The Possible Protective Role of Korean Ginseng on Ochratoxicosis: with Special References on Chromosomal Aberrations in Rats.

Somaia A. Nada, Mahmoud S. Arbid and Hassan A.I. Ramadan[1]

Pharmacology Department & [1]Cell Biology Department
National Research Center, Dokki, Cairo, Egypt.

ABSTRACT

Ochratoxin A (OA) is a potent mycotoxin causing considerable health hazard and economic losses. OA is of concern as it is hepato-nephrotoxic, mutagenic, and carcinogenic to a great variety of animals. LD_{50} of crude OA was 8.5 mg/ kg.b.w., i.p. The clinical symptoms, mortalities and necropsy were recorded in rats injected with OA (LD_{50}, i.p.) during 10 days of daily treatment. Ginseng treatments (20 mg / kg. b.w., i.p.) : before, mixed with, or after OA dose, completely prevented the mortality in rats. OA-treated animals showed microcytic normochromic anaemia, lucocytosis, hypoproteinaemia and elevation of serum ALT, AST, AP, urea, and creatinine values. These findings were declined near the normal levels when ginseng injected with OA. OA (1/5 LD_{50}) induced chromosomal aberrations (65.66%) compared to the control. When ginseng given 10 min before OA injection, chromosomal aberrations were reduced to be 31.66% compared to OA-treated animals. In conclusion: ginseng has a protective effect against ochratoxicosis, it has anti-genotoxic activity and it can repair the chromosomal damage induced by ochratoxin A.

Key words : Ochratoxicosis, Chromosomal aberrations, Mycotoxins, Ochratoxin A, Korean ginseng, Protective effect of *Panax ginseng,* Rat

Introduction

Ochratoxin A (OA) is a highly toxic and encountered secondary fungal metabolite produced by toxigenic strains of *Penicillium* and *Aspergillus,* frequently contaminate foods and feeds causing considerable health hazard and economic losses (Lu *et al.,* 1995). This mycotoxins is known to be hepato-nephrotoxic, teratogenic, carcinogenic, genotoxic and mutagenic to human and most of animal species (Follmann *et al.,* 1995, *Xiao et al.,* 1996).

Ochratoxin A exert its toxic effect through three major effects: (i) inhibition of ATP synthesis (Marquardt and Frohlich, 1992); (ii) enhanced lipid peroxidation in which the free radicals or active oxygen species are involved (Omar *et al.,* 1990) and (iii) inhibition of protein synthesis due to inhibition of phenylalanine tRNA synthetase (Creppy *et al.,* 1984).

Creppy *et al.,* (1985) and Kane *et al.,* (1986) resulted that the mice injected with OA caused

DNA-single strand breaks in the tissues of liver, kidney and spleen.

Pfohl-Leszkowicz (1993) measured OA-DNA adduct formation in different organs (kidney, liver, and spleen) the authors indicated that the kidney was the main target organ of the genotoxicity and carcinogenicity of OA.

It was found that OA induce chromosomal aberrations on X chromosome in human peripheral lymphocytes in patients suffering from endemic nephropathy (Monolova *et al.*, 1990). OA also has been implicated as a causative factors in the fetal renal disease endemic Balkan nephropathy in humans (Krogh *et al.*, 1987) .

Several different strategies were done for controlling or neutralizing the toxic effect including proper storage, modification of the diet to enhance hydrolysis or reduced absorption in gastrointestinal tract and the use of feeding regimen that minimize the mycotoxins effect.

Panax ginseng is a medical plant grown in South Korea and recently used for medical purposes in Egypt. The active principle is ginseng which was reported to repair the damaged tissues of the kidney (Hattori *et al.*, 1989) liver (Yamamoto *et al.*, 1993), brain (Okamura *et al.*, 1994), endothelial cells of myocardium (Zhan *et al.*, 1994). Moreover, it can reduce pulmonary damage induced by free radicals (Sohn *et al.*, 1993).

Panax ginseng was reported previously to reduced ochratoxicosis in rats and it minimized the toxic effect of OA on liver and kidney tissues (Nada *et. al.*, 1996). In another study, Amra *et al.*, (1996) found that *P. ginseng* significantly reduced serum OA concentration and increased the renal and hepatic level of DNA and RNA contents compared to OA-treated rats.

The objective of the present study was to minimize the chromosomal aberrations induced by ochratoxicosis using ginseng in rats.

