ORIGINAL ARTICLE

Effects of milk thistle seed against aflatoxin B_1 in broiler model

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Background: Consumption of aflatoxin B_1 (AFB_1) contaminated products can pose a risk of development of various diseases in human and animals due to radical production. The scope of this work is to evaluate the efficacy of milk thistle seed (MTS), as a radical scavenger, on serum biochemistry, lipid profile and liver enzymes against AFB_1 in broiler chickens contaminated with AFB_1. **Materials and Methods:** The effect of nine experimental treatments (3 × 3 factorial design) was assessed using 216 one-d-old Ross 308 male broiler chicks in a randomized complete design with four replicates of six birds for each dietary treatments: Control (T1), 250 ppb AFB_1 (T2), 500 ppb AFB_1 (T3), 0.5% MTS (T4), 0.5% MTS Plus 250 ppb AFB_1 (T5), 0.5% MTS Plus 500 ppb AFB1 (T6), 1.0% MTS (T7), 1.0% MTS Plus 250 ppb AFB_1 (T8), and 1.0% MTS Plus 500 ppb AFB_1 (T9). The individual and combined effects of dietary AFB_1 and MTS on serum biochemistry factors (Glucose, Calcium, Phosphorus, Iron, Creatinine, and Uric acid), lipid profile (Triglyceride, Cholesterol, Low density lipoprotein (LDL), and High density lipoprotein (HDL)) and liver enzymes aspartate amino-transferase and alanine amino-transaminase (ALT) in broilers were evaluated at 21 days of age. Also, statistical packages Macros-1.002 (2010) were used to perform the above analysis on computer. **Results:** Consumption of 500 ppb AFB_1 in to the diet significantly decreased HDL (58.13 ± 2.65), Calcium (7.11 ± 0.13), and Glucose (197.1 ± 7.42) compared to the control group (85.12 ± 1.95, 9.45 ± 0.17 and 223.1 ± 6.61, respectively), ($P < 0.05$). In contrast, it significantly increased creatinine (2.25 ± 0.011) and AST (244.51 ± 4.91). Using MTS together with AFB_1 significantly reduced the effect of AFB_1on the above parameters. **Conclusion:** MTS can provide protection against the negative effects of AFB_1 on broiler chicks.

Key words: Aflatoxin B_1 (AFB_1), fatty acid, liver enzymes, milk thistle seed (MTS), serum biochemistry

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INTRODCTION

Mycotoxins are secondary toxic metabolites that are produced by fungi growing on food products, such as corn, peanut, and wheat, among others.[1] Exposure to mycotoxins occurs predominantly by the ingestion of contaminated cereals such as corn, wheat, peanuts and sorghum, as well as other raw materials used in the preparation of animal feed.[2] Aflatoxicosis in poultry can cause disease and increased mortality.[3,4] Aflatoxins (AFs) are produced by fungi of the genus *Aspergillus*, particularly *A. flavus, A. parasiticus, and A. nomius*.[5] Seventeen metabolites have been identified as aflatoxins, among them AFB_1 exhibits the highest toxigenic effects and being the most commonly found metabolite in cereals.[6] Biochemically, aflatoxins affect energy, carbohydrates lipids, nucleic acids, and protein metabolism.[7] Their biological effects include carcinogenicity, mutagenicity, teratogenicity, and hepatotoxicity caused by oxidative damage.[8]

Determination of biochemical toxic effects of AFs is important for diagnosis of toxicosis in broilers.[9] AF toxicity in broilers has been manifested by decreased serum concentrations of total protein, albumin, total cholesterol,[4] uric acid,[10] and increased hepatic enzyme activities such as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT).[11,12] In Japanese quail, reduction has been seen in the levels of serum total protein, albumin, globulin, glucose, cholesterol, ALT and elevation of AST and Gamma-glutamyltransferase (GGT) and variable *Alkaline phosphatase* (ALP) levels observed in toxin treated groups.[12] Also, the activity of serum enzymes such as AST has been extensively used as a measure of aflatoxin toxicity in chickens.[13]

Silymarin, an extract from the seeds of milk thistle (*Silybum marianum*), is a complex of four flavonolignans that have strong antioxidant and free radical scavenging activity.[14] It has been shown that milk thistle seed (MTS) protects birds against adverse effects of Aflatoxin B_1 (AFB_1).[14] Milk thistle extracts have been used to treat

