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Arti Ghabru

Department of Basic Sciences, Dr. Y.S.Parmar Horticulture and Forestry, Nauni, Solan-173230, Himachal Pradesh, India

Shivani Chauhan

Department of Microbiology, COBS, CSK HPKV, Palampur-176062, Himachal Pradesh, India

C Varshneya

Retd Dean, COVAS, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, CSK HPKV, Palampur-176062, Himachal Pradesh, India

Effect of Seabuckthorn leaves on Antioxidant and Microsomal Enzymes in poultry birds

Arti Ghabru*, Shivani Chauhan, C Varshneya

ABSTRACT

In vivo studies on broiler birds were carried out to evaluate effect of aflatoxin and seabuckthorn leaves on microsomal enzyme system, antioxidant enzymes and biochemical parameters i.e. serum triglyceride, total plasma protein, aminopyrine demethylase, aniline hydroxylase, NADPH cytochrome P450 reductase, catalase, LPO, superoxide dismutase, GSH, blood urea nitrogen (BUN) and creatinine levels in poultry. The poultry birds were divided into six groups containing six birds each. Aflatoxin (400 ppb) and seabuckthorn leaves (10000ppm) was administered continuously in poultry feed. Aflatoxin increased serum triglyceride, blood urea nitrogen and creatinine levels where as seabuckthorn leaves supplementation at 10000ppm significantly decreased triglyceride ($P<0.05$), blood urea nitrogen ($P<0.05$) and creatinine levels in birds. Toxin decreased liver, kidney and blood superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) activity, whereas, seabuckthorn leaves (SBTL) increased the activity of these enzymes as compared to control group. The level of lipid peroxidation was significantly increased in the toxin exposed group and decreased in case of SBTL. The activity of Aminopyrine demethylase and Aniline hydroxylase increased, while the activity of NADPH cytochrome P450 reductase is decreased in case of toxin group whereas in case of seabuckthorn leaves exposed group showed no significant change in case of aminopyrine demethylase and NADPH cytochrome P450 reductase, however, the activity of Aniline hydroxylase decreased. On the basis of present study, it could be concluded that the seabuckthorn leaves reduced the effect of Aflatoxin which produced oxidative stress by altering the levels of antioxidant enzymes of liver and kidney in adult poultry birds.

Keywords: Aminopyrine demethylase, NADPH cytochrome P450 reductase, Aniline hydroxylase, Superoxide dismutase, Catalase, Reduced glutathione and Seabuckthorn.

INTRODUCTION

Hippophae rhamnoides L., belongs to family *Elaeagnaceae*, commonly known as seabuckthorn growing in North-West Himalayas at high altitude (7000-15,000 feet) and thorny shrub. The natural habitat of Seabuckthorn extends widely in China, Mongolia, Russia and most parts of Northern Europe. In India the plant inhabits dry temperate region and high altitude regions of Himachal Pradesh, Jammu and Kashmir and Uttarakhand. Seabuckthorn has recently gained in interest for its nutritional and medicinal values [7, 20]. Fruits and leaves are considered to be good source of large number of bioactive substances such as vitamins, trace elements, amino acids, β -carotene, zeaxanthin, lycopene, flavonoids, folic acid, fatty acid, tannic acid etc. These substances are mainly responsible for various pharmacological activities. The phytochemical composition of Seabuckthorn has been found to vary with the origin, climate and method of extraction [4, 20]. Its fruit is source of nearly 190 bioactive substances, whereas its oil has nearly 106 such components [21]. There is an ample quantity of quality vitamins in Seabuckthorn fruit and leaves. The mineral contents of Seabuckthorn make the shrub most important. Polyphenols present in Seabuckthorn has antioxidant properties and can be used against the damaging effect of free radicals [5, 10, 18, 19].

The present day intensive animal husbandry practices have made poultry birds susceptible to various environmental contaminants including mycotoxins. The mycotoxins contamination causes 25% of total poultry feed loss. Out of mycotoxins, aflatoxin is responsible for substantial production losses in the poultry industry. The contamination of animal feed is practically unavoidable under moist conditions of midhills of Himachal Pradesh. During recent years nutraceutical properties of seabuckthorn leaves are being exploited in poultry as feed substitute [3] indicating that 3% substitution of crude protein offers better results in performance of broilers. The present studies were conducted to evaluate effect of seabuckthorn leaves on aflatoxin induced toxicity and antioxidant potential of seabuckthorn leaves.

