Int J Pharm Bio Sci 2013 July; 4(3): (P) 1036 - 1042

Research Article Pharmacology

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PROTECTIVE ROLE OF A COMBINATION OF POLYHERBAL COMPOUNDS NEPHTONE AND IMMUPLUS AGAINST OXIDATIVE STRESS IN LIVER AND KIDNEY DUE TO OCHRATOXIN IN BROILERS

M K SRIKANTH[1], M JEEVANA LATHA[2] AND A GOPALA REDDY[1]

Department of [1]Pharmacology & Toxicology and [2]Pathology,
College of Veterinary Science, Rajendranagar, Hyderabad 500 030, India

ABSTRACT

The antioxidant potential of polyherbal compounds namely, nephtone and immuplus was assessed for therapeutic management of an experimental model of oxidative stress induced by ochratoxin, at a toxic level of 2.5 ppm in feed. A total of ninety sexed male broiler chicks (Cobb strain) of day old age were procured for the study. The chicks were randomly divided into six groups consisting of fifteen in each group. Group 1 is supplied with normal basal diet from day 1 up to 42nd day. Group 2 is administered with nephtone@ 1.2 ml / 15 birds for 1 – 2 weeks, 2.4 ml / 15 birds for 3 – 4 weeks and 4.8 ml / 15 birds for 5 – 6 weeks. Group 3 was given Immuplus@ 75mg / 15 birds for 1 – 4 weeks, 150mg / 15 birds for 5 – 6 weeks. Group 4 was administered with Ochratoxin@ 2.5 ppm in feed for 42 days. Group 5 was given Ochratoxin@ 2.5 ppm for first 28 days followed by basal diet from 29 – 42 days. Group 6 birds received ocharatoxin@ 2.5 ppm upto 42 days from day one along with a combination of nephtone and immuplus dosage as mentioned group 2 and group 3 respectively. Antioxidant defense profile (GSH-Px, GSH-R, catalase) was evaluated in blood and GSH, and TBARS was evaluated in liver. Serum parameters like ALT, BUN and total protein were evaluated as biomarkers of hepatic damage and kidney damage. Antioxidant enzyme levels and biochemical parameters were significantly altered in the ochratoxin toxic control groups. These parameters were normal in the controls (groups 1, 2, 3 and group 6 that was given immuplus therapeutically.

M K SRIKANTH
Department of Pharmacology & Toxicology College of Veterinary Science, Rajendranagar, Hyderabad 500 030, India

**Corresponding author*

INTRODUCTION

Mycotoxicosis represents one of the serious problems in poultry and other animals. Natural mycotoxicosis is often caused by exposure to a combination of mycotoxins especially under field conditions as a result of multiple grain sources in poultry feed manufacture. (1) Bueno*et al*(2)observed that among various mycotoxins, aflatoxin, ochratoxin, zearalenone, T-2 toxin, vomitoxin, citrinin and fumonisins are the most common toxins affecting poultry. Kidneys are the main target organs of OTA. OTA is known to affect multiple sites of the nephron; acute exposure mainly affects the proximal parts, while chronic exposure leads predominantly to damage of the proximal tubule. Epidemiological studies implicate OTA with endemic nephropathy, a progressive renal fibrosis, and tumours of the urinary tract. (3) Phenolic compounds act as antioxidants with mechanisms involving both free radical scavenging and metal chelation, and have been shown to be more effective antioxidants *in vitro* than vitamins E and C on molar basis. (4)Khopde*et al*(5) observed that *amla* (*Phyllanthusemblica*) was a more potent antioxidant than vitamin C. Eugenol and ursolic acid obtained from *Ocimum sanctum* have been shown to possess significant antioxidant effect. (6) Dillon *et al* (7) reported that garlic extracts act as potent antioxidants. Kamashi*et al* (8) observed that zinc (80 mg/kg feed) had prophylactic and therapeutic potential against the oxidative damage caused by salinomycin toxic dose (120 mg/kg feed). The bark extracts of *AnadenantheramacrocarpaBrenas*(*Fabaceae*), *Astromiumurundeuva* Engl. (*Anacardiaceae*), *Mimosa verrucosa*Benth (*Fabaceae*) and *Sideroxylonobturifolium* T.D. Penn. (*Sapotaceae*) were found effective in reducing the production of thiobarbituric acid reactive substances (TBARS). (9)