Materials and Methods

Crude Ochratoxin A (OA) was prepared from *Aspergillus ochraceus* (NRRL 3174) and quantitatively determined by the method of Golinski and Chelkowski (1978). LD_{50} of OA was calculated (Karber, 1941). The doses of 1/2 LD_{50} (for experiment I) and1/5 LD_{50} (for experiment II) were injected intraperitoneally (i.p.) which equivalent to 4.25 mg and 1.7 mg/kg.b.w. respectively. Each dose was dissolved in 10 ml of 0.1 M $NaHCO_3$. Ginseng was obtained from EIPICO Company, 10th Ramadan, Egypt. The dose used was 20 mg/kg. b.w./10 ml saline, i.p. (Yokozawa *et al.*, 1995), as the scheduled time of administration in experimental design.

Animals

Fifty eight albino Sprague-Dawley rats weighing 120±5g were used in this study. They were allowed free access to food and water, which were free from OA.

Experimental design

Experiment I: thirty-six rats were divided into 6 equal groups (6 rats each). Rats were injected (i.p.) for 10 successive days as follows: **Group 1,** was the control group, half of the group injected with 10 ml 0.1 M $NaHCO_3$ / kg b. w. and the other half with saline. **Group 2,** was given ginseng 20 mg/ kg b.w.; **Group 3,** was injected with OA LD_{50} (4.25 mg/kg b.w.); **Group 4,** ginseng was administered 10 min before of OA injection ; **Group 5,** ginseng was administered 10 min after OA injection. **Group 6,** ginseng was administered mixed with OA injection. The protective value of ginseng was determined by observation of clinical symptoms and mortality rate and by determination of the haematological (Jain,1986) and serum concentration of transaminases (ALT & AST), alkaline phosphatase (A.P), total protein(T.P), urea, and creatinine were determined on a clinical chemistry analyzer (Gilford Impact 400E , Ciba Coming Diagnostics Corp., Gilford Systes, Oberlin, OH 44774).

Two blood samples were obtained from retro-orbital venous plexus of each rat. The first sample collected in a clean tube containing EDTA anticoagulant for haematological determination and the second for serum preparation for biochemical analysis.

Experiment II: this experiment was conducted for studying chromosomal aberration depending upon the good results obtained from experiment I and we used here 1/5 LD_{50} to produce the toxic effect without mortalities in OA-treated group. Twenty-four rats were divided into 4 equal groups (6 rats each). Rats were injected (i.p.) for 10 successive days as follows: **Group 1,** the control group; **Group 2,** administered ginseng; **Group 3,** injected with OA 1/5 LD_{50} (1.7 mg/kg b.w.) Group 4, ginseng was administered 10 min before of OA injection. Colchicine, 6 mg/kg b.w. was injected i.p. 2 hours before sacrifice the animals. The cells of femur bone marrow were suspended in saline, treated with a hypotonic solution (0.075 M KCl) at 37℃ for 15 minutes, fixed in cold Carnoy's solution (methanol : acetic acid = 3:1) three times, and spread on clean glass slides. After complete drying, slides were stained with 10% Giemsa in phosphate buffer (pH 6.8). Tracing chromosome damage in at least 50 metaphase figures per animal monitored the effect of different treatments on meiosis.

The obtained results from the two experiments were analyzed statistically using ANOVA and LSD for the means in haematological and biochemical analysis, and the 2 X 2 Contingency table for chromosomal aberrations (Snedecor and Cochran, 1967).

Results and Discussion

The calculated LD_{50} of crude OA was 8.5 mg/kg b.w., i.p. The clinical symptoms and mortalities for the rats injected with LD_{50} of OA lost their appetite and appeared severely dehydrated. Diarrohoea of a yellowish color was also observed. Three rats died from the group treated with OA after 3 days of the start of the experiment and no one died from the three groups treated with ginseng and OA.

Postmortem findings observed on dead animals revealed general abdominal congestion, hydroperitoneum, hydrothorax and hyperatrophy of the kidney and liver. The intestine was distended with watery frothy yellowish fluid.

Results of the present study showed that administration of 1/2 LD_{50} of OA for 10 days increased mortalities in rats. Treatment with ginseng before, mixed with or after OA administration completely prevented mortalities in rats. OA induced mortality has been attributed to drop in blood pressure and heart rate producing shock-like responcse as reported by Richardi and Huff (1983). On the other hand, ginseng acts as antioxidant through enhancement of glutathione level in the body and acts as scavenger of free radicals (Chung *et al.*, 1993).

