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Toxicol. Int.Vol. 16, No. 1, 2009 pp 63-68

Oxidative stress due to ochratoxin and T-2 toxin either alone or in combination and evaluation of protective role of *Curcuma longa, Zingiber officinale*, toxichek and activated charcoal

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Abstract

A study was conducted on poultry to evaluate the oxidative stress due to ochratoxin and T-2 either alone or in combination. Broiler chicks were fed OTA @2ppm,T-2 @ 1ppm, *Zingiber officinale* (rhizome shade dried and powdered @ 0.5%, *Curcuma longa* rhizome @ 0.5%, toxichek @ 0.1% and activated charcoal @ 0.4% in feed in combinations of 20 groups. The study revealed a significant ($p < 0.05$) increase in the activities of glutathione peroxidase, glutathione reductase, catalase and TBARS in toxic groups (OTA, T-2 and OTA+T-2). Results of the present study suggest that OTA and T-2 either alone or in combination induce oxidative stress and supplementation of *Curcuma longa*, *Zingiber officinale*, toxichek and activated charcoal in order showed amelioration by stabilizing the antioxidant defenses.

Keywords: Activated charcoal (AC), ochratoxin (OTA), trichothecene (T-2).

Introduction

Mycotoxins can generate free radicals and cause lipid peroxidation with subsequent damage to the biological membranes, proteins and DNA. This could lead to apoptosis and other cytotoxic effects. Ochratoxin A (OTA) is a naturally occurring mycotoxin produced by *Aspergillus* fungi and experimental feeding of OTA has been shown to have detrimental effect on growing chicks (Raju and Dewagowda, 2000). The T-2 toxin is produced by several species of the genus *Fusarium* that are found in many cereals, feeds and vegetables. Research has shown that T-2 toxin causes reduction in feed consumption and weight gain (Huff *et al*., 1988). Several adsorbents were tried to reduce the incidence uses of mycotoxicoses, but studies regarding the use of herbs to counteract mycotoxicoses are limited. Many plants are known to exhibit antioxidant potential. *Curcuma longa* (turmeric), a member of *Zingiberaceae*, has number of biological activities (Joe *et al.*, 2004). *Zingiber officinale* (ginger), belonging to family *Zingiberaceae*, is one of the most widely used spices and has antimicrobial, antioxidant, antitumour and platelet aggregation activities (Youkosekiwa *et al.,* 2000). Activated charcoal (AC) is known to be the most economical adsorbent used against mycotoxicoses (Edrington *et al*., 1997). Polyherbal premix is largely used in industry to combat mycotoxicosis. A herbal premix toxichek (containing *Azadiractha indica*, *Trachispermum ammi* and *Andrographis paniculata*) was used for the study. It is not unusual to use multiple grain sources in poultry and livestock diets. Thus, the possibility exists for feeding diets contaminated with both OTA and T-2 toxin. Therefore, this study was planned to investigate the oxidative stress in broiler chicks fed with diets containing OTA @ 2ppm and T-2 @1ppm singly and in combination from day one to six weeks of age (42nd day), and to evaluate certain herbs and adsorbents to prevent the toxicity.

Materials and Methods

A total of three hundred sexed male broiler chicks (Cobb strain) of day old age were procured for the study. The chicks were randomly divided

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into twenty groups consisting of fifteen in each group. All the groups were maintained as per the following feeding schedule for 6 weeks (42 days)

