was selected on the basis of previous research in which 2 groups of growing lambs orally administrated with (15 ug and 30 ug/kg BW every other day respectively) Fernandez et al., 1996)

Group 3: Fed the basal diet containing 133 mg/kg diet garlic macerate and treated orally with AFB_1 (25 ug/kg BW.) every other day.

Group 4: Fed the basal diet containing panax ginseng extract powder at a dose of 18.9 mg/kg diet and treated orally with AFB_1 (25ug/kg BW) every other day.

Group 5: Fed the basal diet containing garlic macerate only at a dose of 133 mg/kg diet (G5) and.

Group 6: Fed the basal diet containing panax ginseng powder at a dose of 18.9 mg/kg diet (G6).

Aflatoxin B1 was purchased from Sigma Chemical Co. The LD_{50} of AFB_1 when given orally to rabbits is 0.3 mg/kg. b.wt. (Cheeke and Shull, 1985). AFB_1 was first dissolved in acetone then in corn oil to give a concentration of 300 ug/ml. The mixture was not used for several days, but was shaken frequently to allow the acetone to

evaporate. The dose of AFB_1 was calculated according to the animals body weight on the day before dosing. the proper dose (s) for each animal was given orally with the help of a syringe directly into the oesopharyngeal regions. Garlic macerate which has no adverse effect on the flavour of cooked meat, was obtainnd from kahira pharmaceutical and Chemical Industries Company, Cairo, Egypt. Panax ginseng extract powder as stimulating and general tonic was obtained according to the recommendation of the Arab Drug Company, Cairo, Egypt.

Blood samples were collected from the ear vein of all animal every other week throughout the 10 week experimental period. Blood samples were obtained in the morning before access to feed and water and were placed immediately on ice. Heparin was used as an anticoagulant but, in part of sample, it was withheld to obtain serum. Serum was obtained by centrifugation of samples at 860 xg for 20 min, and was stored at – 20°C until used for analyses. Noncoagulated blood was tested, shortly after collection, for hemoglobin (Hb), total erythrocyte count (TEC), packed cells volume (PCV) and total leukocyte count (TLC). Blood Hb concentration was determined by cyanomethemglobin procedure (Wintrobe, 1967). Erythrocytes were counted on AO Bright line hemocytometer using a light microscope at 40-10 magnification. Blood samples were diluted at 200 times by physiological saline (0.9% sodium chloride solution) before counting. Micro wintrobe hematocrit tubes and hematocrit centrifuge were used to determine the packed cells volume (PCV, or hematocrit value). Blood was centrifuged for 5 min at 4000 rpm and the PCV volume was obtained by reading the packed cells volume on the graduated hematocrit tubes. Leukocytes were counted on AO Bright line hemocytometer using a light microscope at 10-10 magnification after diluting blood samples to 20 times with a diluting fluid (1% acetic acid solution and little of leishman's stain) before counting, (Hepler, 1966). Stored serum samples were analysed for total protein (TP) by the Biuret method according to Henry et al., (1974) . Albumin (A) concentration was determined by the method of Doumas et al., (1997) . Globulin (G) concentration was calculated as the differentce between total protein and Albumin. Serum cholesterol was determined according to the method of Watson, (1960) while plasma glucose content was estimated according to Hyvarinen and Nikkila, (1962) urea and creatinine were determined in serum by the methods of Patton and Crouch , (1977) and Henry et al (1974) respectively . Serum total bilirubin was measured using the method of Walters and Gerade, (1970) the activities of serum aspartate aminotransferase (AST) alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957) . Alkaline phosphatase (ALP) activity was determined according to the method of Kind and King (1954) . Data were analysed by the least

squares analysis because of the unequal subclass numbers. Analysis system (SAS), (1986) was used. Means were statistically compared using least significance Difference (LSD) at 0.05 significance level.

RESULTS

Treatment with 25 µglkg. b.wt, of AFB_1 caused significant decrease ($p<0.05$) in Hb, TEC and PCV in the negative control while in group 3,4 AFB_1 had no significant effect on these parameters. Garlic macerate and Panax Ginseng in group 5,6 respectively caused significant increase ($P<0.05$) in Hb and TEC during the treatment period. In general, garlic macerate and panax ginseng counteracted the harmful effects of AFB_1 on Hb, TEC and PCV. Total leukocyte counts were not significantly affected by garlic macerate or panax ginseng or AFB_1 (Table 1).

Data represented in table 2 showed that serum TP and A declined significantly ($P<0.05$) in the negative control group and the harmful effect of AFB_1 in G3, G4 was alleviated. Treatment with AFB_1 and / or garlic macerate or panax ginseng had no significant effects on serum globulin as compared with the positive control group.

