Haryana Vet. (June, 2018) **57 (1)**, 1-5

Research Article

EFFECT OF SEABUCKTHORN (*HIPPOPHAE RHAMNOIDES*) OIL ON AFLATOXIN INDUCED HEPATOTOXICITY IN BROILER CHICKENS

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ABSTRACT

The aim of this study was to evaluate the hepatoprotective activity of seabuckthorn (SBT) oil against toxicity induced by aflatoxin B_1 (AFB_1) in broiler chickens.The experiment included 210 broiler chickens (30 birds per group) divided into 7 groups viz. Control (T_1), aflatoxin (AF) (T_2), aflatoxin+Glucomannan(GM)(T_3), aflatoxin + SBT oil @0.5ml/kg b.w (T_4), Aflatoxin + SBT oil @ 1.0ml/kg b.w (T_5), only SBT oil treated group@0.5ml/kgb.w (T_6) and SBT oil treated group@ 1.0 ml/kg b.w (T_7). GM was mixed with the feed @ 1g/ kg whereas, AF was added @ 400ppb in feed for duration of 28 days. AF treatment significantly ($P<0.05$) reduced weight gain and increased feed conversion ratio (FCR). SBT oil as well as GM significantly ($P<0.05$) improved the growth performance. AF treatment produced a significant ($P<0.05$) decrease in total serum proteins, albumin, globulin, and significant ($P<0.05$) increase in serumaspartate amino transaminase (AST), alanine amino transaminase (ALT),lactate dehydrogenase (LDH) and total serum bilirubin. However, SBT oil as well as GM significantly ($P<0.05$) restored these biochemical parameters to normal levels. The histopathological lesions in the liver were severe in AF treated group whereas least severe in SBToil treated groups. On the basis of the growth performance, biochemical parameters and histopathology of liver, it could be concluded that oral supplementation of seabuckthorn oil provide protection against aflatoxicosis in broilers.

Key Words: Aflatoxicosis, Broilers, Hepatoprotective, Seabuckthorn,

Aflatoxins are produced by the strains of *Aspergillus flavus* and *Aspergillus parasiticus* and can be present as contaminants in a variety of food and feedstuffs. Aflatoxin B_1 is the most prevalent form and has potent hepatotoxic, carcinogenic, genotoxic, immunotoxic and other adverse effects in many animal species including poultry (Beg *et al.,* 2006). Seabuckthorn (*Hippophae rhamnoides*) plant parts are considered to be a good source of large number of bioactive substances like vitamins (A, C, E, K, riboflavin, folic acid), carotenoids (carotene, lycopene), phytosterols (ergosterol, stigmasterol, lansterol, amyrins), organic acids (malic acid, oxalic acid), polyunsaturated fatty acids and some essential amino acids (Beveridge *et al.,* 1999). The flavones of seabuckthorn have antioxidant, anti-ulcerogenic and hepato-protective actions, and its berry oil is reported to inhibit platelet aggregation and prevent thrombosis (Cheng *et al.,* 2003). Seabuckthorn oil extracted from fruits and seeds contains high levels of beneficial unsaturated fatty acids (omega-3, 6, 7), natural antioxidants and vitamins (E, K), carotenoids, as well as phytosterols (Beveridge *et al.,* 1999). During recent years, nutraceutical properties of seabuckthorn leaves are being utilised in poultry as feed substitute indicating that 3% substitution of crude protein offers better results in performance of broilers (Ambatkar, 2009). However, the role of seabuckthorn oil in protecting liver from aflatoxins has not been assessed so far. So, this study was conducted to examine the protective effect of seabuckthorn oil on the aflatoxin induced liver damage.

MATERIALS AND METHODS

Aspergillus flavus (MTCC-9367) culture was obtained from Institute of Microbial Technology, Chandigarh, India for the production of aflatoxin. The fungus was maintained by subculturing in potato dextrose agar (PDA) medium for 10 days interval to ensure its viability (Shotwell *et al*., 1966). The aflatoxin was produced on rice (Shotwell *et al*., 1966). One hundred grams of rice was taken in 500 mL conical flask and soaked in 50 mL of water for 2 h with frequent shaking. Then the flasks were autoclaved at 15 psi/15 min, cooled at room temperature and inoculated with *Aspergillus flavus* MTCC 9367 culture. The flasks were kept at 25°C temperature in slanting position with vigorous shaking for 4 to 6 times a day to prevent clumping and also for better production of aflatoxin. After 48 h, the mould growth was observed as white spots on the surface of the rice grains. On 10th day, the mouldy rice was briefly steamed for 5 min to kill the spores and was dried overnight at 250°C in hot air oven. The mouldy rice was grounded to a fine powder.

