Antioxidant Potential of Sea Buckthorn (*Hippophaer hamnoides*) and Glucomannan on T-2 Toxin-induced Oxidative Stress in Poultry

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Received: March 2024 *51/24* *Accepted: November 2024*

ABSTRACT

This study was undertaken to investigate the antioxidative effect of the sea buckthorn berries (*Hippophae rhamnoides*) and glucomannan (a mycotoxin binder) against T-2 toxin-induced oxidative stress. The T-2 toxin used was produced by growing *Fusarium sporotrichioides* and added at one ppm. Sea buckthorn berries were mixed in the feed at 400 and 800 ppm. The glucomannan was added at 1g per kg feed. The T-2 toxin significantly ($P < 0.05$) increased the lipid peroxidation in the liver and RBCs and catalase in RBCs. A significant ($P < 0.05$) reduction of GSH in the liver and RBCs was observed in the toxin group. However, the supplementation of sea buckthorn and glucomannan significantly ($P < 0.05$) restored the T-2 toxin-induced oxidative stress parameters in poultry.

Keywords: Sea buckthorn, glucomannan, T-2 toxin, poultry.

INTRODUCTION

Trichothecenes are the secondary fungal metabolites originating especially from Fusarium moulds that contaminate almost all crops before harvesting. The 12,13-epoxy trichothecenes, such as T-2 toxin, occur widely in nature, especially in contaminated grains (WHO, 1990). T-2 toxin inhibits DNA, RNA and protein synthesis in eukaryotic cells, which affects the cell cycle and induces apoptosis both in vivo and in vitro (Sudakin, 2003). T-2 toxin increases the rate of lipid peroxidation in the liver and lowers the activity of the glutathione redox system (Rizzo *et al*., 1994). However, it is not clear whether the T-2 toxin accelerates the formation of oxygen-free radicals or impairs the activity of the antioxidant defence system.

The effective methods of mycotoxins detoxification include herbal mould inhibitors, chemical detoxification and application of mineral clays (McDaniel, 1991). The fruits of sea buckthorn (*Hippophae rhamnoides*) are used as a source of herbal medicine, health food and for natural skin care in Europe and Asia. The people use sea buckthorn berries for the treatment of hypertension, digestive system, skin diseases and erosion of the uterus and inflammation of genital organs (Geetha and Asheesh, 2011).

A previous study reported that the sea buckthorn berries contain carotenoids, progesin, flavoxanthin, cryptoxanthin, violaxanthin, neoxanthin, vitamin C, vitamin K and vitamin E (Chauhan and Varshneya, 2012). The alcoholic extract of whole berries of sea buckthorn inhibited the Fenton reaction and also radiation-induced hydroxyl and nitro blue tetrazolium reduction, generating superoxide radicals mediated peroxidation of liver (Goel *et al*., 2002).

Modified glucomannan supplementation is beneficial in reducing the individual and combined adverse effects of aflatoxin, ochratoxin and T-2 toxin in broilers on the body weights, antibody titres, serum

biochemical and haematological parameters (Raju and Devegowda, 2000). The sea buckthorn protected the T-2 toxin-induced immunosuppression (Ramasamy *et al.*, 2010). However, the ability of sea buckthorn berries (Hippophae rhamnoides) and glucomannan to alleviate the adverse effects of T-2 toxin present in feed on oxidative stress remains to be explored.

MATERIALS AND METHODS

Seventy numbers of day-old broiler chicks (30 – 40g) were procured from a hatchery (Uttam Poultry Breeding Farm, Ghurkari, Kangra, H.P) and housed in battery brooders with ad libitum supply of feed and water. They were randomly distributed into seven groups, as below:

Group	Treatment	No. of Birds
I	Control	10
II	T-2 toxin	10
III	T-2 toxin + Glucomannan (GM)	10
IV	T-2 toxin + Seabuckthorn (SBT) 400 ppm	10
V	T-2 toxin + Seabuckthorn 800 ppm	10
VI	T-2 toxin + Seabuckthorn 400 ppm + Glucomannan	10
VII	T-2 toxin + Seabuckthorn 800 ppm + Glucomannan	10