The plasma glucose concentration declined significatly ($P<0.05$) in the negative control group treated with AFB_1.

Table 1: Least square means means (±SEM) blood hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV) and total leukocyte count (TLC) during treatment of male rabbits with aflatoxin B_1 (AFB_1) and garlic or ginseng.

Hematological parameters				
Groups	*Hb gm/dl*	*TEC cells x 10^6 ul*	*PCV %*	*TLC cells x 10^3 ul*
+ VE control	b 12.34	b 5.03	ab 41.5	ab 7.21
-VE control	c 10.29	c 4.18	c 34.3	ab 7.28
AFB_1 + Garlic	cd 11.57	cd 4.53	b 39.0	ab 7.39
AFB_1 + Ginseng	cd 11.64	cd 4.69	ab 40.5	ab 7.19
Garlic only	a 13.66	a 5.35	ab 42.3	bc 6.97
Ginseng only	ab 12.79	a 5.27	ab 41.7	ab 7.23
Least square means ± SEM	0.41	0.19	0.93	0.7

Abcd : Similar or partially superscripts for mean values in the same raw indicate non-significant differences .
Abcd : Different superscripts indicate significant differences ($p < 0.05$) .

Table 2:
Laest square means (±SEM) of serum total protein (TP), albumin (A), globulin (G) urea and creatinine concentration during treatment of rabbits with aflatoxin B_1 (AFB_1) and Garlic or Ginseng.

Serum parameters					
Groups	*TP gm/dl*	*A gm/dl*	*G gm/dl*	*Urea mg/dl*	*Creatinine mg/dl*
+ ve control	ab 7.18	ab 3.31	ab 3.87	54.6	1.242
- ve control	c 6.72	d 3.06	b 3.66	57.9	1.260
AFB_1 + Garlic	bc 7.04	bc 3.20	ab 3.85	54.3	1.256
AFB_1 + Ginseng	bc 7.02	bc 3.22	ab 3.80	56.3	1.233
Garlic only	a 7.35	a 3.37	d 4	53.7	1.198
Ginseng only	ab 7.12	b 3.27	ab 3.86	54.5	1.239
Leas square means ± SEM	0.14	0.07	0.13	1.50	0.019

Abc : Similar or partially superscripts for mean values in the same raw indicate non-significant differences .
Abc : Different superscripts indicate significant differences ($p < 0.05$) .

Table 3: least square means (± SEM) of plasma glucose, serum cholesterol, total bilirubin, asparatate aminotransferase (AST), alanilne aminotransferase (ALT) and alkaline phosphotase (ALP) during treatment of male rabbits with aflatoxin B_1 (AFB_1) and garlic or ginseng.

Biochemical parameters						
Groups	Glucose mg/dl	Cholesterol mg/dl	Bilirubin mg/dl	AST U/l	ALT U/l	ALP U/l
+ ve control	b 100.3	b 52.8	c 0.211	c 31.7	e 44.3	cd 7.03
+ ve control	c 80.7	a 69.7	ab 0.237	a 68.7	a 74.9	a 7.91
AFB_1 + Garlic	cd 96.6	bc 53.9	a 0.220	b 47.2	d 57.9	bc 7.08
AFB_1 + Ginseng	cd 95.7	bc 56.3	a 0.219	ab 43.5	d 54.7	d 6.97
Garlie only	ab 101.5	dc 49.3	c 0.209	c 32.7	ce 43.7	cd 7.04
Ginseng only	ab 99.8	dc 50.5	c 0.207	c 33.2	ce 46.2	bc 7.08
±SEM	1.23	0.41	0.004	3.06	2.11	0.03

Abcd : Similar or partially superscripts for mean values in the same raw indicate non-significant differences .
Abcd : Different superscripts indicate significant differences ($p < 0.05$) .

The harmful effect of AFB_1 had no effect in the presence of garlic macerate or panax ginseng in G3 and G4 respectively which able to prevent the hazardous effect of AFB_1. Treatment with AFB_1 caused significant increase ($P<0.05$) in cholesterol and total bilirubin in the negative control group as compared to the positive control group (Table, 3). Garlic macerate and panax ginseng caused decrease in cholesterol and total bilirubin and couteracted these hazardous effect of AFB_1 on G3 and G4 respectively.

The activities of serum asparate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly ($P<0.05$) induced by treatment with AFB_1 as shown in the negative control group and table 3. Treatment with garlic macerate or or panax ginseng alone as shown in G5 and G6 respectively had no effect on AST, ALT and ALP activities and alleviated the AFB_1 - induced induction in serum AST, ALT and ALP activities in G3 and G4 respectively. A possible explanation for the alleviation effect of garlic macerate and panax ginseng of AFB_1 on the activities of AST, ALT.