Haematological changes induced by OA injection was illustrated in Tables (1&2). Haemoglobine concentration (Hb), PCV and RBC count were significantly decreased than control in rats treated with OA. Meanwhile, ginseng treated animals showed significant increase in PCV value and RBC count comparing to the other treated groups.

The combined injection with ginseng and OA generally increased Hb, PCV, and RBC count significantly compared to OAb alone. Rats treated with OA 10min prior ginseng administration had lower PCV and RBC values than control rats. However, the levels of HB, PCV and RBC in rats of combined treatments did not altered (Table 1).

Table (1): Haemograme of rats after 10 days of treatment with ginseng (20mg/Kg. b.w., i.p.) given before, after, or mixed with ochratoxin A (OA) (1/2 LD50 = 4.25 mg / Kg.b.w., i.p.) [Ginseng + OA ; OA + Ginseng; Ginseng X OA] respectively. Means ± SE , n = 6 / group.

Groups	Hb (g %)	PCV (%)	RBC X 10^6 / µl.	MCV (fl)	MCH (%)	MCHC (pg)
Control	A 13.89 ± 0.33	A 42.62 ± 1.65	A 5.84 ± 0.18	A 73.50 ± 1.04	A 23.8 ± 0.48	A 32.50 ± 0.65
Ginseng	A 15.5 ± 0.65	B 47.82 ± 0.21	B 6.32 ± 0.43	AC 76.3 ± 1.72	A 24.63 ± 1.26	A 32.40 ± 0.41
OA	B 10.78 ± 0.55	C 31.05 ± 1.00	C 4.53 ± 0.12	B 67.30 ± 0.74	A 24.30 ± 0.74	A 34.48 ± 1.50
Ginseng + OA	A 13.85 ± 0.25	A 40.62 ± 1.30	A 5.53 ± 0.16	A 72.63 ± 1.12	A 24.53 ± 0.50	A 34.50 ± 0.66
OA + Ginseng	A 12.39 ± 0.33	D 35.62 ± .95	C 4.95 ± 0.19	A 72.48 ± 0.93	A 23.61 ± 0.54	A 34.61 ± 0.33
Ginseng X OA	A 12.04 ± 0.45	AD 38.70 ± 1.34	A 5.20 ± 0.26	C 77.25 ± 1.54	A 23.20 ± 0.34	A 33.4 ± 0.87

Means with the same letter are not significantly different at $p < 0.05$.

Blood indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) did not changed by ginseng alone, while MCV in OA treated animals was reduced significantly and it markedly increased in ginseng mixed with OA group. Moreover, MCH and MCHC did not affected by the different treatments. The resultant microcytic normochromic anaemia in rats given OA alone, this probably due to many factors as: chelation of iron by OA (Omar *et al.,* 1990 , 1991), protein deficiency (Creppy *et al.,* 1984), impairment of proximal renal function (Hald, 1991) which may lead to inhibition of erythropoietin hormone (Jain, 1986).

Leucocytosis was observed in OA and all the combined treatments with ginseng and OA (Table 2). However, OA treatment caused severe lymphocytosis, neutrophilia, monocytosis, eosinophilia and basophlia. The same results were found in the three treated groups with ginseng and OA (Table 2). The obtained leucocytosis mainly due to lymphocytosis and neutrophilia (Nada *et al.,* 1996). All the haematological figures were changed approximmately due the toxic effect of OA on pluripotent stem cell activity in the bone marrow. On the other hand, ginseng alone or in combination with OA (ginseng + OA and ginseng mixed with OA) caused an increase in RBC count. This findings indicated that ginseng could to enhancement of erythropoiesis (Woo *et al.,* 1993). Rats injected with OA (1/2 LD_{50}) showed a significant elevation in ALT, AST, AP, urea and creatinine (Table 3). These values were diminished and declined near the normal values when ginseng injected with OA. The observed elevation of enzymes activities (ALT, AST and AP) in OA treated animals probably was due to hepatotoxic effect of OA. Similar findings have been reported previously (Nada, 1986). There is some evidence from the literature that OA produces bone changes in young chickens (Huff *et al.,* 1980) which could also a contributory factor to increase AP activity. Combined administration of ginseng with OA minimized the effect of OA as far as serum enzymes ALT, AST and AP are concerned. Serum total protiens were slightly decreased in different experimental groups treated with OA alone or in combination with ginseng (Table 3). The early onset of this mild hypoproteinaemia would suggest albumin loss probably from the kidneys of rats or due to inhibition of hepatic protein synthesis through competitive inhibition of phenylalanyl-tRNA synthetase enzyme (Creppy *et al.,* 1984). Values of urea and creatinine were elevated in OA-treated rats. This increase seems to be due to distruction of some body tissues by OA and development of kidney lesions. Elevation of urea and uric acid were reported in mice and broiler chicks, respectively, exposed to OA (Boorman *et al.,* 1984 ; Kubena *et al.,* 1983).

