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Research Article

ANTIOXIDANT EFFICACY OF SEABUCKTHORN (HIPPOPHAE RHAMNOIDES L.) LEAVES AGAINST OCHRATOXIN A-INDUCED TOXICITY IN LIVER AND KIDNEY TISSUES OF JAPANESE QUAIL CHICKS

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ABSTRACT

The present investigation was designed to study the antioxidant effect of seabuckthorn (SBT) (*Hippophae rhamnoides* L.) against ochratoxin A (OTA) induced oxidative stress in liver and kidneys of Japanese quail. Day-old quail chicks were divided into seven groups and fed a basal quail chick mash containing 2% SBT leaf powder (Group SX), OTA at a dietary level of 3 ppm (Group OX), 25 ppm L-β-phenylalanine plus 3 ppm OTA (Group OP), 2% dietary level of SBT leaf powder plus 3 ppm OTA (Group OS), SBT-leaf soxhlet extract at a level of 10%/litre of drinking water plus 3 ppm OTA (Group OSS), SBT-leaf plain water extract at the level of 10%/litre of drinking water plus 3 ppm OTA (Group OSP) and a standard toxin-free feed (Group CX, control) for 21 days. Addition of OTA at 3 ppm in the diet led to higher lipid peroxidase levels in the liver and kidney tissues in quail chicks of OX group whereas the peroxidase levels were found better in SBT combination groups. Similarly, catalase level was found the lowest in group OX as compared to other groups. In conclusion, antioxidant potential of seabuckthorn partially protected OTA induced oxidative damage in Japanese quail chicks.

Key words: Ochratoxin A, seabuckthorn, Japanese quail, antioxidant activity

Ochratoxin A (OTA) is a naturally occurring mycotoxin produced by *Aspergillus ochraceus* and *Penicillium viridicatum* (Pitt and Hocking, 1997). It is a nephrotoxic, hepatotoxic, teratogenic and carcinogenic agent to animals and possibly to humans (EL- Khour and Atoui, 2010). It is more toxic than other ochratoxins (ochratoxins B and C) and has been reported to be even more toxic than aflatoxin B_1 (Samson, 1992). The toxicity of OTA may be due to: inhibition of ATP production, inhibition of protein synthesis and promotion of membrane lipid peroxidation (Marquardt and Frohlich, 1992; Hoehler *et al.,* 1997). OTA causes significant loss to poultry industry due to reduced weight gain, impaired feed efficiency, reduced egg production and quality (Page *et al*., 1980). L-β-phenylalanine is known to have ameliorative effects against OTA toxicity. OTA competitively inhibits tRNA phenylalanine synthetases, as a consequence the protein synthesis is interrupted (Dirheimer and Creppy, 1991). Since the inhibition is competitive to phenylalanine, it can be reversed by an excess of this amino acid.

Seabuckthorn (SBT; *Hippophae rhamnoides* L., Elaegnaceae) is a thorny nitrogen fixing deciduous shrub, native to Europe and Asia (Rousi, 1971). All parts of the plant are considered to be a good source of a large number of bioactive substances. The fruit and leaf extract of *H. rhamnoides* improves the antioxidant defense system of cells by increasing the intracellular GSH levels and inhibiting reactive oxygen species (ROS) production. Its leaves are rich in flavonoids, tannins and triterpenes (Kallio *et al*., 2002) and possess antioxidant and immunomodulatory (Geetha *et al*., 2003) and anti-stress and anti-inflammatory activity (Ganju *et al*., 2005). SBT leaf aqueous extract has been shown to have a potent adaptogenic activity with no toxicity in rats (Saggu *et al*., 2007). The aim of present study was to evaluate the antioxidant potential of *H. rhamnoides* leaves in OTA induced oxidative stress in Japanese quail.