The blood samples were drawn from wing vein of birds on 28th day (4th week) and 42nd day (6th week) for assay of glutathione peroxidase (GSH-PX) (Paglia and Valentine, 1967) glutathione reductase (GSH-R) (Raghuramulu, 1983) and catalase (Britton and Machlly, 1956). The TBARS (Subramaniam *et al*., 1988) estimation was done by using liver homogenate at the end of the 6th week. Brooding was done for 3 weeks using incandescent bulbs. Feed and water was provided *ad libitum*. Feeding was done in linear trough feeders. Water was supplied through the system of nipple drinkers.T-2 toxin was produced by growing *Fusarium sporotrichoides* (*F. tricintum*) NRRL 3299 strain on broken wheat using Burmeister (1971) method on broken wheat soaked with 35 ml of water and autoclaved at (15 Psi, 121°C, 20 min). Broken wheat was inoculated with spore suspension made from culture on oat meal agar. The broken wheat was incubated for 6 weeks by shaking twice a day. The fungus was killed by autoclaving the broken wheat (15 Psi, 121°C, 20 min). Fermented broken wheat was dried, powdered and stored in a dark place. The concentration of T-2 toxin in culture was estimated by TLC according to Romer *et al*. (1978). Ochratoxin A (OTA) was produced from *Aspergillus ochraceus* obtained from IMTC (Institute of Microbiological Technology, Chandigarh). The culture was grown on oat meal agar slants at 28°C for 2 weeks. Flaked wheat was inoculated with a loopful of *A. ochraceus*. The flasks were incubated in dark at room temperature (28-32°C) for 14 days shaking twice a day. Later, at the end of incubation time, fungus was killed by autoclaving (15 psi, 121°C, 15 min) and dried overnight at 50°C in hot air oven, and powdered. Ochratoxin was extracted and quantified using column chromatography and TLC, respectively as per AOAC (1995). The results were analyzed statistically using one way ANOVA, followed by Duncan's multiple test (Snedecor and Cochran, 1994).

Group	Treatment
1.	Basal diet control (1-42 days)
2.	Ginger control (@ 0.5% in feed) (1-42 days)
3.	Curcuma control (@ 0.5% in feed) (1-42 days)
4.	Toxichek control (@0.1% in feed) (1-42days)
5.	AC @ 0.4% in feed (1-42 days)
6.	AC @ 0.4% in feed (1-42 days)
7.	OTA (@2ppm) + ginger (@0.5%in feed (1-42days)
8.	OTA(@2 ppm in feed) + Curcuma (@0.5%)in feed (1-42days)
9.	OTA(@2 ppm in feed) + Toxicheck (@0.1%) in feed (1-42 days)
10.	OTA(@2 ppm in feed) +AC(@ 0.4%) in feed (1-42days)
11.	T-2 toxin (@ 1 ppm in feed) (1-42 days)
12.	T-2 toxin (@ 1 ppm in feed) + ginger (@ 0.5%) in feed (1-42days)
13.	T-2 toxin (@ 1 ppm in feed) + Curcuma @0.5% in feed (1-42days)
14.	T-2 toxin (@ 1ppm in feed) +Toxicheck @0.1% in feed (1-42days)
15.	T-2 toxin (@ 1 ppm in feed) +AC(@0.4%) in feed (1-42days)
16.	T-2 toxin(@1 ppm in feed)+AC(@ 0.4%) in feed (1-42days)
17.	OTA (@2ppm in feed) +T-2 toxin (@1ppm in feed) + Ginger @ 0.5% in feed (1-42days)
18.	OTA (@2ppm in feed) + T-2 toxin (@ 1ppm in feed) + Curcuma @ 0.5% in feed (1-42days)
19.	OTA (@2ppm in feed) + T-2 toxin (@1ppm in feed) + Toxicheck @ 0.1% in feed (1-42days)
20.	OTA (@ 2ppm in feed) + T-2 toxin (@1ppm in feed) + AC @0.4% in feed (1-42days)

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Results and discussion

Mycotoxins generate excess of free radicals in the form of reactive oxygen species (ROS) or lipid peroxides leading to antioxidant / perooxidant imbalance causing oxidative stress with subsequent lipid peroxidation and damage to the biological molecules. In the present study, the activities of GSH-PX, GSH-R, TBARS and catalase in groups 6, 11 and 16 were increased significantly (Table 1-4), which is attributed to increased free radical production. These results are in accordance with Hoehler and Donald (1996). Treatment with *Curcuma* reduced the concentration of antioxidant enzymes in groups 8, 13 and 18 as compared to the toxic control groups 6, 11 and 16. This effect is attributed to its antioxidant potential, which is in accordance with Venkatesan (1998) and Venkatesan *et al*. (2000). Curcumin, the active principle in *Curcuma*, has been identified as a potent inducer of haemoxygenase-1 (HO-1), a redox sensitive protein that provides protection against various forms of stress (Balogun *et al*., 2003). Treatment with AC also showed amelioration in groups 10, 15 and 20. The observed effectiveness of charcoal suggests that OTA and T-2 are better adsorbed and therefore becomes unavailable for gastrointestinal absorption. The results of the present study showed that treatment with ginger exhibited better amelioration in groups 7, 12 and 17 compared to *Curcuma* and PHC. Ginger scavenges super oxide and hydroxyl radicals *in vitro* (Cao *et al*., 1993) and inhibits lipid peroxidation (Zhouy and Xu, 1992). Treatment with PHC also showed amelioration in groups 9, 14 and 19 similar to *Curcuma* when compared to toxic control groups. Results of the study indicate that amelioration is due to normalizing the activities of antioxidant enzymes by the herbs. The beneficial effect of toxichek is attributed to the synergistic herbs present in the preparation namely, *Azadirachta indica* (Satish *et al*., 2007), *Tachyspermum ammi* (Singh and Singh, 2005) and *Andrographis paniculata* (Rukshamani *et al*., 2007).