Cytogenetic study: ochratoxin A caused a 16.7 fold increase in the incidence of total as well as structural changes and mitosis disruption abnormalities (65.66%) in the examined bone marrow cells (Fig. 1). The types of chromatid aberrations induced were mainly gap, break, centric fusion, deletion, ring and numerical aberrations in the form of hypoploidy. The incidence of these abnormalities were significantly minimized approximately to the half (31.66%) when ginseng administered concurrently

Table (2): Total WBC and differential leucocytic counts (No X 10^6 / µl.) of rats after 10 days of treatment with ginseng (20mg/Kgb.w., i.p.) given before, after, or mixed with ochratoxin A (OA) (1/2 LD50 = 4.25 mg / Kg.b.w., i.p.) [Ginseng + OA; OA + Ginseng; Ginseng X OA] respectively. Means ± SE , n = 6 / group.

Groups	WBC	Lymphoctes	Neutrophile	Monocytes	Eosinophile	Basophile
Control	A 8.91 ± 0.44	A 6.00 ± 0.29	A 2.00 ± 0.09	A 0.72 ± 0.10	A 0.14 ± 0.01	A 0.038 ± 0.02
Ginseng	A 7.88 ± 0.36	B 4.78 ± 0.31	A 2.04 ± 0.07	A 0.81 ± 0.06	B 0.23 ± .01	A 0.031 ± 0.01
OA	B 15.61 ± 0.23	C 8.3 ± 0.12	B 4.7 ± 0.22	B 2.28 ± 0.03	C 0.33 ± 0.02	B 0.00
Ginseng + OA	C 11.87 ± 0.21	B 5.01 ± 0.06	B 5.40 ± 1.63	A 0.83 ± 0.07	C 0.36 ± 0.03	C 0.14 ± 0.01
OA + Ginseng	C 10.36 ± 0.18	B 4.75 ± 0.33	B 4.73 ± 0.44	A 0.91 ± 0.15	D 0.45 ± 0.01	B 0.00
Ginseng X OA	C 11.27 ± 0.14	B 4.92 ± 0.11	B 5.24 ± 0.25	A 0.77 ± 0.06	E 0.51 ± 0.02	D 0.02 ± 0.01

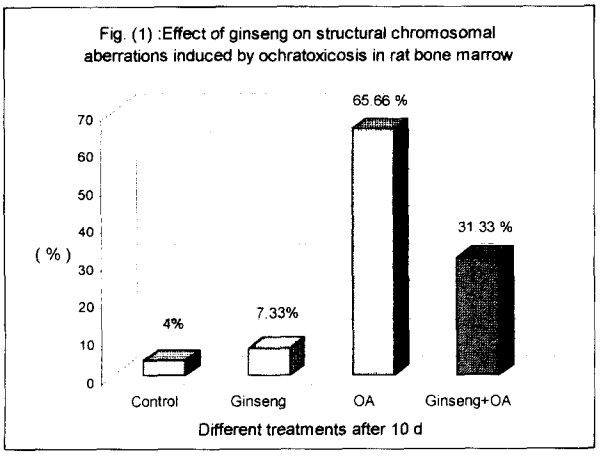
Means with the same letter are not significantly different at $p < 0.05$.

Table (3): Serum biochemistry of rats after 10 days of treatment with ginseng (20mg/Kgb.w., i.p.) given before, after, or mixed with ochratoxin A (OA) (1/2 LD50 = 4.25 mg / Kg.b.w., i.p.) [Ginseng + OA ; OA + Ginseng; Ginseng X OA] respectively. Means ± SE , n = 6 / group.