Estimation of aflatoxin: The aflatoxin content in the mouldy rice powder was estimated by thin layer chromatography at Animal Feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu.

Collection of plant material*:* The seeds of seabuckthorn were collected from Highland Agricultural Research & Extension Centre, Farm Science Centre, CSKHPKV, Kukumseri, Lahaul and Spiti, India. Seabuckthorn seed oil was extracted from seeds by manual extraction.

Chemicals: The purified glucomannan (GM) powder was procured from the Neospark, Drug and Chemicals Private Limited, Hyderabad, Andhra Pradesh (India) as a complimentary sample.

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Table 1
Effect of dietary seabuckthorn oil supplementation and glucomannan on body weight (g), FCR and organ weight (g) in broilers during test period

Treatments	Body weight (g)	Feed conversion ratio	organ weight (g)		
			Liver	Kidneys	Bursa of Fabricius
Control(T1)	1364±43.13[a]	1.84±0.05[a]	20.00±0.36[a]	2.00±0.08[a]	4.13±0.08[a]
Aflatoxin(T2)	1006±42.08[b]	2.20±0.08[b]	37.67±0.92[b]	2.06±0.07[a]	2.75±0.14[b]
AF+GM (T3)	1227±45.96[a]	1.97±0.07[ab]	26.83±1.52[a]	1.88±0.06[a]	4.15±0.37[a]
AF +SBT oil I(T4)	1221±31.45[a]	2.02±0.06[ab]	24.16±1.42[a]	2.01±0.13[a]	3.80±0.23[a]
AF +SBT oil II(T5)	1284±41.62[a]	1.93±0.06[ab]	25.16±1.25[a]	1.91±0.07[a]	3.86±0.11[a]
SBT oil I(T6)	1444±37.4 [a]	1.85±0.03[a]	23.00±0.82[a]	1.71±0.08[a]	4.51±0.16[a]
SBT oil II(T7)	1395±35.70 [a]	1.86±0.04[a]	22.50±1.03[a]	1.91±0.70[a]	4.53±0.12[a]

AF = Aflatoxin (400ppb), GM =Glucomannan (1g/kg feed), SBT oil I = Seabuckthorn seed oil @ 0.5ml/kg body weight, SBT oil II =Seabuckthorn seed oil @ 1.0ml/kg body weight.

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

Experimental design: A total of 210 day-old broiler chicks were procured from a local hatchery. 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 seven groups with 30 birds in each group.The chicks were acclimatized for 2 weeks before treatment. The birds were divided inseven groups viz. Control (T_1), Aflatoxin (AF) (T_2), Aflatoxin + Glucomannan (T_3), Aflatoxin + SBT oil @0.5ml/kg (T_4), Aflatoxin + SBT oil @ 1.0ml/kg (T_5), only SBT oil treated group@0.5ml/kg (T_6) and SBT oil treated group@ 1.0 ml/kg (T_7). GM was mixed with the feed @ 1g/ kg whereas, AF was added @ 400ppb in feed for duration of 28 days.

Sample Collection: The blood was collected in a sterilized vial without any anticoagulant for serum separation. The serum was used for estimating different biochemical parameters such as total proteins (TP), albumin, globulin, bilirubin, aspartate amino transaminase (AST), alanine amino transaminase (ALT) and lactate dehydrogenase (LDH) by using the diagnostic kits (Erba diagnostics, Manheim, Germany and Span Diagnostics, Surat, India) in a semi-automated blood chemistry analyzer (Auto Chemistry System, Bayer Diagnostics).The liver was thoroughly examined for macroscopic lesions and representative tissue samples were collected in 10% buffered formalin for histopathological examination.