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diseases.[15] These extracts are also used to successfully treat hepatitis patients.[16] They have hepatoprotective, antiinflammatory, cytoprotective, and anticarcinogenic effects.[16] Keeping in view the medicinal value of milk thistle, this study was conducted to investigate the efficacy of MTS on serum biochemistry, lipid profile, and liver enzymes in broiler chickens contaminated with AFB_1 during an early stage of exposure (21 days). In our knowledge, this is the first work on the effect of AF in 21-day-old broilers and assessment of MTS on its adverse effects.

MATERIALS AND METHODS

Production of aflatoxin on rice

To produce AF, *A. flavus* obtained from fungi collection of the Centre of Scientific and Industrial Research Organization in Iran, PTCC NO:5004 (IR111). AFB_1 production performed via fermentation of rice by the method of Shotwell *et al*., (1966) with minor modifications by Oguz.[4,17] Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. The AF content in rice powder was analyzed by the method of Shotwell *et al*., (1966) and measured on a thin layer chromatography (TLC) fluorometric densitometer (Camag-III, Basel, Switzer-land) on the TLC spots.[17] The AF within the rice powder consisted of 60 ppm AFB_1.

Experimental design and diets

The individual and combined effects of dietary AFB_1 and MTS on serum biochemistry factors, fatty acid and liver enzymes of broilers were evaluated at 21 days of age. Dietary treatments included a 3 × 3 factorial arrangement with three levels of AFB_1 (0, 250, and 500 ppb), and three levels of MTS (0%, 0.5%, and 1.0%) were incorporated into the basal diet (corn, wheat, and soybean meal). The effect of nine experimental treatments was assessed using 216 one-d-old Ross 308 male broiler chicks in a randomized complete design with four replicates of six birds for each dietary treatments: Control (T1), 250 ppb AFB_1 (T2), 500 ppb AFB_1 (T3), 0.5% MTS (T4), 0.5% MTS Plus 250 ppb AFB_1 (T5), 0.5% MTS Plus 500 ppb AFB_1 (T6), 1.0% MTS (T7), 1.0% MTS Plus 250 ppb AFB_1 (T8), 1.0% MTS Plus 500 ppb AFB_1 (T9). Basal diet was formulated and compounded to meet the nutrient requirements of broiler chicks based on Ross recommendation strain during the whole period of experiment without inclusion of either aflatoxin or binder [Table 1].

Biochemical parameters, lipid profile, and liver enzymes

When the chicks reached to three weeks of age, blood samples from two chicks of each treatment was collected by puncturing the brachial vein. The blood sample was allowed to stand for one hour and centrifuged at a speed of 3,000 rpm for 10 minutes. The clear serum was collected in sterilized disposable plastic tubes and stored at −20ºC. The serum samples were analyzed for serum biochemistry factors (Glucose, Calcium (Ca), Phosphorus, Iron, Creatinine, and Uric acid), lipid profile (Triglyceride, Cholesterol, Low-density lipoprotein (LDL), and High-density lipoprotein (HDL)) and liver enzymes (AST and ALT), were measured by biochemical test.

Statistical analysis

The data was statistically analyzed with the standard procedures of analysis of variance (ANOVA), using on 3 × 3 factorial with completely randomized design as suggested by Andrea Onofri (2010).[18] The statistical packages Macros-1.002 (2010) were used to perform the above analysis on computer.[18]

RESULTS

The effects of AFB_1 and MTS on serum biochemistry (Phosphorus, Iron and Uric Acid), liver profile (triglyceride, cholesterol, LDL, and ALT) are shown in Tables 2 and 3. Feeding AF caused significant decrease in serum glucose, calcium and HDL after 21 days compared with the control group (T1) [Table 2]. AFB_1 (500 ppb) had an adverse effect

Table 1: Composition of the starter, grower, and finisher diets fed to broilers (as fed)

