

## AMELIORATIVE EFFECT OF SEABUCKTHORN (*HIPPOPHAE RHAMNOIDES*) PULP EXTRACT AGAINST AFLATOXIN INDUCED HEPATOTOXICITY IN POULTRY

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**Abstract:** This study evaluated the hepatoprotective activity of seabuckthorn (SBT) pulp against toxicity induced by aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in broiler chickens. The experiment included 120 broilers (30 birds per group) divided into 4 groups viz. Control (group I), Aflatoxin (AF) (Group II), Aflatoxin + SBT pulp extract (Group III) and Only SBT pulp extract group (Group IV). Aflatoxin fed @400ppb and SBT pulp aqua methanolic extract (70%) was fed @1000ppm in feed for a duration of 28 days. The toxicity of AFB<sub>1</sub>@ 400ppb in group II lead to significant decrease (P < 0.001) in total serum proteins (TP) and specifically reduced albumin. Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum Lactate dehydrogenase activity (LDH) and Serum Total Bilirubin (TB) activity increased significantly in group II as compared to all other groups. In the group of poultry birds treated with AFB<sub>1</sub>@400ppb and 70% aqua methanolic extract of SBT pulp @1000ppm (GroupIII) the biochemical parameters like AST, ALT, LDH, Total bilirubin (TB), serum triglycerides were restored to their normal levels as compared to Group II. These findings suggest that SBT pulp has a potent hepatoprotective activity.

**Keywords:** Seabuckthorn, Aflatoxicoses, Broilers, Hepatotoxicity.

### INTRODUCTION

*Seabuckthorn* has been reported to contain more than 200 compounds in the seeds, pulp, fruit, and leaves. These compounds include fat soluble vitamins (A, K, E), 22 fatty acids, 42 lipids, organic acids, amino acids, carbohydrates, vitamins C, B<sub>1</sub>, B<sub>2</sub>, folic acid and flavonoids, phenols, terpenes, tannins and minerals [1-2]. A wide spectrum of pharmacological effects of *Seabuckthorn* have been recently reported, including antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue repair [3-13]. *Seabuckthorn* reduces liver damage by antioxidant activity making it hepatoprotective [14-16]. *Seabuckthorn* oil has a cytoprotective action on liver damage induced by toxic chemicals

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such as carbon tetrachloride, acetaminophen, and ethyl alcohol [17]. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a secondary metabolite of the *Aspergillus flavus* and *Aspergillus parasiticus* fungi and is found in grains and other foods and feed stuffs as a natural contaminant. It is a potent liver toxin and an extremely potent mutagen having teratogenic effects and causing hepatocellular hyperplasia, hepatic necrosis, cirrhosis, biliary hyperplasia, and acute liver damage in animals [18]. The tropical climate with hot and humid condition prevailing in India with poor harvesting of crops during monsoon season, inadequate drying and storage facilities make feed stuff susceptible to fungal contamination resulting in greater economic losses to poultry industry through aflatoxicosis [19]. There are no readily available, scientifically proven therapeutic options, and this study was designed to determine the efficacy of *Seabuckthorn* pulp as a hepatoprotective agent against aflatoxin-induced liver damage in poultry chickens.

### MATERIAL AND METHODS

**Plant Material and Preparation of Leaves Extract** The seabuckthorn pulp was collected from Agricultural Research Extension Centre, Kukumseri, Lahaul (H.P) during the month of September. Seabuckthorn pulp was shade dried and pulp extract was prepared.

**Aflatoxin (AF) production:** The aflatoxin was produced from the pure culture of *Aspergillus parasiticus* NRRL 2999. The culture was maintained on potato dextrose agar (PDA) for about 7 days at 25<sup>0</sup>c before harvesting mould spores. The spore suspension was used for production of aflatoxin on rice as per the method by Shotwell *et al* [34]. The toxin produced in the rice was extracted and analyzed by thin layer chromatography (TLC) [33]. The rice powder was incorporated into basal diet to provide the required, 400 ppb of aflatoxin in feed.

**Experimental design:** 120, day-old broiler chicks were procured from Uttam Hatchery, Mataur Distt: Kangra (H.P). The birds were maintained at standard conditions of temperature, humidity, and light on a standard pellet diet with water ad libitum. This study was approved by the institutional animal ethical committee. The birds were randomly divided in 4 groups with 30 birds in each group. Chickens were housed and maintained under a constant temperature with free access to food and drinking water. The chickens were acclimatized for 2 weeks before treatment. Group I served as Control, Group II received 400ppb of Aflatoxin B<sub>1</sub> in feed, Group III received 400 ppb of Aflatoxin B<sub>1</sub> in feed and seabuckthorn pulp aquamethanolic extract @1000ppm in feed. Group IV received seabuckthorn pulp aquamethanolic extract @1000ppm in feed.

**Sample Collection:** The blood was collected by wing vein puncture, and serum was used for estimating biochemical parameters: total proteins (TP), albumin, Total serum bilirubin

Concentrations, Serum aspartate amino transaminase (**AST**), and alanine amino transaminase (**ALT**), Lactate Dehydrogenase (**LDH**). The liver was collected for macroscopic and histopathological examination in 10% buffered formaline.

**Statistical analysis:** The data was analyzed by analysis of variance test using the Graph Pad InStat version 3.00 for windows (GraphPad Software, San Diego, California, USA, ([www.Graphpad.com](http://www.Graphpad.com))) and the significant differences between mean values were determined using Turkey-kramer Multiple comparison test. The data were presented as Mean±SE. The inter group comparisons were made at 5% level of significance.