Studies by Hasinoff *et al*. (1990) and Omar *et al*. (1990) suggested that OTA-iron chelate catalyses the formation of ROS and this complex stimulates lipid peroxidation in the presence of NADPH-cytochrome P-450 reductase system. T-2 toxins have also shown to stimulate lipid peroxidation in rat liver along with depletion of hepatic glutathione (GSH) (Rizzo *et al*., 1994). Hoehler and Donald (1996) reported that T-2 stimulates lipid peroxidation due to increased free radical generation. Further, they are lipid soluble and therefore can cross cell membranes and subsequently induce lipid peroxidation.

Treatment with *Curcuma* showed lower levels of TBARS in treated groups compared to its corresponding toxic controls (6, 11 and 16) and the results are in accordance with the report of Asai and Miyazawa (2001), who examined the effect of curcumin on lipid peroxidation in rats. Naidu and Thippeswamy (2002) showed the effect of curcumin on copper-iron-induced lipid peroxidation of human LDL and found 40 to 80% inhibition of LDL oxidation. Further, curcumin significantly inhibited both initiation and propagation phases of LDL oxidation. Dietary curcuminoids have lipid lowering potency due to alterations in fatty acid metabolism. Treatment with toxichek showed good amelioration in groups 9, 14 and 19 compared to individual agents indicating that combination of herbs had synergistic antioxidant potential in lowering the levels of lipid peroxidation. Treatment with AC also showed reduced levels, but with lesser efficacy compared to other treatments, suggesting that AC is a good adsorbent but has no biological activity. Supplementation of diet with ginger showed significantly reduced levels of TBARS as compared to toxic control groups 6, 11 and 16. These results are in accordance with the report of Ahmed *et al*. (1999), who studied the influence of dietary ginger on oxidative stress induced by malathion in rats. Results of ginger supplementation suggest that ginger scavenges ROS by maintaining the levels of antioxidant enzymes and its beneficial effects may be attributed to the zingerone components present in ginger.

In conclusion, the results of present investigation enunciated that OTA/T-2 and their combination exerted toxicity by generation of free radicals (ROS). Supplementations of *Curcuma*, Toxichek and ginger countered the adverse effects of OTA+T-2 toxicity, while AC has lower amelioration effect compared to herbs in study.

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Table 1: Glutathione peroxidase (GSH-Px) activity (units/ml) in different groups of broiler chicks