Feed Stuffs	Starter period (1-14 day)	Grower period (14-21 day)
Corn	54.43	50.42
Soybean meal (44% CP)	35	30.29
Wheat	–	10
Fish meal (60% CP)	3.07	2.04
Soybean Fat	3.29	3.57
Dicalcium phosphate	1.73	1.47
Oyster shell	1.16	1.04
Mineral Premix[1]	0.5	0.5
Vitamin premix[2]	0.5	0.5
Salt	0.2	0.2
DL-methionine	0.35	0.28
L-lysine	0.24	0.19
Analyzed values		
ME (Kcal Per kg)	2980	3050
CP (Percent)	22	20
Lys (Percent)	1.43	1.24
Met+Cys (Percent)	1.07	0.95
Thr (Percent)	0.31	0.28
Ca (Percent)	1.05	0.90
P (Percent)	0.52	0.45

Provided at the following rates per kilogram of diet: Mn (from $MnSO_4$-H_2O), 0.63 mg; Zn (from ZnO), 0.52 mg; Fe (from $FeSO_4$-$7H_2O$), 22 mg; Cu (from $CuSO_4$-$5H_2O$), 3 mg; I (from Ca $(IO_3)_2$-H_2O), 0.63 mg; Se, 0.08 mg (from sodium selenite), Provided at the following rates per kilogram of diet: 3,400 IU vitamin A, 800 IU vitamin D_3, 11 IU vitamin E, 0.74 mg vitamin B_1, 4.3 mg vitamin B_2, 0.4 mg vitamin B_3, 1.6 mg vitamin B_6, 0.41 mg vitamin B_{12}, 1.8 mg vitamin K_3, 0.6 mg folic acid, 1.8 mg H_2, 200 mg Choline chloride

Table 2: Effect of aflatoxin B_1 (AFB_1) and Milk thistle seeds (MTS) on serum biochemistry

Treatment		Glucose	Calcium	Phosphorus	Iron	Creatinine	Uric acid
Aflatoxin (ppb)	MTS (Percent)	(mole/L)	(mmole/L)	(mmole/L)	(mole/L)	(μmole/L)	(mmole/L)
0	0	223.1±6.61^{b}	9.45±0.17^{a}	6.59±0.23	459.5±6.05	0.58±0.027^{b}	5.57±0.71
250	0	236.5±7.21^{ab}	8.15±0.21^{ab}	6.25±0.29	494.1±5.61	1.81±0.024^{a}	6.41±0.61
500	0	197.1±7.42^{c}	7.11±0.13^{b}	6.31±0.31	409.1±5.35	2.25±0.011^{a}	7.51±0.21
0	0.5	254.1±6.91^{a}	9.41±0.12^{ab}	7.11±0.33	412.1±7.11	0.63±0.013^{b}	5.35±0.21
250	0.5	245.5±7.14^{a}	9.35±0.22^{ab}	6.67±0.31	268.1±5.71	1.74±0.024^{a}	5.35±0.51
500	0.5	239.5±7.24^{ab}	8.35±0.12^{ab}	6.91±0.24	394.5±4.85	1.71±0.031^{a}	6.11±0.52
0	1.0	249.5±6.81^{a}	9.31±0.25^{ab}	7.75±0.26	319.2±7.21	0.49±0.024^{b}	4.81±0.53
250	1.0	254.1±7.33^{a}	9.21±0.24^{ab}	6.35±0.32	317.5±4.65	1.31±0.025^{ab}	6.42±0.52
500	1.0	249.5±7.45^{a}	8.31±0.17^{ab}	6.65±0.28	261.5±4.37	2.03±0.018^{a}	6.31±0.32
±SEM[1]		7.48	0.21	0.31	5.98	0.027	0.54
P value		0.006	0.042	0.49	0.33	0.041	0.74

[(a-c)]Means within a column lacking a common superscript differ significantly ($P<0.05$), [1]Pooled standard error of the mean, NS=Non significant

Table 3: Effect of aflatoxin B_1 (AFB_1) and Milk thistle seeds (MTS) on fatty acid and liver enzymes of blood plasma at the end of period (21 days)