Parameters Groups	ALT (I U/ ml)	AST (I U/ ml)	Alk. Phosph K.A. / dl.	T.P (g /dl)	Urea (mg /dl)	Creatinine (mg /dl)
Control	A 48.4 ± 2.56	A 77.34 ± 1.08	A 12.9 ± 2.13	A 7.84 ± 2.10	A 57.34 ± 1.54	A 0.63 ± 0.049
Ginseng	B 39.8 ± 1.80	A 79.82 ± 2.36	A 10.54 ± 2.51	A 8.93 ± 2.33	B 47.32 ± 2.01	B 0.86 ± 0.069
OA	C 89.50 ± 1.50	B 159.45 ± 3.21	B 47.64 ± 3.78	A 5.32 ± 1.09	C 164.78 ± 2.54	C 3.27 ± 0.077
Ginseng + OA	AD 51.50 ± 2.40	C 90.63 ± 1.98	C 18.60 ± 1.14	A 7.38 ± 1.18	D 63.80 ± 1.30	B 0.84 ± 0.042
OA + Ginseng	D 59.3 ± 2.93	D 129.56 ± 3.26	C 23.1 ± 1.98	A 6.87 ± 0.98	E 76.21 ± 1.22	D 1.76 ± 0.013
Ginseng X OA	AD 53.40 ± 1.66	E 117.8 ± 2.04	C 20.80 ± 2.06	A 6.82 ± 2.26	E 73.92 ± 1.05	B 0.94 ± 0.018

Means with the same letter are not significantly different at $p < 0.05$.

with OA (Fig. 1, 2a, 2b). Ginseng injection produced only a marginal increase in the frequency of abnormalities, which was never significant, and hence ginseng dose can' t be deemed clastogenic (Fig. 1). No chromosomal- type aberrations were observed in all treated groups, except infrequent centric fusion, ring, and sticky chromosomes were found in the bone marrow cells. In the present study, OA was investigated to induce abnormalities in the mitotic and meiotic metaphase chromosomes. It is suggested that the electrophilic metabolites of OA form adduct with DNA or produce replacement-type mutations. Follmann *et al.,* (1995) found that OA and its metabolite ochratoxin induced sister chromatid exchange in cultured porcine urinary bladder epithelial cells the authors concluded that OA is genotoxic in this vitro system and its metabolite ochratoxin is toxic at higher concentrations. When ginseng administered i.p. concurrently with OA, the incidence of chromosomal aberration was significantly minimized. The protective effect of ginseng was most marked in mitotic chromosomes followed by meiotic chromosomes. Ginseng may achieve its anti-genotoxic effect via blocks and shunts in the OA-metabolic pathway. Ginseng mediated cellular repair and scavenging of the mutagenic radicals could also occur (Mei *et al.,* 1994). Moreover, the pretreatment with *Panax ginseng* (water or alkaloidal fractions) reduced cell damage caused by gamma-rays in mice specially damage to DNA molecules and it plays an important role in the repair or regeneration



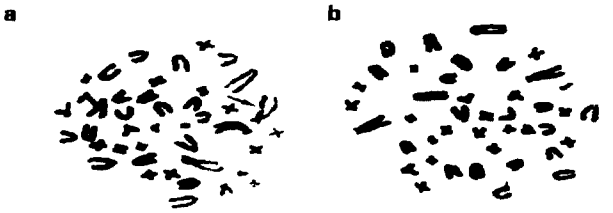


Fig.(2): Metaphase spreads from rat bone marrow treated with, Ochratoxin A (a), Ginseng + Ochratoxin A (b). The arrow shows the breakage effect induced by Ochratoxin A.

process of damaged cells (Kim *et al.,* 1993). Bose and Sinha, (1994) and Dramshila *et al.,* (1994) found that vitamin C and retinol were minimized chromosomal abnormalities and gross morphology of the sperm head of mice induced by OA oral administration. The present work investigated that, ginseng has a protective effect against ochratoxicosis, it has anti-genotoxic activity and it can repair the chromosomal damage induced by ochratoxin A.

References

Amra, H.A., El-Deeb, M.K. and Nada S.A. (1996): Reduction of ochratoxin A level in rat serum by L-Methionine and Ginseng, *J. Union Arab Biol.,* **5**, 95 - 107.