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MATERIALS AND METHODS

Collection of SBT Leaves and Preparation of Different Extracts: SBT leaves were collected from the orchards of Krishi Vigyan Kendra, Kukumseri, Lahaul and Spiti, Himachal Pradesh. Leaves were shade dried and ground to fine powder. The leaves were submitted for flavonoid estimation to the Department of Biotechnology, Amrita University, Kollam, Kerala and were found to contain flavonoids such as quercetin (0.020%), kaempferol (0.015%) and isorhamnetin (0.15%). Soxhlet extract of SBT leaves was made using Soxhlet apparatus. Leaf powder (100 g) wrapped in thimble made up of coarse filter paper was kept in this apparatus and one liter of water was kept in the assembly. The extraction was carried out using powdered leaf by refluxing with water at 65°C for 5 hours. Then final volume of the extract collected from round bottom flask was cooled and subsequently stored at 4°C. To make plain water extract, 100g of leaf powder was soaked in one liter of distilled water. The mixture was allowed to stir with the help of electric stirrer for 24 hours. Thereafter extract was first filtered with coarse filter paper and then with Whatman filter paper. Final volume of the extract was stored at 4°C. Cold water extract was also tested for the isolation of any pathogenic bacteria and was found negative.

Ochratoxin A and L-β-phenylalanine: OTA was produced from the fungus *A. ochraceous* (NRRL-3174) on maize and the culture material with known level of OTA was supplied by Prof. G. E. Rottinghaus, University of Missouri, Columbia, USA on gratis. A pure compound of L-β- phenylalanine (Hi-Media, Mumbai, India) was used for the study.

Experimental Birds and Feeding Schedule: The present study was conducted using 315, day-old Japanese quail chicks procured from the Central Poultry Development Organization, Chandigarh. The animal care and experimental protocol were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Prior to arrival of quail birds, the room, cages, trays and cage stands were first washed, disinfected and then sterilized. The room was fumigated with formaldehyde gas, just 2 days prior to arrival of quail chicks.

The quail chicks were maintained on *ad lib* chick mash (Quail mash) from day 1 until the end of the experiment. Feed was autoclaved before feeding or mixing with OTA culture material(s) and SBT powder. Pre-boiled drinking water was provided during the experiment. Before feeding, representative samples of chick mash were submitted to the Animal Feed Analytical and Quality Control Laboratory, Namakkal, Tamil Nadu, India for analysis of common mycotoxins.

Experimental Design: The quail chicks (n=315) were randomly divided into 7 groups i.e. CX, SX, OX, OP, OS, OSS and OSP with 45 birds in each group. In each group, there were three replicate pens of 15 quail each. Each dietary treatment was started from day 1 and continued up to 21 days for the study of antioxidant activity in various groups (Table 1).

Antioxidant Status: Liver and kidney (approx. 200 mg each) tissues were individually collected from four birds at necropsy from each group at 7, 14, 21 days post feeding (DPF), stored at -20°C and further homogenized (IKA homogenizer, Germany) in 1:10 volume of ice-cold 100 mM PBS (pH 7.4) over the ice particulates in the glass tubes. The homogenate was

Table 1
Various treatments in different groups of quail chicks from day 1 to 21

Group	Treatment	Total level of culture material/leaf powder/ SBT leaf extract achieved	Dosing level of material (s) used
CX	Chick mash alone	0%	0
SX	SBT-leaf powder alone	2%	20 g/kg SBT
OX	OTA alone	3.75%	3 ppm OTA
OP	OTA + Phe	3.75% + 0.025%	3 ppm OTA + 25 ppm Phe
OS	OTA + SBT-leaf powder	3.75% + 2%	3 ppm OTA + 20 g/kg SBT
OSP	OTA + SBTe	3.75% + SBT-leaf powder in distilled water (10% w/v)	3 ppm OTA + SBTe in drinking water (10% v/v)
OSS	OTA + SBTse	3.75% + SBT-leaf powder in distilled water (10% w/v)	3 ppm + SBTse in drinking water (10% v/v)