Correspondence:

Arti Ghabru

Department of Basic Sciences, Dr. Y.S.Parmar Horticulture and Forestry, Nauni, Solan-173230, Himachal Pradesh, India

Email: arti.adore[at]gmail.com

MATERIALS AND METHODS

Aflatoxin production

The aflatoxin was produced on rice [17]. One hundred grams of rice was taken in flask and soaked overnight in 75 ml of water. Inoculate the flask with *Aspergillus flavus* (MTCC 6672). Add 30 ml of sterile water to each flask and incubate the flasks at 28°C for 15 days. Whitish moldy growth was observed on the surface of the rice which later turned yellow followed by green color after 48 hrs of inoculation. The moldy rice was steamed at 100°C for 1 hrs to kill the spores by drying in hot air oven at 60 °C overnight. The dried culture was analyzed for aflatoxin content.

Mycotoxin estimation

The aflatoxin content was estimated by chromatographic techniques [16] at Veterinary College and Research Institute, Namakkal, Tamil Nadu and aflatoxin level was further confirmed from Feed Analysis Lab (Skylark Lab, Pathankot, Punjab).

Collection of plant material

The seabuckthorn leaves were collected from Agricultural Research Extension Centre, Kukumseri, Lahaul-Spiti.

Preparation of leaves powder

The leaves were dried in shade and ground to fine powder. The

powder was used for preparation of aqua methanolic extract of seabuckthorn and then lyophilized and mixed in broiler feed to achieve the dietary level at 10,000 ppm.

Management

The experimental animal house was thoroughly cleaned and disinfected. The chicks were reared in battery cage system throughout the experiment. The chicks were vaccinated with 0.05 ml of New Castle disease vaccine through intra ocular route on days six and fourteen. The chicks were provided standard poultry feed obtained from the Department of Animal Nutrition, COVAS, CSK HPKV, Palampur (H.P.). The feed and water were provided *ad libitum* during the entire period of experimentation. The broiler starter feed was provided up to day 35 of age and thereafter birds were fed finisher feed up to end of the experiment.

Experimental protocol

The day-old broiler chicks were procured from Uttam Hatchery, Mataur, Distt Kangra (H.P). On the day of arrival, the chicks were examined for any abnormality and ill health. They were acclimatized in the new environment for two weeks. All the experiments detailed in this study were conducted according to the guidelines of Institutional Animal Ethical Committee. Approval of the Institutional Ethic committee was taken (IAEC-Registration No. 259, CSK HPKV, Palampur letter no. QSD/ VSR/COVAS/11/IAEC/-51-71 dated: 25.4.2011).

Table 1: Experimental design to evaluate the effect of seabuckthorn (*Hippophae rhamnoides*) leaves extract and glucomannan on toxic effect of aflatoxin in broiler chickens

| Group | Toxin / SBT/GM | No. of birds | Dose of SBT (ppm) | Dose of GM (g/kg feed) | Mycotoxin (AF) in feed (ppb) |
|-------|----------------|--------------|-------------------|------------------------|------------------------------|
| I | Control | 6 | - | - | - |
| II | SBT | 6 | 10000 | - | - |
| III | Aflatoxin | 6 | - | - | 400 |
| IV | AF + SBT+GM | 6 | 10000 | 1 | 400 |
| V | AF + SBT | 6 | 10000 | - | 400 |
| VI | AF +GM | 6 | - | 1 | 400 |

AF- Aflatoxin, SBT – Seabuckthorn, GM - Glucomannan

1. In vivo antioxidant activity in blood and tissues

Superoxide dismutase (SOD), lipid peroxidation, GSH, Microsomal enzyme Aminopyrine N- demethylase and catalase activities were carried out by standard methods [2, 11, 13, 14]. NADPH-cytochrome P450-reductase and Aniline hydroxylase activities were determined by standard methods [6, 12].

2. Biochemical parameters

Estimation of various biochemical parameters i.e. triglyceride were done by using Siemens kit. Total serum proteins were estimated by Biuret method [8].

Statistical analysis

The data were analyzed using Graph Pad InStat for windows (Graph Pad Software, San Diego, California, USA) and the significant difference between means was determined using Tukey-Kramer multiple comparison test.

RESULTS

In the present study Aflatoxin treatment significantly (P<0.05) increased serum triglycerides as compared to the control. The supplementation of seabuckthorn leaves at 10000 ppm significantly (P<0.05) decreased triglycerides. Toxin treatment significantly (P<0.05) increased the blood urea nitrogen (BUN) level while seabuckthorn leaves supplementation at 10000 ppm level decreased. Increase in serum creatinine levels produced by Aflatoxin treatment was decreased by Seabuckthorn leaves. The concentration of MDA indicating lipid peroxidation increased by toxin in kidney, liver and blood whereas the level of blood glutathione (GSH) significantly decreased in toxin exposed birds. GSH level was found to increase in seabuckthorn treated group (group II) than all other groups except group VI where an increase is noticed in GSH level of liver tissue. In overall trend seabuckthorn restored the GSH level near to normal, which was decreased by the administration of aflatoxin in broilers diet, which is evident from the GSH level of group III. The combined

supplementation of glucomannan and seabuckthorn also restored the GSH level close to normal.