## RESULTS AND DISCUSSION

**Internal organ weight (Liver):** A significant increase in the weight of liver was observed in aflatoxin treated group (Group II) in comparison to all other treatments. Group III (AF+SBT pulp) showed the internal organ (liver) weight value near to Group I (Table 1). These findings suggest a protective effect of SBT pulp against the deleterious hepatic effects of AFB<sub>1</sub>. The enlargement of liver may be due to the interference in lipid transport and lipid synthesis so that more accumulation of lipids inside the hepatocytes occurs [20]. Significant ( $P < 0.001$ ) increase in the relative weights of liver in birds fed aflatoxin contaminated feed as observed in the present study has been reported earlier with Aflatoxin [21]. This result may be due to the hepatotoxic effect of AF, resulting in appreciable changes in the functioning and gross appearance of liver [20].

**Table 1. Effect of dietary seabuckthorn leaves aquamethanolic (70%) extract supplementation on internal Organ weight (Liver) (g) in broilers**

GROUPS	CONTROL	AF	AF+SBT pulp	SBT pulp
LIVER	19.54 <sup>a</sup> ±0.756	36.47 <sup>b</sup> ±0.715	25.57 <sup>c</sup> ±1.494	20.71 <sup>ac</sup> ±0.680

Mean values ± S.E. (n=30), the means with same superscripts in between columns do not differ significantly at 5% level

**Biochemical profile:** The results of biochemical studies are depicted in Table 2. The AFB<sub>1</sub> determined a significant reduction of TP, albumins and increased the AST, ALT and LDH activity ( $P < 0.001$ ) in the group II (AFB<sub>1</sub> treated) in comparison with the control group. Administration of SBT pulp along with AF (Group-III) restored these values to normal levels. In present study, significant ( $P < 0.05$ ) increase in the serum total bilirubin was observed in aflatoxin group (Group II) whereas in all other groups it came out to be in normal range, suggesting the hepatoprotective effect of SBT pulp aquamethanolic extract. A significant

increase may be attributed to the hepatotoxicity caused by the aflatoxin. As the serum bilirubin is the product resulted from the metabolism of spent haemoglobin and produced in the liver. This reflects the status of liver health in animal [30]. High activity of AST, ALT, LDH, and total bilirubin were indicative of hepatocellular destruction and dysfunction [30,31]. Hence, normal values of total bilirubin in Group III (AF+SBT leaves) shows that SBT has hepatoprotective action [32]. A mechanism of action of the AFB<sub>1</sub> is related to the inhibition of protein synthesis, which was previously reported [26]. Additionally, a decrease in the concentration of whole plasma proteins and in albumin have been proposed as indicators of the alteration in protein synthesis observed in aflatoxicosis. This damage might be used as an indicator in early diagnosis of aflatoxicosis and thus preventive measures could be adopted [27]. ALT activity significantly increased in the AFB<sub>1</sub>-treated birds whereas the ALT activity was restored to normal levels in SBT treated group (Group-III). It indicates that SBT pulp was successful in ameliorating the hepatotoxic activity of aflatoxin. A similar trend of observations was reported in case of AST and LDH activity.

**Table 2. Effects of diets containing seabuckthorn (SBT) leaves, aflatoxin B1 (AFB1), or both, on total proteins (TP), albumins (ALB), globulins (GLB), alanine amino transaminase (ALT), and aspartate amino transaminase (AST), LDH, Total bilirubin**

GROUPS	Total Protein (g/dl)	Total Albumin (g/dl)	ALT (U/L)	AST (U/L)	LDH (U/L)	Total bilirubin (mg/dl)
<b>CONTROL</b>	3.41±0.154 <sup>a</sup>	1.73±0.176 <sup>a</sup>	172.67 <sup>a</sup> ±6.82	68.5 <sup>a</sup> ±5.84	474.89 <sup>a</sup> ±18.91	0.426 <sup>a</sup> ±0.0424
<b>AF</b>	1.99±0.101 <sup>b</sup>	1.28±.173 <sup>b</sup>	247.17 <sup>b</sup> ±16.54	147.16 <sup>b</sup> ±16.87	677.95 <sup>b</sup> ±45.41	.98 <sup>b</sup> ±0.107
<b>AF+SBT Leaves</b>	3.06±0.184 <sup>a</sup>	1.56±.149 <sup>a</sup>	188.83 <sup>a</sup> ±7.95	87.83 <sup>ac</sup> ±5.46	507.11 <sup>a</sup> ±43.02	0.77 <sup>c</sup> ±0.099
<b>SBT Leaves</b>	3.64±.159 <sup>c</sup>	1.96±.187 <sup>a</sup>	162.0 <sup>a</sup> ±5.23	53.33 <sup>ac</sup> ±6.48	396.37 <sup>d</sup> ±16.56	0.41 <sup>a</sup> ±0.032

Mean values ± S.E. (n=30), the means with same superscripts in between columns do not differ significantly at 5% level

### CONCLUSION

The hepatoprotective potential of Seabuckthorn pulp aqua methanolic extract (70%) was evaluated in aflatoxin induced hepatotoxicity. Birds treated with SBT pulp aqua methanolic extract showed improved biochemical parameters indicating amelioration by the SBT pulp extract. The extract proved to be hepatoprotective as it ameliorated the hepatotoxicity induced by aflatoxin B<sub>1</sub> in experimental birds.

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