The T-2 toxin was produced in the wheat using Fusarium sporotrichioides MTCC 2081 and quantified by using thin-layer chromatography (Tapia, 1985). The experiments were performed in accordance with the guidelines of the Institutional Animal Ethical Committee of the College of Veterinary and Animal Sciences, CSKHP Agricultural University, Palampur, Himachal Pradesh, India. Broiler mash fed to chickens in this study was tested at the Animal Feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute, Namakkal-637 001, Tamil Nadu (India) to ascertain that the feed was free of aflatoxins and T-2 toxin. The ripe berries of sea buckthorn were collected from the Agricultural Research Extension Centre, Kukumseri, Lahaul (Himachal Pradesh), India. The berries were shade-dried and ground to obtain a fine powder. The purified glucomannan powder was procured from the Neospark, Drugs and Chemicals Private Limited, Hyderabad—500 082 Andhra Pradesh, India.

The T-2 toxin culture material, glucomannan, and sea buckthorn berry powder were incorporated into the broiler mash at 1 ppm, 1g per kg feed, and 400 or 800 ppm, respectively, for 28 days. The birds were vaccinated against Newcastle disease (NDV) at 7 and 14 days of age by intraocularly administering one drop (106 EID50/bird) of Newcastle disease virus Lasota strain vaccine (Venkeys Biological Limited, India).

Antioxidative Parameters

Estimation of different oxidative stress-related biochemical profiles in erythrocytes and liver was carried out. Absorbance of all the estimations was read, using a single beam VIS and UV- VIS Spectrophotometer (UV 5704 SS, ECIL, India).

Oxidative Status in Erythrocytes

The erythrocyte lysate was used for the estimation of different oxidative stress-related parameters. The lipid peroxidation in erythrocyte lysate was measured in terms of malondialdehyde (MDA)production by the method of Shafiq-Ur-Rehman (1984). The reduced glutathione (GSH) was assayed by the 5,5’ dithiobis (2-nitrobenzoic acid) (DTNB)

method of Prins and Loos (1969). The catalase activity was estimated in erythrocytes by the spectrophotometric method as described by Bermeyer (1983).

Oxidative Status in Liver

Preparation of Tissue Homogenate

The 10% liver homogenate was prepared by trituration in a mortar and pestle to determine the antioxidant parameters. The extent of lipid peroxidation was expressed in terms of MDA (malondialdehyde) production and determined by the Thio Barbituric Acid (TBA) method of Shafiq-Ur-Rehman (1984). GSH was assessed by estimating free-SH groups, using DTNB method of Sedlak and Lindsay (1968).

Statistical Analysis

The data were analyzed using Graph Pad Instat version 6.00 for windows (Graph Pad Software, San Diego, California, USA, and www. Graphpad.com.) and the significant differences between mean values were determined using Tukey – Kramer Multiple comparisons test. The comparison was made at 5% levels of significance.

RESULTS

Estimation of oxidative stress-related biochemical profile was carried out in erythrocytes and liver tissues on day 28 of post-treatment. A significant ($P < 0.05$) increase in erythrocytic lipid peroxidation was observed in birds treated with T-2 toxin alone as compared to healthy controls. However, there was a significant ($P < 0.05$) decrease in the lipid peroxidation in all other treatment groups as compared to the T-2 toxin-treated group. The group treated with SBT 800 ppm plus GM plus toxin revealed ($P < 0.05$) reduced values of the lipid peroxidation in red blood cells when compared to those in the healthy control group.

The significant ($P < 0.05$) decrease in reduced glutathione was noted in RBCs of the T-2 toxin-treated group as compared to healthy controls. The significant ($P < 0.05$) increase in reduced glutathione was observed in the rest of the treatment groups except the toxin plus GM treated group when compared with the toxin alone treated group. The birds treated with SBT 800 ppm plus GM plus toxin revealed significantly ($P < 0.05$) increased values of the reduced glutathione in RBCs when compared to the healthy control group.