Seabuckthorn supplementation significantly increased ($P < 0.05$) the level of superoxide dismutase and catalase activity in poultry birds whereas toxin significantly ($P < 0.05$) decreased the level. Seabuckthorn has lowered the lipid peroxidation in comparison to

control. The dietary intake of aflatoxin decreased ($P > 0.05$) the total plasma protein in broilers. There was significant increase in the protein levels in birds receiving seabuckthorn alone. Seabuckthorn leaves showed significant ($P < 0.05$) variation in aniline hydroxylase and NADPH cytochrome P450 reductase activities. Aflatoxin significantly ($P < 0.05$) effect the activity of microsomal enzymes.

Table 2: Effect of dietary seabuckthorn leaves supplementation on Creatinine (mg/dl), BUN (mg/dl) and triglyceride (mg/dl) levels in broilers

| Sr. No. | Groups | Creatinine (mg/ dl) | BUN (mg/ dl) | Triglycerides (mg/ dl) |
|---------|-----------------------------|--------------------------|--------------------------|----------------------------|
| 1 | Control | 0.84 ± 0.08 ^a | 5.01 ± 0.35 ^a | 65.78 ± 1.62 ^a |
| 2 | SBT (10000ppm) | 0.81 ± 0.25 ^a | 2.84 ± 0.20 ^b | 49.11 ± 1.14 ^b |
| 3 | Toxin (400ppb) | 1.64 ± 0.17 ^b | 8.20 ± 0.10 ^c | 161.20 ± 1.27 ^c |
| 4 | SBT+Toxin+ Glucamannan (GM) | 0.95 ± 0.22 ^a | 5.78 ± 0.10 ^a | 64.38 ± 2.60 ^a |
| 5 | Toxin+SBT | 1.04 ± 0.07 ^a | 6.21 ± 0.07 ^d | 86.67 ± 1.14 ^d |
| 6 | GM+Toxin | 0.84 ± 0.09 ^a | 5.74 ± 0.18 ^a | 85.24 ± 3.33 ^e |

Values are expressed as Mean ± SEM. (n=6), mean values having same superscripts do not differ significantly at 5 % level.

Table 3: Effect of sub-acute oral exposure of Aflatoxin on Total plasma protein in adult poultry birds

| Sr. No. | Groups | Total Plasma Protein (mg/dl) |
|---------|----------------------------|------------------------------|
| 1 | Control | 5.50 ± 0.76 ^a |
| 2 | SBT (10000ppm) | 5.47 ± 0.19 ^a |
| 3 | Toxin (400ppb) | 7.67 ± 0.43 ^b |
| 4 | SBT+Toxin+Glucamannan (GM) | 4.91 ± 0.12 ^a |
| 5 | Toxin+SBT | 4.30 ± 0.40 ^a |
| 6 | GM+Toxin | 5.22 ± 0.30 ^a |

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

Table 4: Effect of sub-acute oral exposure of Aflatoxin on superoxide dismutase in adult poultry birds

| Sr. No. | Groups | Superoxide dismutase (units/ mg protein) | | |
|---------|-----------------------|--|--------------------------|--------------------------|
| | | Liver | Kidney | Blood |
| 1 | Control | 1.09 ± 0.03 ^a | 3.91 ± 0.21 ^a | 1.33 ± 0.10 ^a |
| 2 | SBT (10000ppm) | 1.40 ± 0.14 ^b | 4.24 ± 0.38 ^a | 1.99 ± 0.09 ^b |
| 3 | Toxin (400ppb) | 0.44 ± 0.05 ^c | 2.80 ± 0.22 ^a | 0.93 ± 0.07 ^a |
| 4 | SBT+Toxin+Glucamannan | 0.97 ± 0.05 ^a | 4.40 ± 0.39 ^a | 1.65 ± 0.06 ^a |
| 5 | Toxin+SBT | 1.04 ± 0.06 ^a | 4.11 ± 0.31 ^a | 1.54 ± 0.10 ^a |
| 6 | GM+Toxin | 1.18 ± 0.13 ^a | 3.91 ± 0.33 ^a | 1.48 ± 0.15 ^a |

Values are expressed as Mean ± SEM. (n=6), mean values bearing same superscripts do not differ significantly at 5 % level.