And ALP in plasma is that these garlic macerate or panax ginseng may inhibit the liver damage induced by AFB_1.

Serum urea and creatinine were insignificantly increased by aflatoxin treatment (Table, 2). Garlic or panax ginseng caused a slight decrease in serum urea and creatinine and alleviated the AFB_1 induced increase in these parameters during the treatment periods.

Table 1: Least square means means (±SEM) blood hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV) and total leukocyte count (TLC) during treatment of male rabbits with aflatoxin B_1 (AFB_1) and garlic or ginseng.

DISCUSSION

Aflatoxicosis is primarily a hepatic disease cause liver damage and having produced sufficient evidence of carcinogenicity in experimental animals to be identified as carcinogen Mandel et al., 1987 and Shen et al., (1994).

The results shows that concentration of cholesterol and bilirubin increased significantly ($p<0.05$) in negative control rabbits that exposed to AFB_1 only than in the positive control one. The other groups of rabbits that received garlic or panax ginsesg with AFB_1 and also those received garlic or panax ginseng only showed a marked ($p<0.05$) decrease values in serum level of cholesterol and total bilirubin compared to the negative control group, (Table, 3). The elevated serum cholesterol and bilirubin in rabbits exposed to aflatoxin supported the findings of Harvey et al (1991),

Edrington et al (1994) in lambs, in broiler chicks Abdelhamid et al (1994) and Dwivedi et al (1993), and Mehta et al (1993), in rats. The elevations in serum cholesterol levels indicated that liver metabolism and transport of these metabolites are disrupted due to the hepatic damage induced by aflatoxin, (Huff et al., 1986). The induction in the total bilirubin indicates that AFB_1 caused malfunction in the liver (El-zahar et al., 1996). Also Clifford and Ress (1967) reported that the elevation in plasma bilirubin concentration could be due to the of the onset of peripheral necrosis The results in (Table, 3) showed that plasma glucose declined significantly ($p<0.05$) during the treatment with AFB_1 this decrease was not detected in G3 & G4 that givin AFB_1 beside garlic or panax ginseng respectively a garlic or ginseng alone in G5 or G6 and this result in agreement with previous studies that showed a decrease in plasma glucose of growing pigs treated with aflatoxin B_1, Harvey et al. (1994) and Edrington et al (1994), in lambs Harvey et al (1991) , Harvey et al (1999) in growing crossbred barrows, Schell et al., (1993), in rats Mehta et al (1993) and in brioler chicks Abdelhamid et al (1994). Also, Dwivedi et al (1993) reported that administration of aflatoxin B1 resulted in a significant decrease in glycogen in rat liver. Results of the present study (Table, 2) indicated that serum urea and creatinine of the treated animals with AFB_1 of the negative control group showed insignificant increase as compared with the + ve control group. similar results were obtained by Abdelhamid et al (1994) who found that aflatoxin treatment caused an increase in blood creatinine in broiler chicks. Garlic or panax ginseng caused a slight decrease in serum urea and creatinine and alleviate the AFB_1 induce in these parameters during the treatment periods. The present results indicate that treatment with 25 ug/kg day AFB_1 caused significant reductions in Hb, TEC and PCV, and these effects on Hb and PCV were alleviated on G3 & G4 which given garlic macerate or panax ginseng recpectively beside AFB_1 (Table, 1), or that given garlic or ginseng alone.The decrease in TEC , Hb and PCV would lower the oxygen supply to different tissues resulting in low energy production. Reduction in hemoglobin content can be related to the decreased size of red blood cells or the impaired biosynthesis of heme in bone marrow (Edrington et al., 1997). Also, the reduction in Hb content may be due to increased rate of destruction or reduction in the rate of formation of TEC. This premise is supported by recorded low TEC in the treated groups (Table, 1). Similar studies also showed a decrease in hematological parameters in the aflatoxin – treated broiler chicks Kubena et al., 1997) and in turkeys (Kececc, et al (1998). The anemia induced by aflatoxin has been described by Tung et al. (1975) as a type of hemolytic anemia characterized by decreased TEC, PCV, Hb and bone marrow hyperplasia. This of anemia could result from inhibition of hematopoiesis, defective hematopoiesis, increased de-