A significant ($P < 0.05$) increase in the catalase activity was also observed in erythrocytes of the T-2 toxin-fed group when compared with those in the healthy controls. Whereas the significantly ($P<0.05$) decreased catalase activity in erythrocytes was observed in the rest of the treatment groups, except toxin plus GM, when compared with the toxin alone-fed group. Also, the birds treated with SBT 800 ppm plus GM plus toxin revealed significantly ($P<0.05$) decreased catalase activity in erythrocytes as compared to the healthy control birds. The results were presented in Figures 1, 2 and 3.

The value of MDA for lipid peroxidation and reduced glutathione in liver tissues is presented in Figures 4 and 5, respectively. The significant ($P<0.05$) increase in the values of MDA for lipid peroxidation was recorded in the liver tissue of T-2 toxin-treated birds when compared with the healthy controls. However, in all other treatment groups, there was a significant ($P < 0.05$) reduction in the lipid peroxidation in liver tissue as compared to the toxin-treated group birds. The birds treated with SBT 800 ppm plus GM plus toxin revealed significantly ($P < 0.05$) reduced values of the LPO as compared to the healthy controls and the rest of the treatment groups.

The reduced glutathione was significantly ($P < 0.05$) decreased in the toxin-treated group as compared to the healthy control. However, the significant ($P < 0.05$) increase in reduced glutathione was seen in the birds treated with

toxin plus SBT 800 ppm level, toxin plus SBT 400 ppm plus GM and toxin plus SBT 800 ppm plus GM when compared with the T-2 toxin-treated group. The significant ($P<0.05$) increase in the reduced glutathione was also observed in birds treated with toxin plus SBT 800 ppm plus GM group when compared to those in the healthy control group.

DISCUSSION

Many of the environmental toxicant induces oxidative damage, leading to cellular damage, in animals and humans. The oxidative damage caused by the feeding of T-2 toxin-contaminated diets could be alleviated by supplementation with sea buckthorn and glucomannan (toxin binder), and simultaneous use of both provided the best results. In the present study, a significant increase in lipid peroxidation and catalase activity and a decrease in reduced glutathione were observed in erythrocytes following T-2 toxin treatment. In the liver, a significant increase in lipid peroxidation and a decrease in reduced glutathione were observed in the T-2 toxin alone-treated group. This indicates the adverse effect of T-2 toxin on antioxidant status in liver and erythrocytes, which reduces the efficiency of the antioxidant defence in the biological system in broiler chicken (Mezes *et al.*, 1998). The significant increase in the LPO in RBC and liver in the present study may be due to the generation of free radicals by T-2 toxin metabolite, causing disturbances in the structure of cellular membranes (Karppanen *et al.*, 1989). Suneja *et al.* (1989) had also shown an increase in the level of liver lipid peroxides in rats fed with T-2 toxin. Lipid peroxidation is one of the factors responsible for the damage and necrosis of the liver, induced by chemical compounds like mycotoxins. Because biological membranes are rich in unsaturated fatty acids, the susceptibility of membranes to peroxide attack occurs frequently.

The reduced glutathione (GSH) status is an important factor involved in the protection against lipid peroxidation, and its concentration is maintained in part through the enzymes participating in its metabolism. GSH is also responsible for the maintenance of the redox status of the cell (Sies, 1999). So, the significant decrease of reduced glutathione in the present study may also be the reason for increased LPO. In male broiler chicks, the hepatic GSH concentration has been reported to decrease after 7 days of oral administration of T-2 toxin (Leal *et al.*, 1999).

When male rats were administered a single dose of DON or T-2 toxin orally, there was a significant decrease in the activity of catalase (Rizzo *et al.*, 1994). These results are contrary to the present results and might be due to differences in catalase metabolism between mammalian species and poultry.