Statistical analysis: The data was analyzed by analysis of variance test using the Graph Pad Instatversion 3.00 for windows (GraphPad Software, SanDiego, California, USA (www.graphpad.com) and the significant differences between mean values were determined using Tukey-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

Growth performance:The effects of SBT oil and GM on growth performance of broiler chicks were studied in terms of body weight gain, feed conversion ratio (FCR) and organ weight of liver, kidneys and bursa of Fabricius (Table 1). The birds fed on aflatoxin (T2) contaminated feed (400 ppb) showed a significant ($P<0.05$) decrease in body weight, feed intake and an increase in FCR. The results of present investigation were in agreement with the observations of Aravind *et al*. (2003) and Raju and Devegowda (2000). Body weight, feed intake and FCR were significantly restored in T3,T4 and T5 groups. The relative weights of liver increased significantly ($P<0.05$) with addition of 400 ppb AF (Table 1), The increase in liver weights as seen in present study were similar to the findings of Perozo and Rivera (2003), against aflatoxicosis in broilers.The increase in liver weight could be attributed to increased lipid deposition in liver due to impaired fat metabolism by aflatoxin (Batina*et al.*, 2005). The bursal weights were found significantly ($P<0.05$) lowered in T2 group following addition of 400ppb aflatoxin in feed (Table 1) as compared to control group (T1). A similar reduction in weights of thymus and bursa in broilers fed with aflatoxin were reported by Smith and Ross (1991). The weights of liver were significantly decreased and bursa were significantly increased ($P<0.05$) in T3, T4 and T5 groups as compared to aflatoxin treated T2 group. These observations are in consonance with the findings of Geetha *et al*. (2003).

Biochemical profile: The dietary intake of aflatoxin significantly decreased ($P<0.05$) the total serum protein,

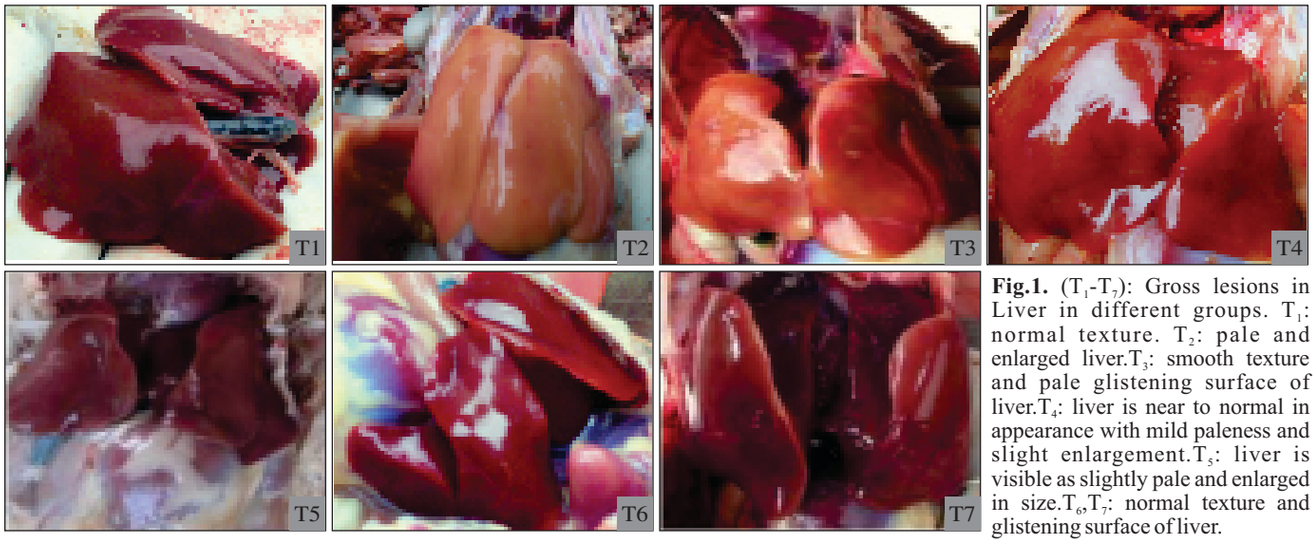

Fig.1. (T_1-T_7): Gross lesions in Liver in different groups. T_1: normal texture. T_2: pale and enlarged liver.T_3: smooth texture and pale glistening surface of liver.T_4: liver is near to normal in appearance with mild paleness and slight enlargement.T_5: liver is visible as slightly pale and enlarged in size.T_6,T_7: normal texture and glistening surface of liver.