struction of red blood cells, or a combination of all three (Huff et al 1986). In addition, the reduction in the blood parameters (PCV, RBC and Hb) may be attributed to a hyperactivity of bone marrow Tung et al., (1975), leading to production of red blood cells with impaired integrity which were easily destructed in the circulation.Results in table 2 showed that total protein (TP) and albumin (A) were significantly reduced by aflatoxin treatment. Some previous studies showed that aflatoxin induced reduction in TP and A (Kubena et al., 1997. Kececci et al, 1998 Harvey et al., 1991 and Edrington et al, 1997) and in lambs, broiler chicks and crossbred pigs. Also Dwivedi et al. (1993) reported that administration of aflatoxin B_1 resulted in a significant decrease in DNA, RNA, and proteins in rat liver and total proteins in serum. The decrease in plasma protein may be due to degeneration of endoplasmic reticulum and inhibition of protein synthesis (Svivastava, 1982). In addition Osuna and Edds (1982) suggested that such effects may be due to the metabolism of aflatoxin in the liver, where it interferes with protein synthesis and RNA production resulting in decreasing albumin and B and g globulin. The observed decrease in plasma proteins could be attributed in part to the damaging effect of aflatoxin on the liver cells, as confirmed by the increase in the activities of serum AST, ALT and ALP (Table, 3) due to their release from the damaged liver cells into circulation. These hazardous effects were alleviated in G3, G4 due to the beneficial influences of garlic and ginseng. Transaminases (AST and ALT) are important and critical enzymes in the biological processes (Harper et al, 1979) which considered as indicators of the hepatic disfunction and damage, (Shakoori et al., 1994). Alkaline phosphatase enzyme (ALP)is a sensitive biomarker to metallic salts since it is a membrane bound enzyme related to the transport of various metabolites (Lakshmi et al., 1994) Coleman (1992) the various isoenzymes of ALP are ubiquitous through the body, although they are mainly present in liver, bone, intestine, kidney, placenta and white blood cells (Tietz, 1976). Aflatoxin administration resulted in a significant increase ($p<0.05$) in the activities of serum AST, ALT and ALP (Table, 3). These results are in agreement with the findings by Harvey et al (1991) Edrington et al (1994) and Fernandez et al (1996) in lambs. The increment of the activities of AST, ALT and ALP in plasma is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream. (Navaro et al., 1993), which gives an indication on the hepatotoxic effect of aflatoxin B_1. Also, Osuna and Edds (1982), reported that the increased values of AST and ALP could be related to the liver necrosis and hepatotoxic effects of AFB_1. In addition, the increase of ALP in serum could be a result of damage of liver cells and bile duct obstruction due to proliferation of its cells and or related to the progressive liver necrosis (Newberne and Butter, 1969), thus, the observed increase in serum AST,

ALT and ALP could be attributed in part to the concomitant hepatic necrosis as induced by AFB_1.

So the present study show that supplementation of diets with garlic and panax ginseng as food additives in rabbit diets had alleviated the toxic effect of AFB_1 (Table, 1, 2 and 3). The beneficial influences of panax ginseng noted in the present study which investigate in previous study (Yun – taikkoo, (2003) who investigated that the prolonged administration of red ginseng extract significantly inhibits the incidence of hepatoma and also proliferation of pulmonary tumors induced by aflatoxin B_1 and urethane. So he repoted its anticarcinogenic effect or human cancer preventive effect of panax ginseng and Berges et al (1953), who observed that the increased alliin content of the garlic powder fed to rats administred with aflatoxin B_1 was associated with a proportional decrease in the number and area of preneoplastic foci. So they suggested that garlic partly exerts its anticarcinogenic effects through increasing enzymes involved in AFB_1 detoxification. So he suggested that garlic had anticarcinogenic effects through increasing enzymes involved in AFB_1 detoxification and Guyonnet et al (2002), found that Diallyl sulfide (DAS) and diallyl disulfide (DADS), two garlic constituents inhibit aflatoxin B1 (AFB_1) initiated carcinogenesis in rat liver. So they decided that allyl sulfides give protection against AFB_1 – induced carcinogensis. So he suggested that garlic constituents inhibit aflatoxin B_1 (AFB_1) – initiated carcinogenesis in rat liver.

From the present results, it can be concluded that exposure of animals to AFB_1 is capable of inducing marked hazardous alterations in some hematological and biochemical characteristics, and enzymatic activities which considered as necessary steps before investigation by sonography and histopthology to perceive any alteration in the liver texture initiated carcinogenesis , using macerate garlic or panax ginseng as feed additives improve the blood constituents through correcting the lipids and proteins disturbance and consequently the other metabolic changes and could be a beneficial way to overcome toxicity of AFB_1 in the intoxicated animals. However, further studies using garlic or panax ginseng as prophylactic agents against the toxicity of AFB_1 in the intoxicated animals might be needed. Histopathological, hematological and endocrinological studies in the same respect, may be need. Accordingly, care must be taken into account to avoid the contamination of feed stuffs with aflatoxin.

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