S. No.	Group	4th week	6th week
1	Basal diet (1 - 42 d)	53.45 ±0.64^{m}	57.40 ±0.39^{n}
2	Ginger (1 - 42 d)	47.34 ±0.36^{j}	53.27 ±0.17^{l}
3	Curcuma (1 - 42 d)	48.20 ±0.44^{k}	52.30 ±0.43^{k}
4	PHC (1 - 42 d)	48.21 ±0.07^{k}	51.14 ±0.17^{j}
5	AC (1 - 42 d)	51.72 ±0.64^{l}	54.60 ±0.28^{m}
6	OTA (1 - 42 d)	61.71 ±0.16^{o}	63.21 ±0.27^{p}
7	OTA + Ginger (1 - 42 d)	38.09 ±0.57^{b}	41.18 ±0.71^{d}
8	OTA + Curcuma (1 - 42 d)	41.84 ±0.30^{e}	43.76 ±0.17^{e}
9	OTA + PHC (1 - 42 d)	40.75 ±0.12^{c}	40.98 ±0.19^{d}
10	OTA + AC (1 - 42 d)	38.34 ±0.14^{b}	39.33 ±0.28^{c}
11	T - 2	60.54 ±0.19^{r}	61.03 ±0.02^{o}
12	T-2 + Ginger (1 - 42 d)	41.55 ±0.21d^{e}	45.77 ±0.33^{g}
13	T-2 + Curcuma (1 - 42 d)	44.85 ±1.14^{h}	50.02 ±0.75^{i}
14	T-2 + PHC (1 - 42 d)	46.08 ±0.02^{i}	48.14 ±0.45^{h}
15	T-2 + AC (1 - 42 d)	31.29 ±0.16^{a}	32.27 ±0.18^{a}
16	OTA + T-2 (1 - 42 d)	64.36 ±0.15^{p}	67.08 ±0.30^{q}
17	OTA + T-2 + Ginger (1 - 42 d)	40.91 ±0.55^{cd}	44.02 ±0.74^{e}
18	OTA + T-2 + Curcuma (1 - 42 d)	42.51 ±0.37^{f}	45.02 ±0.28^{fg}
19	OTA + T-2 + PHC (1 - 42 d)	43.55 ±0.63^{g}	44.26 ±0.17^{ef}
20	OTA + T-2 + AC (1 - 42 d)	34.08 ±0.07^{b}	35.05 ±0.83^{b}

Values are mean + SE of 8 observations;
Means with different alphabets as superscripts differ significantly (P<0.05) ANOVA

Table 2: Catalase activity (moles/sec) in different groups of broiler chicks

S. No.	Group	4th week	6th week
1	Basal diet (1 - 42 d)	2.08 ±0.01^{c}	2.39 ±0.02^{c}
2	Ginger (1 - 42 d)	1.96 ±0.01^{a}	2.31 ±0.01^{b}
3	Curcuma (1 - 42 d)	2.03 ±0.04^{b}	2.19 ±0.03^{a}
4	PHC (1 - 42 d)	2.02 ±0.02^{b}	2.18 ±0.05^{a}
5	AC (1 - 42 d)	2.15 ±0.01^{d}	2.32 ±0.03^{b}
6	OTA (1 - 42 d)	2.99 ±0.03^{j}	3.27 ±0.02^{l}
7	OTA + Ginger (1 - 42 d)	2.59 ±0.06^{fg}	2.70 ±0.01^{gh}
8	OTA + Curcuma (1 - 42 d)	2.44 ±0.02^{eg}	2.57 +0.02^{e}
9	OTA + PHC (1 - 42 d)	2.60 ±0.08^{fg}	2.62 ±0.02^{f}
10	OTA + AC (1 - 42 d)	2.65 ±0.01^{h}	2.73 ±0.02^{hi}
11	T - 2	2.94 ±0.04^{j}	3.05 ±0.01^{k}
12	T-2 + Ginger (1 - 42 d)	2.42 ±0.01^{fe}	2.51 ±0.02^{d}
13	T-2 + Curcuma (1 - 42 d)	2.24 ±0.06^{e}	2.38 ±0.01^{c}
14	T-2 + PHC (1 - 42 d)	2.39 ±0.02^{f}	2.56 ±0.02^{e}
15	T-2 + AC (1 - 42 d)	2.43 ±0.08^{fe}	2.73 ±0.06^{hi}
16	OTA + T-2 (1 - 42 d)	3.06 ±0.01^{k}	3.45 ±0.04^{m}
17	OTA + T-2 + Ginger (1 - 42 d)	2.89 ±0.01^{i}	2.90 ±0.02^{j}
18	OTA + T-2 + Curcuma (1 - 42 d)	2.43 ±0.02^{eg}	2.56 ±0.06^{e}
19	OTA + T-2 + PHC (1 - 42 d)	2.64 ±0.01^{h}	2.68 ±0.02^{g}
20	OTA + T-2 + AC (1 - 42 d)	2.89 ±0.01^{i}	2.89 ±0.02j

Values are mean + SE of 8 observations
Means with different alphabets as superscripts differ significantly (P<0.05) ANOVA

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Table 3: TBARS activity (n moles MDA/mg protein) in different groups of broiler chicks