OTA=Ochratoxin A; Phe=L-β-phenylalanine; SBT=Seabuckthorn; SBTe=SBT-leaf plain water extract; SBTse=SBT-leaf soxhlet extract

centrifuged for 10 min at 3000 rpm and the resulting supernatant was used for estimation of lipid peroxidase (LPO) and catalase (CAT) enzymes. The extent of lipid peroxidation was measured in terms of malondialdehyde (MDA) production by thiobarbituric acid (TBA) method as described by Shafiq-ur-Rehman (1984). The CAT assay was performed according to the method of Aebi (1983). One unit of catalase activity was defined as the amount of enzyme required to decompose 1 μmol of H_2O_2 in 1 min. The protein content of samples was determined using the calorimetric method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Statistical Analysis: Data generated were subjected to statistical test for comparison of mean values among different groups at various intervals using ANOVA and Duncan's Multiple Range Test (Snedecor and Cochran, 1989). All levels of significance were based on the 95% level of probability. All results were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Antioxidant Status: Estimation of LPO in the liver tissue revealed that it was higher in all OTA treated groups (OX, OP, OS, OSS and OSP) throughout the experiment in comparison to control groups (CX, SX) (Table 2). At 7 DPF, the levels of MDA were found to be 57.4% higher in OX group and it was the highest (85.7%) in group OP in comparison to control (CX). However, lower MDA levels in SBT combination groups at 7 DPF indicated some protection against OTA induced oxidative stress. At 21 DPF, the MDA production was significantly higher in group OX (110.5%) in comparison to other combination groups (OP, OS, OSS, and OSP), again reflecting protective efficacy of SBT. Increase in the level of MDA with the advancement of experiment showed that the level of oxidative damage was dependent on the duration of mycotoxin feeding. LPO in the kidney tissue was found to be higher in all OTA treated groups (OX, OP, OS, OSS and OSP), however, the difference was statistically non-significant ($P \leqslant 0.05$) when compared with control groups (CX, SX) at 7 and 21 DPF (Table 2). However, at 14 DPF, there was a significantly higher lipid peroxidation in group OSP (115%) in comparison to control groups (CX, SX).

Liver tissue catalase values were lower in the all the OTA treated groups and were the lowest in group OX throughout the study in comparison to control (Table 2). At 14 and 21 DPF, catalase values in combination groups (OS, OSS, OSP) were comparable to control groups (CX, SX) (Table 2). However, the difference in values in all OTA treated groups was not found statistically significant ($P \leqslant 0.05$) in comparison to control groups (CX, SX) throughout the experiment. Catalase enzyme activity was found to be lower in the kidney tissues of all OTA treated groups at various intervals; however, the percent decrease in the catalase values was the highest in group OX (Table 2).

The results in the present study showed enhanced lipid peroxidation as an effect of OTA which agrees with earlier observations (Rahimtula *et al.,* 1988; Stoev *et al.,* 2002) and could result in structural changes in the cell membranes sufficient to allow an influx of cellular calcium to cause changes in metabolic activity within the cells and ultimately to cause cell necrosis (Orrenius and Bellomo, 1986). Besides, in the presence of prooxidants, ochratoxin uncouples oxidative phosphorylation (Hoehler *et al*., 1997). Petrik *et al*. (2003) demonstrated that a very low concentration of ochratoxin was sufficient to induce apoptosis and oxidative damage to kidney cells in Wistar rats. The results of the present study suggest that supplementation of SBT leaf powder, SBT extracts and phenylalanine along with OTA probably stimulated the antioxidant system in the organs for counteracting the oxidative damage caused by OTA and this protection by SBT might be mediated through the modulation of cellular antioxidant mechanism due to higher amount of flavonoids, carotenoids and vitamins E and C present in its leaves. Our findings are consistent with those of Vijayaraghavan *et al.* (2006) who reported that *H. rhamnoides* leaf extract and *H. rhamnoides* flavones significantly recovered the levels of MDA, GSH and GSSG, showing their protective effect against mustard gas toxicity in rats. Leaf and fruit extracts of *H. rhamnoides* improved the antioxidant