serum albumin and serum globulin in broilers as compared to control group (Table 2). A decrease in concentration of total serum proteins and albumin have been proposed as indicators of the alteration in protein synthesis observed in aflatoxicosis (Jindal *et al.,* 1994; Abo- Norag *et al.,* 1995). The addition of GM and SBT oil to the aflatoxin contaminated diet increased the serum protein, serum albumin and serum globulin in broiler chick as compared to aflatoxin treated group (T2). Geetha *et al.* (2008) and Shashikanth *et al.* (2011) also reported that the rats fed with seabuckthorn extract possessed higher serum protein levels. The seabuckthorn oil treatments at both doses (T4 and T5) and glucomannan caused non-significant(P>0.05) increases in A/G ratio in broiler chicks.

The dietary administration of aflatoxin produced significant increase (P<0.05) in the AST, ALT and ALP level in comparison to control and all other treatment groups (Table 2). Similar to present study, the earlier workers also reported significant elevation in the ALT and AST levels in aflatoxin treated chicks (Kim *et al.,* 2003; Jayabarathi and Mohamudha, 2010). Feeding of GM in aflatoxin treated group (T3) and SBT oil in aflatoxin treated groups (T4 and T5) decreased AST activity in a non-significant manner (P>0.05). The dietary intake of glucomannan and seabuckthorn oil decreased AST activity in a non-significant manner (P>0.05). Basmacoiglu *et al.* (2005) also reported decrease in AST levels following treatment with glucomannan alone and in combination with seabuckthorn in broilers. Treatment with GM and SBT oil at both doses significantly (P<0.05) restored the ALT and ALP levels.

In the present study there was significant increase (P<0.05) in the LDH activity in aflatoxin treated group (T2) as compared to control (T1). Ozyurt *et al.* (2006)