Treatment		Triglycerides	Cholesterol	LDL	HDL	AST	ALT
Aflatoxin (ppb)	MTS (Percent)	(mole/L)	(mole/L)	(mmole/L)	(mmole/L)	(U/L)	(U/L)
0	0	136.11±5.21	141.51±8.27	32.11±5.25	85.12±4.95^{a}	196.11±5.45^{b}	20.11±3.21^{b}
250	0	142.51±6.11	161.52±9.31	37.51±4.24	81.11±3.64^{ab}	222.12±5.17^{ab}	28.55±2.84^{ab}
500	0	152.72±7.44	168.71±7.33	41.25±5.31	58.13±4.65^{b}	244.51±4.91^{a}	36.22±3.93^{a}
0	0.5	133.51±6.31	150.52±9.82	52.25±3.81	89.25±3.15^{a}	207.12±5.39^{b}	21.35±2.91^{ab}
250	0.5	133.54±7.44	151.12±8.21	51.25±4.35	84.51±5.11^{ab}	197.71±4.42^{b}	24.91±3.95^{ab}
500	0.5	139.12±4.22	156.71±7.25	19.51±5.24	70.52±4.85^{ab}	207.51±6.11^{b}	27.92±2.82^{ab}
0	1.0	132.11±6.14	144.52±7.45	34.25±6.56	91.25±3.71^{a}	192.52±4.65^{b}	22.12±3.24^{ab}
250	1.0	125.52±4.23	148.12±7.94	27.75±3.73	88.12±5.67^{ab}	201.12 ±6.91^{b}	22.27±3.12^{ab}
500	1.0	143.12±5.55	143.13±8.27	29.11±4.18	67.11±5.47^{ab}	212.51±4.86^{b}	25.95±3.92^{ab}
±SEM[1]		6.07	8.42	4.84	4.93	5.53	3.44
P value		0.81	0.67	0.052	0.043	0.045	0.51

[(a-b)]Means within a column lacking a common superscript differ significantly ($P<0.05$), [1]Pooled standard error of the mean, NS=Not significant

on liver enzyme (AST and ALT) enhancing the values (244.51 ± 4.91 and 36.22 ± 3.93), respectively, compared to control group (196.11 ± 5.45 and 20.11 ± 3.21) ($P < 0.05$). Furthermore, addition of different levels of AFB_1 alone (250 ppb and 500 ppb), into the diet significantly decreased creatinine (1.81 ± 0.024 and 2.25 ± 0.011, respectively) compared to the control group (0.58 ± 0.027), ($P < 0.05$). Interestingly, MTS supplementation to AF-contaminated feed (500 ppb) significantly increased serum glucose and decreased creatinine and AST levels ($P < 0.05$) compare to AFB_1 (500 ppb). Although, MST declined ALT levels compare to AFB_1 groups (T_2 and T_3) but it was not significant ($P = 0.51$). AF (500 ppb) caused a marked drop in HDL and MTS at 1% alone increased HDL (91.25 ± 2.71) concentration in comparison to control group (85.12 ± 1.95), and MTS supplementation to AF-contaminated feed also enhanced HDL but it was not significant.

DISCUSSION

Our results showed that serum total glucose level was significantly different between groups contaminated with different levels of AFB_1. Previous studies performed with high levels of AF (2.5-5 mg/kg diet) showed significant decreases in serum total glucose, protein, albumin, total cholesterol and uric acid levels.[4,19] The differences between previous studies and our current findings can be related to the AF doses in the diet. In the present study, the use of low AF levels (250 ppb) did not cause any significant changes in the calcium, glucose, HDL, and AST compared to control group.[19] Protein synthesis inhibition in the liver together with other damage to the liver and kidney could induce the biochemical changes during aflatoxicosis.[20] The results also revealed that treatment with MTS prevented changes in AST activity, supporting the idea that these MTS may provide protection against toxic effects of AFB_1. Other authors reported no significant changes in serum biochemistry for the lower dietary AF, such as 100 ppb[21] and 200 ppb[22] in broiler feed. However, when AF levels increased in food up to 300 ppb and more the serum biochemistry was significantly affected and total protein, albumin, and cholesterol levels were decreased.[22] These results agree with data reported by Henry *et al.*, who fed broilers 80 ppm AFB_1 for three weeks and observed an increase in the levels of ALT and AST liver enzymes.[23] Similar results were also observed by Ledoux *et al.*, in experiments with both turkeys and