defense system of the cells by increasing the intracellular GSH levels and inhibiting ROS production. Geetha *et al.* (2008) found that H. *rhamnoides* leaf extract reduced the MDA production in a dose-dependent manner in carbon tetrachloride induced toxicity in rats. Higher doses of SBT extract were found to inhibit the chromium induced increase in plasma MDA levels and restored the reduced glutathione levels to normal levels in rats (Geetha *et al.,* 2003). Renzulli *et al.* (2004) found that rosamarinic acid, a phenolic compound present in *Boraginaceae* species of plant (sage, basil, mint), reduced free radical oxygen and apoptosis of human hepatoma cells induced by aflatoxin B_1 and OTA. In the liver tissue, the catalase values were lower in all OTA treated groups, however, SBT treated groups showed improvement in the enzymatic levels which might be due to improved antioxidant defense system. This finding is consistent with that of Chakraborty and Verma (2010) who also reported that the levels of MDA and catalase in the liver tissue were better in *Emblica officinalis* aqueous extract treated mice compared with ochratoxin group.

The study revealed that the lipid peroxidation decreased in the liver and kidney tissue of Japanese quail chicks fed seabuckthorn along with ochratoxin A which reflects antioxidant potential of this shrub. Similarly, the levels of anti-oxidant enzyme (catalase) were found improved in seabuckthorn treated groups. In conclusion, seabuckthorn has shown some antioxidant activity and partial protection against ochratoxin A induced oxidative stress in birds. Further studies are required to explore fully the protective efficacy of seabuckthorn for safeguarding poultry health against mycotoxin exposure.

Table 2

Effect of seabuckthorn on lipid peroxidase and catalase activity during ochratoxin A induced toxicity in Japanese quail[1]

Organs	Intervals	Lipid peroxidase and catalase activity in different groups					
		CX	SX	OX	OP	OS	OSS	OSP
		Lipid Peroxidase (MDA/g of tissue) activity in groups						
Liver	7 DPF	0.21±0.03^{b}	0.18±0.03^{b} (-14.2%)	0.33±0.05^{ab} (57.4%)	0.39±0.07^{a} (85.7%)	0.23±0.04^{b} (9.5%)	0.32±0.05^{ab} (52.3%)	0.24±0.03^{ab} (14.2%)
	14 DPF	0.46±0.05^{c}	0.56±0.04^{abc} (21.7%)	0.51±0.06^{c} (10.8%)	0.82±0.11^{a} (78.2%)	0.72±0.15^{abc} (56.5%)	0.75±0.07^{ab} (63.0%)	0.80±0.01^{a} (73.9%)
	21 DPF	0.57±0.03^{b}	0.62±0.08^{b} (8.7%)	1.20±0.15^{a} (110.5%)	0.69±0.12^{b} (21%)	1.11±0.09^{a} (94.7%)	0.93±0.06^{ab} (63.1%)	1.09±0.22^{a} (91.2%)
Kidney	7 DPF	0.13±0.01^{a}	0.15±0.04^{a} (15.3%)	0.22±0.06^{a} (69.2%)	0.24±0.09^{a} (84.6%)	0.25±0.07^{a} (92.3%)	0.17±0.02^{a} (30.7%)	0.14±0.03^{a} (7.6%)
	14 DPF	0.20±0.02^{b}	0.35±0.06^{ab} (75.0%)	0.28±0.09^{ab} (40.0%)	0.21±0.02^{b} (5.0%)	0.34±0.06^{ab} (70.0%)	0.26±0.04^{ab} (30.0%)	0.43±0.11^{a} (115.0%)
	21 DPF	0.33±0.06^{a}	0.33±0.02^{a} (0.0%)	0.44±0.03^{a} (33.3%)	0.45±0.04^{a} (36.3%)	0.43±0.04^{a} (30.3%)	0.34±0.06^{a} (3.0%)	0.34±0.07^{a} (3.0%)
		Catalase (U/L) activity in groups						
Liver	7 DPF	17.75±4.10^{a}	17.20±3.46^{a} (-3.0%)	12.90±1.11^{a} (-7.3%)	16.02±4.67^{a} (-9.7%)	12.48±3.62^{a} (-29.6%)	13.11±3.74^{a} (-26.1%)	12.37±3.14^{a} (-30.3%)
	14 DPF	13.92±1.54^{a}	12.89±1.10^{a} (-7.3%)	9.50±1.67^{a} (-31.7%)	8.02±0.87^{a} (-42.3%)	13.64±1.24^{a} (-2.0%)	18.14±6.42^{a} (30.3%)	13.02±4.65^{a} (-6.4%)
	21 DPF	18.21±9.07^{a}	16.93±3.78^{a} (-7.0%)	12.53±1.63^{a} (-31.1%)	18.31±4.85^{a} (0.5%)	13.59±3.24^{a} (-25.3%)	14.44±4.75^{a} (-20.7%)	15.72±1.79^{a} (-13.6%)
Kidney	7 DPF	16.97±1.64^{a}	18.88±3.63^{a} (11.2%)	12.33±1.03^{a} (-27.3%)	12.23±3.88^{a} (-7.9%)	14.82±1.60^{a} (-12.7%)	12.83±1.17^{a} (-24.4%)	13.87±0.90^{a} (-18.4%)
	14 DPF	16.44±2.39^{a}	15.30±2.11^{a} (-6.9%)	7.86±3.27^{a} (-52.1%)	10.11±3.41^{a} (-38.5%)	10.24±2.70^{a} (-37.7%)	9.90±2.04^{a} (-39.8%)	12.07±2.11^{a} (-26.6%)
	21 DPF	26.38±11.86^{a}	23.51±6.53^{a} (-10.9%)	15.96±2.85^{a} (-39.5%)	16.08±3.89^{a} (-31.6%)	12.61±2.67^{a} (-52.2%)	16.16±5.25^{a} (-38.7%)	19.83±1.71^{a} (-24.8%)