MATERIALS AND METHODS

i. Animals

A total of seventy five sexed male broiler chicks (Cobb strain) of day old age were procured for the study. The chicks were randomly divided into five groups consisting of fifteen in each group. The chicks were provided with feed and water *ad libitum* throughout the experiment. All the groups were maintained as per the following feeding schedule for 6 wks (42 days) in order to evaluate the protective effect of poly herbal compound immuplus against experimental ochratoxicosis in broilers.

ii. Experimental procedure

Group 1 received normal basal diet from day 1 to 42nd day. Group 2 received nephtone@ 1.2 ml / 15 birds for 1 – 2 weeks, 2.4 ml / 15 birds for 3 – 4 weeks and 4.8 ml / 15 birds for 5 – 6 weeks. Group 3 received Immuplus@ 75mg / 15 birds for 1 – 4 weeks, 150mg / 15 birds for 5 – 6 weeks. Group 4 was given Ochratoxin@ 2.5 ppm in feed for 42 days. Group 5 was given Ochratoxin@ 2.5 ppm for first 28 days followed by basal diet from 29 – 42 days. Group 6 birds received ocharatoxin@ 2.5 ppm upto 42 days from day one along with a combination of nephtone and immuplus dosage as mentioned group 2 and group 3 respectively.

iii. Collection of samples and Anti oxidant markers

The blood samples were drawn from wing vein on 28th day and 42nd day from the birds (identified by wing band numbers) in each group for assay of glutathione peroxidase (GSH-PX), glutathione reductase (GSH-R) and catalase. Serum samples were separated from the blood. These samples were used for the estimation of alanine aminotransferase (ALT), blood urea nitrogen (BUN), total proteins. The TBARS and glutathione (GSH) concentrations were estimated by using liver homogenate at the end of the 6th wk.

iv. Statistical analysis

The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 12.0. Differences between means were tested using Duncan´s multiple comparison test and significance was set at $P < 0.05$.

RESULTS

i. Gutathione peroxidase (gsh-px)

The GSH-P_x (units/ml) activity in the basal diet control (group 1) was 85.807 ± 2.479, which was significantly ($P < 0.05$) increased in the ochratoxin control groups 4, 5 and 10 to 136.050 ± 2.842, 136.148 ± 0.880 and 135.307 ± 0.799, respectively at the end of 4th wk. The values recorded in groups 2 and 3 were 82.565 ± 0.714 and 82.810 ± 0.402, respectively at the end of 4th wk and 84.217 ± 0.568 and 84.446 ± 2.244, respectively at the end of 6th wk and there was a significant ($P < 0.05$) difference when compared to basal diet control (group 1) at the end of 4th wk and also at the end of 6th wk (87.803 ± 1.823). Discontinuation of ochratoxin in group 5 after 4th wk did not normalize the activity of GSH P_x at the end of 6th wk (132.010 ± 1.811). In group 10, the activity of GSH P_x (105.204 ± 1.602) was decreased significantly ($P< 0.05$) when compared to their respective 4th wk values and that of ochratoxin control (group 4; 149.183 ± 0.868) (Table 1).

ii. Glutathione reductase (GSH-R)

The activity of GSH-R (units/ml) in the basal diet control (group 1) was 43.116 ± 1.762, which was significantly ($P < 0.05$) increased in ochratoxin control groups 4, 5 and 10 (89.802 ± 0.351, 88.707 ± 0.353 and 89.371 ± 0.994, respectively) at the end of 4th wk. The activity in groups 2 and 3 was 40.009 ± 1.333 and 39.296 ± 1.122, respectively at the end of 4th wk and 38.639 ± 1.475 and 38.016 ± 1.413, respectively at the end of 6th wk and there was a significant ($P< 0.05$) difference when compared to basal diet control (group 1) at the end of 4th wk and 6th wk (42.116 ± 1.188). In group 5, discontinuation of ochratoxin feed after 4th wk, showed a significant ($P < 0.05$) decrease when compared to its 4th wk value at the end of 6th wk (80.104 ± 1.128). Also in group 10, the activity of GSH-R (55.049 ± 0.680) was significantly ($P < 0.05$) decreased when compared to their respective 4th wk values and that of group 4 (102.244 ± 0.741) at the end of 6th wk (Table 1).