broilers.[24] The liver is the principal organ for AF toxicity where most aflatoxins are bioactivated to the reactive form, which is known to bind deoxyribonucleic acid (DNA) and proteins, damaging the liver structures.[19,25] Liver function tests, including serum levels of liver enzymes, are used to assess injury to liver cells, the liver's ability to synthesize proteins, and the excretory functions of the liver.[26,27] Elevated serum enzyme test results usually indicate liver injury earlier than other indicators of liver function. The key enzymes are ALT and AST, which are present in liver cells. ALT is liver specific, whereas AST is derived from organs other than the liver. In most cases of liver damage, there are parallel rises in ALT and AST like what has been shown in this research. In the present study, serum AST and ALT activity was significantly increased in birds consuming 500 ppb of AFB_1 [Table 3]. The elevation of AST and ALT may be due to disruption of hepatic cell as a result of necrosis or a consequence of altered membrane permeability.[28] Supplementation with low (0.5) and high (1.0) percent of MTS to the diet resulted normal serum biochemistry on glucose and AST levels. Tedesco *et al*., (2004) found similar results on serum biochemistry in broilers consumed with milk thistle diets alone.[14] In addition, ALT activity in the blood plasma of aflatoxin-treated group supplied with silymarin did not differ from the non-treated control group in their experiment.[14,20] It has been demonstrated that the activation of AFB_1 in human and rat liver is a complex process controlled by multiple cytochrome P450 enzymes.[1,19] Silymarin can inhibit the cytochrome P450 system, and consequently inhibit AFB_1 activation.[14] However, Gordon *et al*., (2006) did not prove the significant decrease of ALT activity in patients suffering from chronic hepatitis when they were given formulas with thistle seeds.[29] Presence of AFB_1 in food chain is associated with decrease in quality and quantity of food and feed materials.[19,20] Almost all of the biochemical changes due to AF presented in this research is in accordance with rat and human.[1,30,31] The hazard of AF to human health may result from direct ingestion of mycotoxins contaminated cereals with fungi as well as secondary contamination through products of animals and poultry with remains of mycotoxins or their metabolites in the animal products. In conclusion, our results suggest that MTS may provide protection against the negative effects of AFB_1 on broiler chicks and indirectly reduce development of liver and kidney disease in human.

CONCLUSION

Results showed that treatment with MTS can be effective in counteracting the negative effects of AFB_1 intoxication on AST, HDL, and calcium in broilers. The results showed that consumption of different levels of MTS from 0.5% to 1.0% in poultry diets may alleviate some of the adverse effects of aflatoxin in growing broilers and more importantly reduce the aflatoxin-contaminated meat used in human food.

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REFERENCES

1. Hussein HS, Brasel JM. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 2001;167:101-34.
2. Chu FS. Mycotoxins: Food contamination, mechanism, carcinogenic potential and preventive measures. Mutat Res 1991;259:291-306.
3. Ibrahim IK, Shareef AM, Al-Joubory KM. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Res Vet Sci 2000;69:119-22.
4. Oguz H, Kececi T, Birdane YO, Onder F, Kurtoglu V. Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during experimental aflatoxicosis. Res Vet Sci 2000;69:89-93.
5. Moss MO. Recent studies of mycotoxins. Symp Ser Soc Appl Microbiol 1998;27:62-76S.
6. IARC. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42 (Supplement 7) International Agency for Research on Cancer, Lyon, France; 1987.
7. Ellis WO, Smith JP, Simpson BK, Oldham JH. Aflatoxin in food: Occurrence, biosynthesis, effects onorganisms, detection and methods of control. Crit Rev Food Sci Nutr 1991;30:403-39.
8. Forrester LM, Neal GE, Judah D, Glancey MJ, Wolf CF. Evidence for involvement of multiple forms of cytochrome P-450 in aflatoxin B1 metabolism in human liver. Proc Natl Acad Sci U S A 1990;87:8306-10.
9. Rosa CA, Miazzo R, Magnoli C, Salvano M, Chiacchiera SM, Ferrero S, *et al*. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poult Sci 2001;80:139-44.
10. Kececi T, Oguz H, Kurtoglu V, Demet O. Effects of polyvinylpolypyrrolidone, synthetic zeolite, and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br Poult Sci 1998;39:452-8.
11. Santurio JM, Mallmann CA, Rosa AP, Appel G, Heer A, Dageförde S, *et al*. Effect of sodium bentonite on the performance and blood variables of broiler chickens intoxicated with aflatoxin. Br Poult Sci 1999;40:115-9.
12. Madheswaran R, Balachandran C, Murali Manohar B. Influence of dietary culture material containing aflatoxin and T(2) toxin on certain serum biochemical constituents in Japanese quail. Mycopathologia 2004;158:337-41.
13. Leeson S, Summers JD. Some nutritional implications of leg problems in poultry. Br Vet J 1988;44:81-92.
14. Tedesco D, Steidler S, Galletti S, Tameni M, Sonzogni O, Ravarotto L. Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chicks. Poult Sci 2004;83:1839-43.
15. Schrieber SJ, Wen Z, Vourvahis M, Smith PC, Fried MW, Kashuba AD, *et al*. The pharmacokinetics of silymarin is altered in patients with hepatitis C virus and nonalcoholic fatty liver disease and correlates with plasma caspase-3/7 activity. Drug Metab Dispos 2008;36:1909-16.
16. Bares JM, Berger J, Nelson JE, Messner DJ, Schildt S, Standish LJ, *et al*. Silybin treatment is associated with reduction in serum ferritin in patients with chronic hepatitis C. J Clin Gastroenterol 2008;42:937-44.