[1]Data are means ± SE of 4 quail each; CX=Control group; SX=Seabuckthorn control; OX=Group kept on ochratoxin A diet only; OP=Group kept on l-phenylalanine and ochratoxin A; OS= Group kept on SBT leaves powder and ochratoxin A; OSP=Group kept on SBT leaves plain water extract and ochratoxin A; OSS=Group kept on SBT leaves soxhlet extract and ochratoxin A; [a-c]Values within rows at each interval (between groups CX, SX, OX ,OP, OS, OSS and OSP) with different superscripts are significantly different by ANOVA ($P \leqslant 0.05$); Figures in parenthesis indicate percent increase or decrease in mean values in different groups in comparison to the control group (CX).

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REFERENCES

Aebi, H.U. (1983). Catalase. In : Methods in Enzymatic Analysis. H.U., Bergmeyer. (edt.), Academic Press, New York.

Chakraborty, D. and Verma, R. (2010). Ameliorative effect of *Emblica officinalis* aqueous extract on ochratoxin-induced lipid peroxidation in the kidney and liver of mice. *International J. Occup. Med. Environ. Hlth.* **23**: 63-73.

Dirheimer, G. and Creppy, E.E. (1991). Mechanism of action of ochratoxin A. *IARC Sci. Publ.* **115**:171-186.

EL-Khour, A. and Atoui, A. (2010). Ochratoxin A: General overview and actual molecular status. *Toxin* **2**: 461-493

Ganju, L., Padwad, Y., Singh, R., Karan, D., Chanda, S., Chopra, M.K., Bhatnagar, P., Kashyap, R. and Sawhney, R.C. (2005). Anti-inflammatory activity of seabuckthorn (*Hippophae rhamnoides*) leaves *Intl. Immunopharmacol.* **5**: 1675-1684.

Geetha, S., Jayamurthy, P., Pal, K., Pandey, S., Kumar, R. and Sawhney, R.C. (2008). Hepatoprotective effects of seabuckthorn (*Hippophae rhamnoides* L.) against carbon tetrachloride induced liver injury in rats**.** *J. Sci. Food Agric.* **88**: 1592-1597.