iii. Catalase

The activity of catalase (moles/sec) in basal diet control (group 1) was 2.474 ± 0.119, which was significantly ($P < 0.05$) increased in the ochratoxin control groups 4, 5 and 10 (5.837 ± 0.065, 5.896 ± 0.146 and 5.859 ± 0.067, respectively) at the end of 4th wk. The activity in groups 2 and 3 was 2.634 ± 0.134 and 2.600 ± 0.102, respectively at the end of 4th wk and 3.120 ± 0.101 and 3.077 ± 0.066, respectively at the end of 6th wk without any significant difference when compared to group 1 at the end of 4th and 6th wk (3.067 ± 0.087). In group 5, discontinuation of ochratoxin at the end of 4th wk did not normalize the activity of catalase at the end of 6th wk (5.529 ± 0.108). However, in group10, the activity of catalase was significantly ($P < 0.05$) reduced when compared with their respective 4th wk values and that of group 4 (6.695 ± 0.193) at the end of 6th wk (Table 1)

iv. Total protein

The concentration of total protein (g/dl) in the basal diet control (group 1) was 4.593 ± 0.150, which was significantly ($P<0.05$) decreased in the ochratoxin control groups 4, 5 and 10 to 2.053 ± 0.134, 2.056 ± 0.018 and 2.59 ± 0.0100, respectively at the end of 4th wk. The values recorded in groups 2 and 3 did not reveal any significant difference when compared to group 1 at the end of 4th wk and 6th wk. In group 5, following discontinuation of ochratoxin feed after 4th wk, the total protein concentration did not normalize at the end of the 6th wk (2.768 ± 0.089). In group 10, the concentration of total proteins was significantly ($P<0.05$) increased (3.924 ± 0.233) at the end

of 6th wk as compared to their respective 4th wk values and that of ochratoxin control (group 4) at the end of 6th wk (1.985 ± 0.013) (Table 2).

v. Alanine transaminase (ALT)

The activity of ALT (IU/L) in the basal diet control (group 1) was 16.989 ± 1.372, which was significantly (P < 0.05) increased in the ochratoxin control groups 4, 5 and 10 to 52.401 ± 2.167, 51.714 ± 2.907 and 48.051 ± 1.001, respectively at the end of 4th wk. The values recorded in groups 2 and 3 did not reveal any significant difference when compared to group 1 at the end of 4th and 6th wk. In group 5, discontinuation of ochratoxin feed after 4th wk did not normalize the ALT activity at the end of the 6th wk (44.971 ± 1.594). However, in group 10, the ALT activity was significantly (P<0.05) reduced (33.966 ± 0.439) as compared to the activity of ochratoxin control (group 4; 65.043 ± 1.976) at the end of 6th wk (Table 2).

vi. Blood urea nitrogen (BUN)

The concentration of BUN (mg/dl) in the basal diet control (group 1) was 7.687 ± 0.511, which was significantly (P < 0.05) lower when compared to ochratoxin control groups 4, 5 and 10 (16.051 ± 0.517, 15.921 ± 0.069 and 16.115 ± 0.130, respectively) at the end of 4th wk. The BUN concentration in therapeutic control groups 2 and 3 was 7.715 ± 0.084 and 7.754 ± 0.098, respectively at the end of 4th wk and 7.937 ± 0.097 and 7.982 ± 0.186, respectively at the end of 6th wk without any significant difference when compared to basal diet control (group 1). However, at the end of 6th wk in group 10, the levels of BUN were significantly (P<0.05) reduced (10.911 ± 0.081) when compared to their respective 4th wk values and ochratoxin control (group 4; 21.077 ± 0.326) at the end of 6th wk (Table 2).

vii. Reduced glutathione (GSH)