S. No.	Group	6th week
1	Basal diet (1 - 42 d)	0.58 ± 0.03^{a}
2	Ginger (1 - 42 d)	0.66 ± 0.02^{bcd}
3	Curcuma (1 - 42 d)	0.63 ± 0.01^{ab}
4	PHC (1 - 42 d)	0.65 ± 0.01^{bc}
5	AC (1 - 42 d)	0.68 ± 0.01^{cde}
6	OTA (1 - 42 d)	0.94 ± 0.01^{l}
7	OTA+Ginger (1 - 42 d)	0.88 ± 0.02^{jk}
8	OTA+Curcuma (1 - 42 d)	0.80 ± 0.02^{g}
9	OTA+PHC (1 - 42 d)	0.82 ± 0.01^{gh}
10	OTA+AC (1 - 42 d)	0.87 ± 0.01^{ijk}
11	T - 2	0.85 ± 0.01^{hijk}
12	T-2+Ginger (1 - 42 d)	0.73 ± 0.01^{f}
13	T-2+Curcuma (1 - 42 d)	0.67 ± 0.01^{bcd}
14	T-2+PHC (1 - 42 d)	0.70 ± 0.02^{def}
15	T-2+AC (1 - 42 d)	0.71 ± 0.01^{ef}
16	OTA+T-2 (1 - 42 d)	1.89 ± 0.01^{m}
17	OTA +T-2 + Ginger (1 - 42 d)	0.85 ± 0.01^{hij}
18	OTA+T-2+Curcuma (1 - 42 d)	0.82 ± 0.01^{gh}
19	OTA+T-2+PHC (1 - 42 d)	0.83 ± 0.01^{ghi}
20	OTA+T-2+AC (1 - 42 d)	0.90 ± 0.01^{k}

Values are mean + SE of 8 observations
Means with different alphabets as superscripts differ significantly ($P<0.05$) ANOVA

Table 4: GSH-R activity (n moles/g of protein) in different groups of broiler chicks

S. No.	Group	4th week	6th week
1	Basal diet (1 - 42 d)	23.02 ± 0.27^{b}	27.22 ± 0.74^{a}
2	Ginger (1 - 42 d)	22.30 ± 0.20^{b}	30.81 ± 0.79^{b}
3	Curcuma (1 - 42 d)	29.35 ± 1.84^{c}	35.44 ± 0.12^{d}
4	PHC (1 - 42 d)	31.38 ± 0.18^{d}	34.58 ± 0.13^{c}
5	AC (1 - 42 d)	19.95 ± 0.62^{a}	26.74 ± 0.60^{a}
6	OTA (1 - 42 d)	60.34 ± 0.02^{n}	66.54 ± 0.06^{m}
7	OTA+Ginger (1-42 d)	39.24 ± 0.06^{f}	41.10 ± 0.26^{g}
8	OTA+Curcuma (1-42 d)	40.44 ± 0.11^{g}	43.58 ± 0.16^{h}
9	OTA+PHC (1-42 d)	40.35 ± 0.15^{g}	43.04 ± 0.03^{h}
10	OTA+AC (1-42 d)	43.13 ± 0.05^{j}	46.02 ± 0.01^{j}
11	T - 2	49.11 ± 0.05^{m}	52.95 ± 0.01^{l}
12	T-2+Ginger (1-42 d)	39.47 ± 0.15^{f}	40.85 ± 0.31^{fg}
13	T-2+Curcuma (1-42 d)	38.13 ± 0.03^{e}	39.32 ± 0.10^{e}
14	T-2+PHC (1-42 d)	38.48 ± 0.15^{e}	40.13 ± 0.01^{f}
15	T-2+AC (1-42 d)	45.37 ± 0.16^{j}	45.36 ± 0.07^{j}
16	OTA+T-2 (1-42 d)	66.02 ± 0.02^{o}	70.66 ± 0.03^{n}
17	OTA+T-2+Ginger (1-42 d)	43.56 ± 0.22^{k}	44.31 ± 0.34^{i}
18	OTA+T-2+Curcuma (1-42 d)	41.50 ± 0.35^{h}	43.17 ± 0.05^{h}
19	OTA+T-2+PHC (1-42 d)	42.39 ± 0.16^{i}	43.54 ± 0.08^{h}
20	OTA+T-2+AC (1-42d)	48.19 ± 0.05^{l}	49.19 ± 0.07^{k}

Values are mean + SE of 8 observations
Means with different alphabets as superscripts differ significantly ($P<0.05$) ANOVA

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Received : 10.09.2008
Accepted: 15.11.2008