Table 5: Effect of sub-acute oral exposure of Aflatoxin on catalase in adult poultry birds

| Sr. No. | Groups | Catalase (µM of H ₂ O ₂ Utilised/ min/ mg protein) | | |
|---------|----------------------------|--|---------------------------|--------------------------|
| | | Liver | Kidney | Blood |
| 1 | Control | 6.55 ± 0.50 ^a | 10.50 ± 0.59 ^a | 2.58 ± 0.35 ^a |
| 2 | SBT (10000ppm) | 9.23 ± 0.69 ^b | 13.80 ± 1.68 ^a | 3.52 ± 0.16 ^b |
| 3 | Toxin (400ppb) | 4.57 ± 0.11 ^a | 6.81 ± 1.36 ^a | 1.26 ± 0.21 ^a |
| 4 | SBT+Toxin+Glucamannan (GM) | 6.08 ± 0.51 ^a | 9.37 ± 0.41 ^a | 2.63 ± 0.27 ^a |
| 5 | Toxin+SBT | 6.96 ± 0.23 ^a | 10.15 ± 0.83 ^a | 2.63 ± 0.40 ^a |
| 6 | GM+Toxin | 7.28 ± 0.88 ^a | 10.29 ± 0.98 ^a | 2.19 ± 0.25 ^a |

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

Table 6: Effect of sub-acute oral exposure of Aflatoxin on blood glutathione in adult poultry birds

| Sr. No. | Groups | GSH (mM/ g) Mean ± SEM | | |
|---------|----------------------------|--------------------------|--------------------------|--------------------------|
| | | Liver | Kidney | Blood |
| 1 | Control | 1.18 ± 0.02 ^a | 1.26 ± 0.13 ^a | 1.60 ± 0.01 ^a |
| 2 | SBT (10000ppm) | 1.44 ± 0.03 ^b | 1.24 ± 0.03 ^b | 1.76 ± 0.01 ^b |
| 3 | Toxin (400ppb) | 0.91 ± 0.03 ^c | 0.87 ± 0.03 ^a | 1.36 ± 0.04 ^c |
| 4 | SBT+Toxin+Glucamannan (GM) | 1.25 ± 0.09 ^a | 1.07 ± 0.06 ^a | 1.45 ± 0.05 ^a |
| 5 | Toxin+SBT | 1.10 ± 0.04 ^a | 1.07 ± 0.06 ^a | 1.49 ± 0.04 ^a |
| 6 | GM+Toxin | 1.01 ± 0.04 ^a | 1.04 ± 0.08 ^a | 1.40 ± 0.04 ^d |

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

Table 7: Effect of sub-acute oral exposure of Aflatoxin on Aminopyrene demethylase (nmole/min/mg protein) in adult poultry birds

| Sr. No. | Groups | Aminopyrene demethylase (nmol/ min/ mg protein) Mean ± SEM | |
|---------|----------------------------|--|--------------------------|
| | | Liver | Kidney |
| 1 | Control | 2.29 ± 0.12 ^a | 1.18 ± 0.04 ^a |
| 2 | SBT (10000ppm) | 2.33 ± 0.19 ^a | 0.92 ± 0.09 ^a |
| 3 | Toxin (400ppb) | 2.68 ± 0.10 ^a | 1.47 ± 0.06 ^a |
| 4 | SBT+Toxin+Glucamannan (GM) | 2.47 ± 0.20 ^a | 1.11 ± 0.09 ^a |
| 5 | Toxin+SBT | 2.51 ± 0.11 ^a | 1.20 ± 0.10 ^a |
| 6 | GM+Toxin | 2.25 ± 0.21 ^a | 1.03 ± 0.07 ^a |

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

Table 8: Effect of sub-acute oral exposure of Aflatoxin on Aniline hydroxylase (nmole/min/mg protein) in adult poultry birds

| Sr. No. | Groups | Aniline hydroxylase (nmol/ min/ mg protein) Mean ± SEM | |
|---------|----------------------------|--|--------------------------|
| | | Liver | Kidney |
| 1 | Control | 0.77 ± 0.04 ^a | 0.49 ± 0.03 ^a |
| 2 | SBT (10000ppm) | 0.44 ± 0.04 ^b | 0.31 ± 0.04 ^a |
| 3 | Toxin (400ppb) | 0.85 ± 0.03 ^a | 0.68 ± 0.03 ^a |
| 4 | SBT+Toxin+Glucamannan (GM) | 0.62 ± 0.06 ^a | 0.61 ± 0.06 ^a |
| 5 | Toxin+SBT | 0.59 ± 0.07 ^a | 0.66 ± 0.07 ^a |
| 6 | GM+Toxin | 0.80 ± 0.11 ^a | 0.55 ± 0.09 ^a |