The GSH concentration (nmoles/g of protein) in the basal diet control (group 1) was 106.8256 ± 0.6108, which was significantly (P<0.05) decreased in groups 4, 5 and 10 to 61.5518 ± 0.3831, 68.2770 ± 0.5361 and 91.7136 ± 1.9914, respectively at the end of 6th wk. The therapeutic control groups 2 and 3 revealed a significant (P<0.05) increase in the activity (120.4052 ± 1.0283 and 117.9982 ± 0.4898, respectively) when compared to the basal diet control at the end of the 6th wk. Group 10 that were supplemented with nephtone, immuplus and nephtone + immuplus, respectively after 4th wk revealed an increase in GSH activity when compared to ochratoxin control (group 4) at the end of the 6th wk (Table 2).

vii. Tiobarbituric acid reactive substances (TBARS)

The TBARS activity (nmoles of MDA / g tissue) in the basal diet control (group 1) was 0.792± 0.10, which was significantly increased in groups 4, 5 and 10 to 1.414 ± 0.13, 1.350 ± 0.10 and 1.185 ± 0.15, respectively at the end of 6th wk. However, the therapeutic control groups 2 and 3 (0.719 ± 0.03 and 0.693 ± 0.03, respectively) did not reveal any significant difference when compared to the basal diet control at the end of 6th wk. (Table 3).

Table 1
GSH- Px, GSH-R, catalase activity in different groups of broiler chicks

S.NO.	Groups	GSH- Px		GSH-R		Catalase	
		4th week	6th week	4th week	6th week	4th week	6th week
1	BASAL DIET CONTROL (1-42d)	85.807 ±2.479^{bA}	87.803±1.823^{aB}	43.116±1.762^{bA}	42.116±1.188^{aB}	2.474±0.119^{bA}	3.067±0.087^{aB}
2	NEPHTONE CONTROL (1-42d)	82.565±0.714^{bA}	84.217±0.568^{aB}	40.009±1.333^{bA}	38.639±1.475^{aB}	2.634±0.134^{bA}	3.120±0.101^{aB}
3	IMMUPLUS CONTROL (1-42d)	82.810±0.402^{bA}	84.446±2.244^{aB}	39.296±1.122^{bA}	38.016±1.413^{aB}	2.600±0.102^{bA}	3.077±0.066^{aB}
4	OCHRATOXIN CONTROL (1-42d)	136.050±2.842^{cA}	149.183±0.868^{eB}	89.802±0.361^{eA}	102.244±0.741^{fB}	5.837±0.065^{cA}	6.695±0.193^{eB}
5	OCHRATOXIN (1-28d) + BASAL DIET (29-42d)	136.148±0.880^{cA}	132.010±1.811^{dB}	88.707±0.353^{eA}	80.104±1.128^{dB}	5.896±0.146^{cA}	5.259±0.108^{dB}
6	10. OCHRATOXIN (1-28d) + NEPHTONE + IMMUPLUS (29-42d)	135.307±0.799^{cA}	105.204±1.602^{cB}	89.371±0.994^{eA}	55.049±0.680^{cB}	5.859±0.067^{c}	4.116±0.057^{cB}

Table 2
Serum parameters in different groups of broiler chicks

S.NO.	Groups	ALT		BUN		Total Protein	
		4th week	6th week	4th week	6th week	4th week	6th week
1	BASAL DIET CONTROL (1-42d)	16.989±1.372^{bA}	24.925±1.553^{bB} A	7.687±0.511^{bA}	8.849±0.226^{aB}	4.593±0.150^{cA}	4.733±0.179^{aB}
2	NEPHTONE CONTROL (1-42d)	19.414±0.900^{bA}	23.048±2.562^{bB} A	7.715±0.084^{bA}	7.937±0.097 ^{aB}	4.530±0.274^{cA}	4.668±0.077^{aB}
3	IMMUPLUS CONTROL (1-42d)	17.230±0.844^{bA}	22.684±1.848^{bA}	7.754±0.098^{bA}	7.982±0.186^{aB}	4.498±0.279^{cA}	4.694±0.165^{aB}
4	OCHRATOXIN CONTROL (1-42d)	52.401±2.167^{cA}	65.043±1.976^{eA}	16.051±0.517^{cA}	21.077±0.326^{eB}	2.053±0.134^{bA}	1.985±0.013^{cB}
5	OCHRATOXIN (1-28d) + BASAL DIET (29-42d)	51.714±2.907^{cA}	44.971±1.594^{dA}	15.921±0.069^{cA}	16.306±0.103^{dB}	2.056±0.018^{bA}	2.768±0.089^{cB}
6	10. OCHRATOXIN (1-28d) + NEPHTONE + IMMUPLUS (29-42d)	48.051±1.001^{cA}	33.966±0.439^{cA}	16.115±0.130^{cA}	10.911±0.081^{cB}	2.059±0.100^{bA}	3.924±0.233^{dB}