Geetha, S., SaiRam, M., Mongia, S.S., Singh, V., Ilavazhagan, G. and Sawhney, R.C. (2003). Evaluation of antioxidant activity of leaf extract of seabuckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats. *Mol. Cellular Biochem.* **87**: 247- 251.

Hoehler, D., Marquardt, R.R. and Frohlich, A.A. (1997). Lipid peroxidation as one mode of action in ochratoxin A toxicity in rats and chicks. *Canadian J. Anim. Sci.* **77**: 287-292.

Kallio, H., Yang, B. and Peippo, P. (2002). Effects of different origins and harvesting time on vitamin C, tocopherols and tocotrienols in seabuckthorn (*Hippophae rhamnoides* L.) berries *J. Agric. Food Chem.* **50**: 6136-6142.

Lowry, O., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.

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

Orrenius, S. and Bellomo, G. (1986). Toxicological implications of perturbation of Ca^{2+} homeostasis in hepatocytes. In: Cheung, W.Y. (edt.), Calcium and Cell Function. Academic Press, Orlando.

Page, R.K., Stewart, G., Wyatt, R., Bush, P., Fletcher, O.J. and Brown, J. (1980). Influence of low level of ochratoxin A on egg production, egg-shell stains, and serum uric acid levels in Leghorn- type hens. *Avian Dis.* **24**: 777-780.

Petrik, K.J., Zanic-Grubisic, T., Barisick, Pepeljnjak, S., Radic, B., Ferencic, Z. and Cepelak, I. (2003). Apoptosis and oxidative stress induced by ochratoxin A in rat kidney. *Arch. Toxicol.* **77**: 685-693.

Pitt, J.I. and Hocking, A.D. (1997). *Fungi and Food Spoilage*. Weimer: Aspen Publishers.

Rahimtula, A.D., Bereziat, J.C., Bussacchini-Griot, V. and Bartsch, H. (1988). Lipid peroxidation as a possible cause of ochratoxin A toxicity. *Biochem. Pharmacol.* **37**: 4469-4477.

Renzulli, C., Galvano, F., Pierdomenico, L., Speroni, E., and Guerra, M.C. (2004). Effect of rosamarinic acid against aflatoxin B_1 and ochratoxin A induced cell damage in a human hepatoma cell line (Hep G2). *J. Appl. Toxicol.* **24**: 289-296.

Rousi, A. (1971). The genus Hippophae L., taxonomic study. *Annal. Bot.* **8**:177-277.

Saggu, S., Divekar, H.M., Gupta, V., Sawhney, R.C., Banerjee, P.K. and Kumar, R. (2007). Adaptogenic and safety evaluation of seabuckthorn (*Hippophae rhamnoides*) leaf extract: A dose dependent study. *Food Chem. Toxicol.* **45**: 609-617.

Samson, R.A. (1992). Mycotoxins: A mycologists perspective. *J. Med. Vet. Mycol. Suppl.* **30**: 9-18.

Shafiq-ur-Rehman. (1984). Lead induced regional lipid peroxidation in brain. *Toxicol. Letters* **21**: 333-337.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods. (8^{th} edn.), Iowa State University Press, Ames, USA.

Stoev, S.D., Koynarsky, V. and Mantle, P.G. (2002). Clinicomorphological studies in chicks fed ochratoxin A while simultaneously developing coccidiosis. *Vet. Res. Commun.* **26**: 189-204.

Vijayaraghavan, R., Gautum, A., Kumar, O., Pant, S.C., Sharma, M., Singh, S., Kumar, H.T.S., Singh, A.K., Nivsarkar, M., Kaushik, M.P., Sawhney, R.C., Chaurasia, O.P., and Prasad, G.B.K.S. (2006). Protective effect of ethanolic and water extract of seabuckthorn against the toxic effect of mustard gas. *Indian J. Exptl. Biol.* **44**: 821-831.