The natural absorbent (glucomannan) has been reported to decrease the detrimental effect of T-2 toxin (Raju and Devegowda, 2000 and Freimund *et al.*, 2003). A significant reduction in the lipid peroxidation in the liver and RBCs of broiler chicken was observed in the present study. However, a non-significant reduction of catalase and an increase in GSH in RBCs and a significant increase in GSH in liver tissues were observed after oral administration of glucomannan in broiler chicken (Weber *et al.*, 2006).

The sea buckthorn at different dietary levels significantly protected against the adverse effect of T-2 toxin on antioxidant status in RBCs and liver tissues of chicken. The reduction in the level of lipid peroxidation and an increase in the level of reduced glutathione were suggestive of protection imparted by sea buckthorn through the modulation of cellular antioxidant mechanisms owing to higher amounts of carotenoids, vitamin E and C present in berries. Previous reports from this laboratory showed that sea buckthorn berries

are rich in phenolic compounds, flavonoids, lycopene, vitamin E, vitamin C, and β-carotene (Chauhan and Varshneya, 2012). Similarly, at higher doses, sea buckthorn extract inhibited the chromium-induced increase in plasma malondialdehyde levels and restored the reduced glutathione levels to normal levels in rats (Geetha *et al.*, 2003).

CONCLUSION

In conclusion, the supplementation of the sea buckthorn and glucomannan in combination showed an additive effect. So, on the basis of these results, it may be correlated that sea buckthorn and glucomannan can be used as protectants in the poultry industry to decrease the economic loss associated with oxidative stress induced by mycotoxins, especially T-2 toxin.

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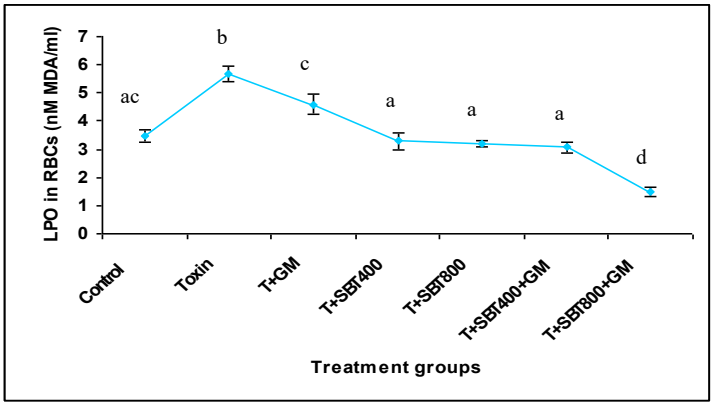
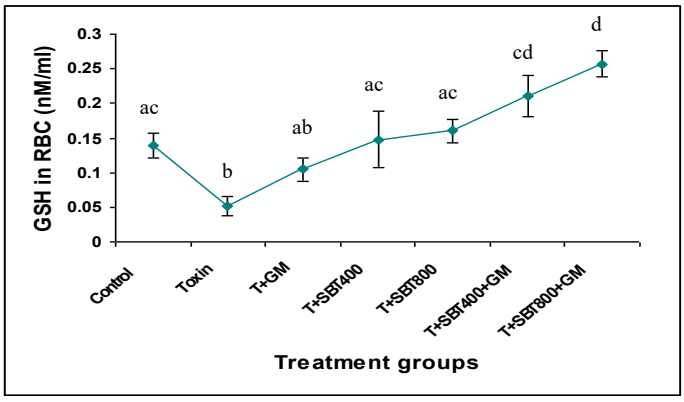


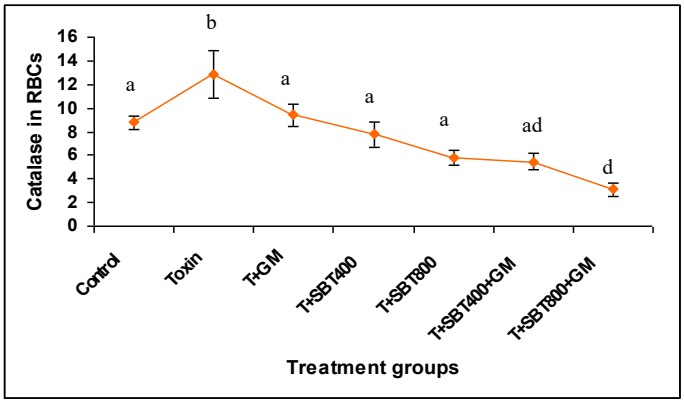
Mean values ± SE (n=6), Means bearing the same superscripts do not differ significantly at 5% level