Table 3
GSH and TBARS activity in liver of broiler chicks

S.NO.	Groups	GSH	TBARS
		6th week	6th week
1	BASAL DIET CONTROL (1-42d)	106.8256±0.6108^{g}	0.792±0.10^{a}
2	IMMUPLUS CONTROL (1-42d)	117.9982±0.4898^{h}	0.693±0.03^{a}
3	OCHRA TOXIN CONTROL (1-42d)	61.5518±0.3831^{a}	1.414±0.13^{b}
4	OCHRATOXIN (1-28d) + BASAL DIET (29-42d)	68.2770±0.5361^{b}	1.350±0.10^{b}
5	OCHRATOXIN + IMMUPLUS (1-42d)	90.3006±0.6102^{e}	1.294±0.17^{b}
6	10. OCHRATOXIN (1-28d) + NEPHTONE + IMMUPLUS (29-42d)	91.7136±1.9914^{ef}	1.185±0.15^{b}

DISCUSSION

The antioxidant defense profile has revealed a significant ($p<0.05$) increase in the activities of GSH-Px, GSH- R and catalase in the toxic controls (groups 4 and 5) at the end of 4th wk and the values of nephtone control group and immuplus control (group 3) was comparable to that of the basal diet control (group 1) at respective time intervals. In groups 10 which received nephtone and immuplus along with ochratoxin throughout the study, revealed the values more or less comparable to those of group 1 at respective observations. The concentration of GSH was significantly reduced and found lowest in the toxic controls (groups 3 and 4) at the end of the 6th wk. The activities of GSH-Px, GSH-R and catalase, and the concentration of GSH were determined as they form the components of antioxidant defense system, which effectively scavenges the reactive oxygen species (ROS) or free radicals. The findings of the present study indicated ongoing free radical induced damage in the biological system, which is a result of imbalance between ROS production and antioxidant defense with subsequent oxidative stress. (10)The prophylactic effect ofnephtone was studied by supplementing these agents along with oxidative stressor (ochratoxin) from day 1 to 42 revealed the antioxidant defenses (GSH-Px and catalase) comparable to those of basal diet, though the activity of GSH-R and concentration of GSH differed slightly compared to basal diet control but with a significant improvement compared to toxic controls. The thiobarbituric acid reacting substances (TBARS) activity assessed at the end of 6th wk revealed the highest value in toxic control group 4. These findings are in accordance with those of Kalendar*et al* (11), who noted raised malondialdehyde (MDA) levels in rats due to doxorubicin toxicity and observed that vit. E + Se supplementation could bring back the MDA levels to normal. Malondialdehyde (MDA) activity is an indicator of lipid peroxidation and membrane damage. Therefore, it was assessed at the end of 6th wk in liver tissue homogenate as an indicator of oxidative stress induced by ochratoxin. In the present study, the reduced TBARS activity in groups 2 and 3 suggest the anti-lipid peroxidation and membrane protectant actions of poly herbal formulations in the study. Hence, in the present study, either the revival of the antioxidant defense profile or maintenance of the antioxidant defenses could be attributed to the antioxidant properties of synergistic herbs in nephtone.