Boorman G.A., Hong H.L., Dieter M.P., Hayes H.T., Poland A.E., Stack M. and Luster M.I. (1984): Myelotoxicity and macrophage alteration in mice exposed to ochratoxin A. *J. Toxicol. Appl. Pharmcol.,* **72,** 304- 312.

Bose S. and Sinha S.P. (1994): Modulation of ochratoxin produced genotoxicity in mice by vitamin C. *Food and Chemical Toxicology*, **32**, 6, 533- 537.

Chung H.Y., Kim K.W., Oura H. Yokozawa T. (1993): Effect of ginsenoside Rb_2 on the antioxidants in senescence- accelerated mice (SAM-R/1). *Proceeding of the 6th International ginseng symposium,* Seoul, Korea .

Creppy, E.E., A. Kane, G. Dirheimer, C. Lafarge-Frayssinet, S. Mousset and C. Frayssinet (1985): Genotoxicity of ochratoxin A in mice: DNA single-strand break evaluation in spleen, liver and kidney, *Toxicol. Lett.,* **28,** 29-35.

Creppy, E.E., Roschenthaler, R. and Dirheimer, G. (1984): Inhibition of protein synthesis in mice by ochratoxin A and its prevention by phenylalanine. *Food Chem. Toxicol.,* **22**, 883-886.

Darmshila K. and Sinha S.P. Kumai D.(1994): Effect of retinol on ochratoxin produced genotoxicity in mice. *Food and Chemical Toxicology,* **32**, 5, 471- 475.

Follmann W., Hillebrand I.E., Creppy E.E. and Bolt H.M. (1995): Sister chromatid exchange frequency in cultured isolated porcine urinary bladder epithelial cells (PUBEC) treated with ochratoxin A and alpha. *Archives of Toxicology,* **69**, 4, 280- 286.

Golinski P. and J. Chelkowski (1978): Spectral behavior of ochratoxin A in different solvents. *J. Assoc. of. Anal. Chem.,* **61,** 3, 586-589.

Hald B.(1991): Porcine nephropathy in Europe mycotoxins, endemic nephropathy and urinary tract tumors, IARC scientific Pub.No. 115, ed. M. Castegnaro, R.Plestina, G. Dirheimer, I.N.Chernozemsky and H. Bartsch. (Oxford:Oxford Univ. Press) pp 49-56.

Hattori T., T. Nagamatsu, M. Ito and Y. Suzuki(1989): Studies on the antinephretic effect of T.J.-8014, a new Japanese medicine and its mechanisms (1): Effects on original type antiGBM nephritis in rats and platelet aggregation, *Jap. J. Pharmacol.* **50,** 4, 477-485.

Jain N.C.(1986): Schalm' s Veterinary Haematology, 4th ed., Lee and Febiger, Philadeliphia.

Jin R., Wan L.L., Mitsuishi I., Kodama K. and Kurashige S. (1994): Immunomodulative effects of Chinese herbs in mice treated with anti-tumor agent cyclophosphamide. *Yakugaku Zasshi. Jul.* **114,** 7, 533- 538.

Kane, A., E.E. Creppy, A. Roth, R. Roschenthaler and G. Dirheimer (1986): Distribution of the [^{3}H]-label from low doses of radioactive ochratoxin A ingested by rats, and evidence for DNA single-strand breaks caused in liver and kidneys. *Arch. Toxicol.,* **58,** 219-224.

Karber G.(1941): Pharmacologische Methoden Zur Auffindung von Arzneeimit-tain und Gifter Wirkungsweise Vor. Dr. Med. Leopold Ther. Wissenschaftliche Varlage Gesse. Gesellschaft. M.B.H.

Kim, S.H., Cho, C.K., Yoo, S.Y., Koh, K.H.,Yun, H.G. and Kim, I.H. (1993): *In vivo* radioprotective activity of *Panax ginseng* and diethyldithiocarbamate. *In vivo,* 7 : 467 - 470.

Krogh, P. (1987): Ochratoxin in food. In: P. Krogh, (ed). Mycotoxins in food. Academic Press Inc. San Diego, CA. pp, 97-121.

Kubena L.F., Huff W.E., Harvey R., Corrier D.E., Pillips T.D. and Crenger C.R.(1988): Ochratoxin A and deoxynivalenol on growing broiler chicks. *Poult. Sci.,* **67**, 2, 253- 260.