Fig. 1: Effect of dietary supplementation of sea buckthorn berries and glucomannan on Lipid peroxidation (nM MDA/ml) in RBC of birds fed with T-2 toxin



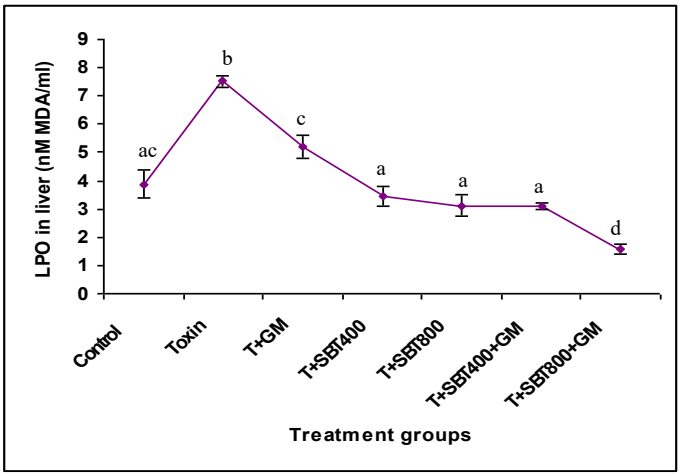
Mean values ± SE (n=6), Means bearing the same superscripts do not differ significantly at 5% level.

Fig. 2: Effect of dietary supplementation of sea buckthorn berries and glucomannan on reduced glutathione (GSH, nM/ml) in erythrocytes of birds fed with T-2 toxin



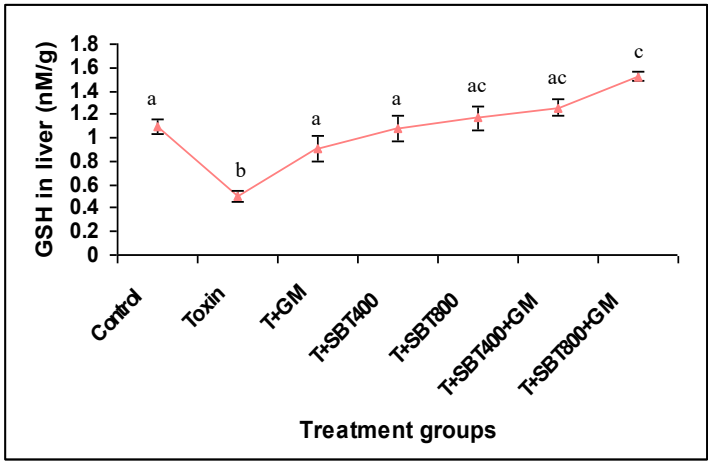
Mean values ± SE (n=6), Means bearing the same superscripts do not differ significantly at 5% level.

Fig. 3: Effect of dietary supplementation of sea buckthorn berries and glucomannan on Catalase (mMH2O2 utilized Min-1 mg-1Hb) in RBC of birds fed with T-2 toxin



Mean values ± SE (n=6), Means bearing the same superscripts do not differ significantly at 5% level.

Fig. 4: Effect of dietary supplementation of sea buckthorn berries and glucomannan on Lipid peroxidation (nM MDA/g) in the liver of birds fed with T-2 toxin



Mean values ± SE (n=6), Means bearing the same superscripts do not differ significantly at 5% level.

Fig. 5: Effect of dietary supplementation of sea buckthorn berries and glucomannan on reduced glutathione (GSH, nM/g) in the liver of birds fed with T-2 toxin