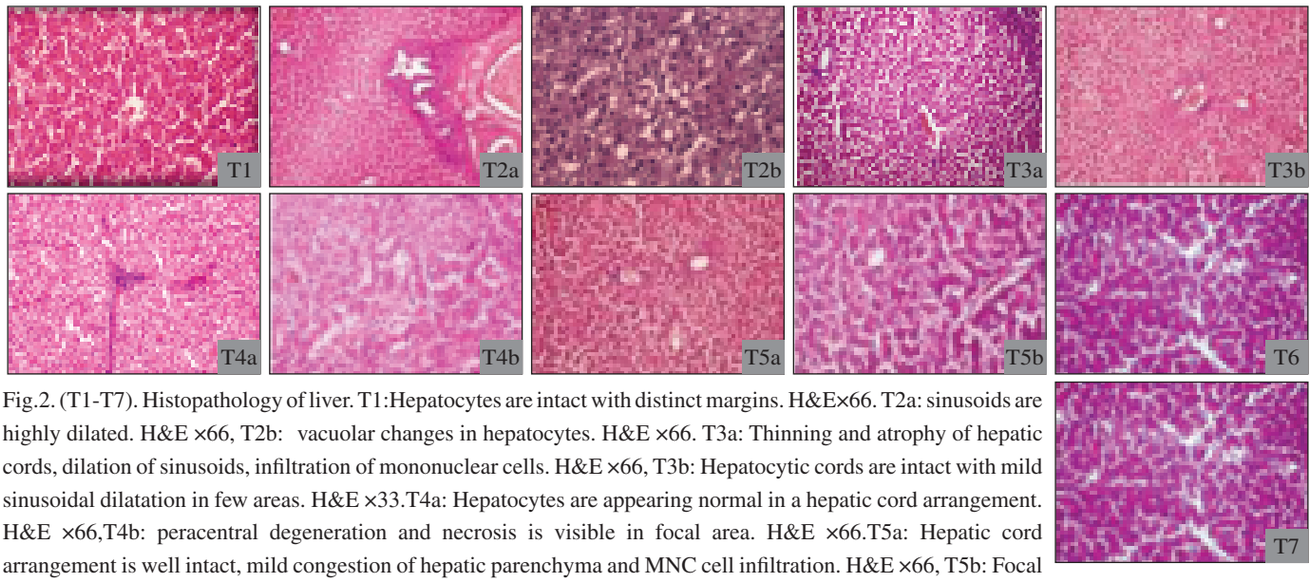

Fig.2. (T1-T7). Histopathology of liver. T1:Hepatocytes are intact with distinct margins. H&E×66. T2a: sinusoids are highly dilated. H&E ×66, T2b: vacuolar changes in hepatocytes. H&E ×66. T3a: Thinning and atrophy of hepatic cords, dilation of sinusoids, infiltration of mononuclear cells. H&E ×66, T3b: Hepatocytic cords are intact with mild sinusoidal dilatation in few areas. H&E ×33.T4a: Hepatocytes are appearing normal in a hepatic cord arrangement. H&E ×66,T4b: peracentral degeneration and necrosis is visible in focal area. H&E ×66.T5a: Hepatic cord arrangement is well intact, mild congestion of hepatic parenchyma and MNC cell infiltration. H&E ×66, T5b: Focal area of necrosis is visible in the liver parenchyma along with mononuclear cell infiltration. H&E ×66.T6, T7: Hepatic cord arrangement is well intact. Hepatocytes seem to be normal in appearance. H&E ×33.

reported that AST, ALT, GGT and LDH usually appear in serum when there is damage on the liver and muscle tissues caused by aflatoxin.The groups treated with aflatoxin and glucomannan (T3), seabuckthorn oil and aflatoxin (T4 and T5) have shown reduction in serum LDH activity significantly as compared to aflatoxin treatedgroup (T2). Serum total bilirubin is the product resulted from the metabolism of haemoglobin and produced in the liver. This reflects the status of liver health in animal. However, in the present study, the total serum bilirubin levels increased significantly ($P<0.05$) in aflatoxin treated group. However, glucomannan (T3) and SBT oil (T4 and T5) restored thenormalized level of the total bilirubin range. The findings of the present studies revealed that the SBT oil has hepatoprotective effect in the broiler birds.

Pathological changes: The gross and histopathological changes in the liver are depicted in the Fig.1 and Fig. 2, respectively. Macroscopically in control group, liver grossly appeared to be normal in texture without any discolouration and enlargement. Grossly, the liver of aflatoxin fed birds (T2) was pale and enlarged in appearance. These observations were in agreement with previous reports (Srivani *et al.,* 2003). In aflatoxin and glucomannan treated group (T3), the liver was found to be slightly pale, icteric, and enlarged in size to a mild extent in comparison to aflatoxin group (T2). Also, moderately dilated liver sinusoidal spaces were observed. In the seabuckthorn oil treated groups at both doses (T4 and T5) along with aflatoxin treated birds, livers of broilers were slightly pale in comparison to normal liver besides slight enlargement. The paleness was comparatively less severe than aflatoxin group (T2) but was more pronounced than only SBT oil treated groups (T6 and T7). In seabuckthorn oil treated groups (T6 and T7) on 28th days post-treatment, the liver was found to be in normal colour and texture.

Histopathologically, in control group (T1) there were well defined margins of hepatocytes with proper arrangement of hepatic cords in hepatic tissue. Hepatocyte degeneration in varying degrees, fibrosis in portal areas and bile duct proliferation were the prominent findings in aflatoxin treated group (T2). In T2 group, many hepatocytes were swollen in periportal and midzonal areas. Some nuclei were pyknotic and there was loss of nuclei in some of the hepatocytes. The fibrosis and bile duct proliferation were prominent in the livers of the birds which were investigated in the aflatoxin treated group (T2). In addition, mild mononuclear cell infiltration was observed in portal areas. These changes in the liver were attributed to the tendency of aflatoxin to produce severe circulatory and degenerative changes in the parenchymotous organs, particularly the liver which is the primary site of protein synthesis. Even though aflatoxin caused degeneration of hepatocytes, the more resistant connective tissue and biliary tissues reacted to toxic injury with proliferative changes. In glucomannan treated group (T3), the cytoplasm of hepatocytes showed mild vacuolation, a focal infiltration with inflammatory cells and moderate hydropic degeneration. The histopathology reflected the protective effect of combined administration of glucomannan that ameliorate aflatoxin in poultry. Similar findings were reported by Gowda *et al.* (2007)