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

Table 9: Effect of sub-acute oral exposure of Aflatoxin on NADPH cytochrome P450 reductase (nmole/min/mg protein) in adult poultry birds

| Sr. No. | Groups | NADPH Cytochrome P450 reductase (nmol/ min/ mg protein) Mean ± SEM | |
|---------|----------------------------|--|---------------------------|
| | | Liver | Kidney |
| 1 | Control | 33.03 ± 0.18 ^a | 29.63 ± 1.07 ^a |
| 2 | SBT (10000ppm) | 35.17 ± 3.02 ^a | 27.81 ± 2.26 ^a |
| 3 | Toxin (400ppb) | 14.79 ± 0.55 ^b | 13.90 ± 0.44 ^b |
| 4 | SBT+Toxin+Glucamannan (GM) | 25.38 ± 1.92 ^c | 24.28 ± 1.38 ^a |
| 5 | Toxin+SBT | 20.54 ± 0.45 ^d | 18.50 ± 3.39 ^c |
| 6 | GM+Toxin | 23.18 ± 1.84 ^e | 21.86 ± 1.51 ^a |

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

Table 10: Effect of sub-acute oral exposure of aflatoxin on antioxidant enzyme lipid peroxidation in liver, kidney and blood in adult poultry birds

| Sr. No. | Groups | LPO (nM MDA/ g) Mean ± SEM | | |
|---------|-----------------------|----------------------------|--------------------------|--------------------------|
| | | Liver | Kidney | Blood |
| 1 | Control | 6.25 ± 0.70 ^a | 2.95 ± 0.25 ^a | 0.69 ± 0.03 ^a |
| 2 | SBT (10000ppm) | 6.82 ± 0.83 ^b | 2.58 ± 0.59 ^b | 0.53 ± 0.06 ^a |
| 3 | Toxin (400ppb) | 10.59 ± 1.16 ^a | 4.95 ± 0.51 ^a | 0.82 ± 0.08 ^a |
| 4 | SBT+Toxin+Glucamannan | 5.59 ± 0.43 ^a | 4.80 ± 0.38 ^a | 0.59 ± 0.03 ^a |
| 5 | Toxin+SBT | 5.96 ± 0.20 ^a | 4.45 ± 0.37 ^a | 0.53 ± 0.02 ^a |
| 6 | GM+Toxin | 6.69 ± 0.31 ^a | 3.90 ± 0.43 ^a | 0.63 ± 0.09 ^a |

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

DISCUSSION

The main objective of the studies was to examine the effect of seabuckthorn leaves on the growth of poultry birds. Increased in BUN, creatinine, triglyceride and total protein content in serum of poultry birds fed with aflatoxin (100ppm) was in accordance with earlier reports [9]. The concentration of MDA indicating lipid peroxidation increased by toxin in kidney, liver and blood whereas the level of blood glutathione (GSH) significantly decreased in toxin exposed birds. GSH level was found to increase in seabuckthorn treated group. Similar antioxidant activity of seabuckthorn with respect to increase in hepatic GSH level was observed by earlier workers [22]. In earlier studies reported an increase in the level of GSH with the glucomannan treatment in aflatoxicosis in rabbits [31].

Increased lipid peroxidation on feeding of aflatoxin supplemented diet was in accordance with Gowda *et al.* [28]. This increase in peroxide level in liver was also due to aflatoxin induced decrease in activity of superoxide dismutase, catalase, glutathione peroxidase and reductase [25]. Seabuckthorn has lowered the lipid peroxidation in comparison to control. These findings on the oxidative stress were identical to that of Dubey *et al.* [30] who used rat brain. The antioxidant action is due to the presence of tocopherol, carotenoids and flavones in SBT leaves, which prevents lipid peroxidation and lipid auto oxidation of liposomes [22, 27].

Decrease in the total protein has also been noticed earlier [9, 23, 28, 32, 33]. The decrease in protein level could be due to impaired hepatic functions which reduces protein synthesis in the body [26]. There was significant increase in the protein levels in birds receiving seabuckthorn alone. Geetha *et al.* [29] and Shashikanth *et al.* [24] reported that the rats fed with seabuckthorn extract possessed higher serum protein levels.

Aflatoxin B1 induced toxicity and carcinogenicity can be distorted by feeding diets with various phytochemicals [1].

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