Li, X., J.X. Chen and J.J. Sun(1990): Protective effect of *Panax notoginseng* saponins on experimental myocardial injury induced by ischemia and reperfusion in rats. *Chung. Kuo. Yao. Li. Hsueh. Pao,* **11,** 1, 26-29.

Lu, P., Marquardt, R.R. and Kierek-Jaszczuk, D. (1995) : Immunochemical identification of fungi using polyclonal antibodies raised in rabbits to exoantigens from *Aspergillus ochraceus. Lett. Appl. Micro.,* **20**, 41 - 45.

Manolova Y., Manolov G., Parvanova L., Petkova B.T., Castegnaro M. and Chernozemsky I.N. (1990) : Induction of characteristic chromosomal aberrations, particularly X-trisomy, in cultured human lymphocytes treated by ochratoxin A, a mycotoxin implicated in Balkan endemic nephropathy. *Mutation Research Fundmental and molecular mechanisms of mutagenesis* **231,** 2, 143- 149.

Marquardt, R.R. and Frohlich, A.A. (1992): A review of recent advances in understading ochratoxicosis. *J. Anim.Sci.,* **70,** 3968 - 3988.

Mei, B., Wang, Y.F., Wu, J.X. and Chen, W.Z. (1994): Protective effects of ginsenosides on oxygen free radical induced damages of cultured vascular endothelial cells *in vitro. Yao. Husch. Hsuch-Pao,* **29,** 11, 801-808.

Nada ,S.A. , Hussein, A.A. , EL-Deeb, M. K. and Arbid, M. S. (1996): Comparative study of ginseng *(Panax ginseng)* and L-Methionien on ochratoxicosis in rats. *J. Egypt. Soc. Toxicol,* **16,** 49 - 55.

Okamura N., K. Kobayashi, A. Akaike and A. Yagn (1994) : Protective effect of ginseng saponine against impaired brain growth in neonatal rats exposed to ethanol. *Biochem. Pharmacol. Bull.,*

17, 2,270-274.

Omar, R. F., Hasinoff, B. B., Mejilla, F. and Rahimtula, A. D. (1990): Mechanism of Ochratoxin A stimulated lipid peroxidation. *Biochem.Pharmacol.,* **40,** 1183- 1191

Omar R.F., Rahimtula A.D. and Bartsch H.(1991): Role of cytochrome P-450 in ochratoxin A-stimulated lipid peroxidation. *J. Biochem.Toxicol.,* **6,** 203 - 209.

Pfohl-Leszkowicz, A., Grosse, Y., Kane, A., Creppy, E.E., and Dirheimer, G. (1993): Differential DNA adduct formation and disappearance in three mouse tissues after treatment with the mycotoxin ochratoxin A, *Mutation Res.,* **289,** 265-273.

Richardi J.C. and Huff W.E. (1983): Effect of acute ochratoxicosis on blood pressure and heart rate of broiler chickens. *Poult. Sci.,* **62,** 2164-2168.

Snedecor G.W. and Cochran W.G.(1967): Statistical Methods. 6th. ed. The Iowa State Univ. Press, Ames, Iowa, USA.

Sohn H. O., H.B. Lim, Y. G. Lee, D.W. Lee and Y.I. Kim (1993): Effect of subchronic administration of antioxidants against cigarette smoke exposure in rats. *Arch. Toxicol.*, **67,** 10, 667-73.

Woo, Y.M., Lee H.W. Kim J.P. (1993): The effect of ginseng on the postoperative nutritional status and immune functions of gastric carcinoma patients. Proceeding of the 6th International ginseng symposium, Seoul, Korea .

Xiao, H., Madhyastha, S., Marquardt, R.R., Li, S., Vodela, J.K., Frohlich, A.A. and Kemppainen, B.W. (1996) : Toxicity of ochratoxin A , its opened lactone form and several of its analogs : structure - activity relashonships. *Toxicol.Appl. Pharmacol.,* **137**, 182 - 193.

Yamamoto M., Miki S., Deguchi H., Uemiya M., Uemura T. and Kumagai A. (1993): Combined effect of red ginseng with XIAO-CHAI-HU-TANG in patients with chronic hepatitis. Proceeding of the 6th International ginseng Symposium, Seoul , Korea, **57**- 60.

Zhan Y., X.H. XO and Y.P. Jiang(1994): Protective effects of ginsenoside on myocardic ischemic and reperfusion injuries. *Chang. Hua. T. Hsueh. Tsa. Chih.,* **74,** 626-28.