17. Shotwell OL, Hesseltine CV, Stubblefield RD, Sorenson WG. Production of aflatoxin on rice. Appl Microb 1966;14:425-8.
18. *Onofri A*. Department of Agriculture and Environmental Sciences, University of Perugia, Borgo XX Giugno,. 74-06121 Perugia, Italy. 2010.
19. Yunus AW, Razzazi-Fazeli E, Bohm J. Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: A review of history and contemporary issues. Toxins 2011;3:566-90.
20. Wu JW, Lin LC, Hung SC, Lin CH, Chi CW, Tsai TH. Hepatobiliary excretion of silibinin in normal and liver cirrhotic rats. Drug Metab Dispos 2008;36:589-96.
21. Matur E, Ergul E, Akyazi I, Eraslan E, Cirakli ZT. The effects of Saccharomyces cerevisiae extract on the weight of some organs, liver, and pancreatic digestive enzyme activity in breeder hens fed diets contaminated with aflatoxins. Poult Sci 2010;89:2213-20.
22. Johri TS, Sadagopan VR, Shrivastava HP, Majum-Dar S. Effect of aflatoxin on the performance of purebred chicks. Indian Anim Sci 1990;60:1246-8.
23. Henry MH, Wyatt RD, Fletcher OJ. The toxicity of purified fumonisin B1 in broiler chicks. Poult Sci 2000;79:1378-84.
24. Ledoux DR, Brown TP, Weibking TS, Rottinghaus GE. Fumonisin toxicity in broiler chicks. J Vet Diagn Invest 1992;4:330-3.
25. Miazzo R, Peralta MF, Magnoli C, Salvano M, Ferrero S, Chiacchiera SM, *et al*. Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumunisin. Poult Sci 2005;84:1-8.
26. Pratt DS, Kaplan MM. Evaluation of abnormal liver enzyme results in asymptomatic patients. N Engl J Med 2000;342:1266-71.
27. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. CMAJ 2005;172:367-79.
28. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology 2008;245:194-205.
29. Gordon A, Hobbs DA, Bowden DS, Bailey MJ, Mitchell J, Fransis AJ, *et al.* Effects of Silybummarianum on serum hepatitis C virus RNA, alanine aminotransferase levels and well-being in patients with chronic hepatitis C. J Gastroenterol Hepatol 2006;21:275-80.
30. Sharma V, Gupta R, Sharma S. Effect of oral administration of ethanolic root extract of Tinospora cordifolia on aflatoxin B1-induced toxicity in swiss albino mice. J Nat Pharm 2011;2:125-32.
31. Sheen LY, Wu CC, Lii CK, Tsai SJ. Effect of diallyl sulfide and diallyl disulfide, the active principles of garlic, on the aflatoxin B1-induced DNA damage in primary rat hepatocytes. Toxicol Lett 2001;122:45-52.

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