Table 2
Effect of dietary seabuckthorn oil supplementation and glucomannan on biochemical parameters

Group	Serum protein	Albumin	Globulin	A/G ratio	AST	ALT	ALP	LDH	Total Bilirubin
Control (T1)	4.37 ±0.29^{a}	1.60 ±0.04^{a}	2.77 ±0.29^{a}	0.624 ±0.08	167.67 ±5.25^{a}	52.30 ±3.18^{a}	471.83 ±8.44^{a}	450.62 ±32.87^{a}	0.43 ±0.044^{a}
AF (T2)	2.98 ±0.15^{b}	1.34 ± 0.06^{b}	1.63 ±0.08^{b}	0.838 ±0.07	201.11 ±10.94^{b}	73.33 ±2.18^{b}	705.16 ±20.23^{b}	523.89 ±34.03^{b}	1.14 ±0.17^{b}
AF+GM (T3)	3.57 ±0.17^{ab}	1.42 ±0.02^{ab}	1.74 ±0.16^{b}	0.986 ±0.11	179.00 ±3.67^{ab}	57.50 ±4.46^{a}	518.50 ±41.76^{a}	410.47 ±17.38^{ac}	0.83 ±0.08^{a}
AF+SBT oil I(T4)	3.60 ±0.23^{ab}	1.63 ±0.65^{a}	1.66 ±0.23^{b}	1.025 ±0.13	183.00 ±2.83^{ab}	50.83 ±2.99^{a}	479.16 ±16.41^{a}	509.19 ±18.76^{ab}	0.83 ±0.14^{a}
AF+SBToi l II(T5)	3.52 ±0.16^{ab}	1.47 ±0.04^{ab}	1.79 ±0.16^{ab}	0.843 ±0.06	180.00 ±3.64^{ab}	52.5 ±1.87^{a}	463.50 ±15.99^{a}	498.17 ±16.04^{ab}	0.77 ±0.09^{a}
SBT oil I(T6)	4.27 ±0.26^{ac}	2.04 ±0.15^{c}	2.19 ±0.39^{a}	1.12 ±0.22	153.83 ±5.51^{ac}	43.16 ±4.68^{a}	507.00 ±19.33^{a}	390.67 ±17.99^{ad}	0.38 ±0.05^{a}
SBT oil II(T7)	4.56 ±0.26^{ac}	2.07 ±0.15^{c}	2.47 ±0.34^{a}	0.963 ±0.18	162.00 ±5.23^{ac}	44.25 ±2.93^{a}	517.00 ±17.66^{a}	393.19 ±15.42^{ad}	0.41 ±0.03^{a}

AF = Aflatoxin (400ppb), GM =Glucomannan (1g/kg feed), SBT oil I = Seabuckthorn seed oil @ 0.5ml/kg body weight, SBT oil II =Seabuckthorn seed oil @ 1.0ml/kg body weight.

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

AST= aspartate amino transaminase, ALT = alanine amino transaminase, ALP = alkaline phosphatase, LDH = lactate dehydrogenase

while observing mild to moderate lesions in the liver of aflatoxin at 1000 ppb level with GM dosed birds. In SBT oil treated groups (T4 and T5), there were focal areas of peracentral necrosis, slight infiltration of mononuclear cells and increased cytoplasmic granularity. There was also mild degree of sinusoidal dilation. The pathological changes were less severe than aflatoxin treated group (T2) indicating a protective effect of seabuckthorn oil on the liver against hepatic injuries caused by aflatoxin. Similar results were reported by Azuma *et al.* (2003) in broilers treated with seabuckthorn oil. In SBT oil treated groups (T6 and T7), the hepatic cord arrangement was well intact with apparently no microscopic lesion in the hepatocytes .The potential hepatoprotective role of SBT oil may be associated with its antioxidant constituents such as selenium, carotene, tocopherol, phenolic compounds, and vitamin E and C working individually or in synergy (Azuma *et al.,* 2003). On the basis of growth performance, biochemical, gross and histopathological changes, it could be concluded that SBT oil has hepatoprotective effect in poultry aflatoxicosis.

ACKNOWLEDGEMENTS

Authors are thankful to Dean, COVAS, CSKHPKV for providing necessary facilities for carrying out the research work.

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