In this study, the ALT activity has revealed a significant increase in the toxic controls (groups 4 and 5) at the end of the 4th wk and the values of nephtone control (group 2) and immuplus control (group 3) was comparable to those of the basal diet control (group 1) at respective time intervals. Groups 6 which received a combination of nephtone and immuplus along with ochratoxin throughout the study, revealed slightly higher values when compared to those of group 1 but remained significantly lesser when compared to toxic controls (groups 3 and 4) at respective observations. These results are in accordance with the reports of Horovitz*et al*(12) and Kamashi*et al*(8), who reported similar findings in different models of stress injury. The prophylactic effect of nephtone and immuplus was studied by supplementing these agents along with stressor (ochratoxin) from day 1 to 42 (group 6) revealed slightly increased ALT activity comparable to that of basal diet control. Hence, in this study, the prophylactic and therapeutic effects of immuplus in stabilizing the ALT activity could be due to the antioxidant and membrane stabilizing potential of synergistic herbs on liver. The protein profile was studied by assessing total protein in the plasma of birds. The study revealed a significant decrease in the concentrations of total protein (groups 4 and 5) at the end of the 4th and 6th wk. The values of nephtone control (group 2) and immuplus control (group 3) was comparable to those of the basal diet control (group 1) at respective

time intervals. The concentrations of total proteins was assessed as they serve as indicators of hepatic damage and in such cases, lower concentration of proteins, albumins and globulins is seen, which could be attributed to the reduced capacity of the liver to synthesize them. Reduced hepatic synthesis is not unexpected because of potential of OTA to inhibit protein synthesis both *in vitro* and *in vivo*.

REFERENCES

1. Prakash GC, Nalini TS, Vijayasarathi SK and Umakantha B. Pathomorphological studies to evaluate the effect of combination of aflatoxin and ochratoxinA in broiler chickens. Indian Journal of Animal Sciences 72: 418-420 (2002).
2. Bueno DJ, Salvano M, Silva JD, Gonzalez SN and Oliver G.Mycotoxins : diagnosis and prevention in poultry. BoletinMicologico16 : 23-36, (2001)
3. Barisic, LadaRumora, JozsefPetnik, IvanaCepelak and TihanaZanicGrubisic.Ochratoxin A Induces apoptosis in LCC-PK1 cells via JNK and P38 MAPK Activation. CroaticaChemicaActa 78: 385-392, (2005)
4. Rice-Evans CA, Mller NJ and Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science 2 : 152-159, (1997).
5. 5.Khopde SM, Priyadarshini KI, Mohan H, Gawandi VB, Satav JG, YakhmiJV, Banavaliker MM, Biyani MK and Mittal JP. Characterizing the antioxidant activity of *amla*(*Phyllanthusemblica*) extract. Current Science 81 : 185-190, (2001).
6. 6.Balanehru S and Nagarajan B. Protective effect of oleanolic acid and livosolic acid against lipid peroxidation. Biochemistry International 24 : 981-990, (1991).
7. 7. Dillon S A, Lowe G M, Billington D and Rahman K. Dietary supplementation with aged garlic extract reduces plasma and urine concentrations of 8-isoprostaglandin F2X in smoking and non smoking men and women. Journal of Nutrition 132 : 168-171, (2002).
8. 8. Kamashi K, Reddy AG, Reddy KS and Reddy VR. Evaluation of zinc against salinomycin toxicity in broilers. Indian Journal of Physiology and Pharmacology48 : 89-95, (2004).
9. 9. Desmarchelier C, LisboaRomadR, Conssio J and Ciccia G. Antioxidant and free radical scavenging activities in extracts from medicinal trees used in the 'Caatinga' region in northeastern Brazil. Journal of Ethnopharmacology67 : 69-77, (1999).
10. 10. Bandyopadhyay U, Das D and Banerjee RK. Reactive oxygen species : oxidative damage and pathogenesis. Current Science 77 : 658-665, 199
11. 11.Kalendar Y, Yel M and Kalendar S. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats : The effects of vitamin E and Catechin. Toxicology209 : 39-45, (2005).
12. 12. Horovitz CT, Avidar V, Bogin E, Schloesbeng A, Shkap I, Weisman Y and Eayed MN. Enzyme profile in blood and tissues of chicken fed various levels of Monensin. Journal of Veterinary Medicine 35 : 473-480, (1998).