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Spirulina platensis Inhibits Aflatoxin B_1 Induced Biochemical Changes in Male Swiss Albino Mice

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ABSTRACT

Aflatoxins (AF) are harmful metabolites produced by Aspergillums species principally by *Aspergillus. flavus and Aspergillus parasiticus*. Aflatoxins are hepatotoxic, teratogenic, mutagenic and carcinogenic. The main objective of the current study was to evaluate protective effects of *Spirulina platensis* extract against aflatoxin B_1 (AFB_1) induced biochemical changes in male Swiss albino mice. Randomly 25 healthy inbred mice were allocated into five groups, each having 5 mice. Group I (Control group), mice received normal diet. Group II mice received 100 mg/kg/day of *S. platensis* extract. Group III mice received 200 µg/kg/day of AFB_1. Group IV mice received *S. platensis* extract 100 mg/kg/day and 200 µg/kg/day of AFB_1. Group V mice received *S. platensis* extract 200 mg/kg/day and 200 µg/kg/day of AFB_1 for 28 days. Levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), globulin, albumin and total plasma protein were analyzed in blood samples using an automated biochemistry analyser. Data analysis was done using one way ANOVA with Tukey's Honestly Significantly Differenced (HSD) post-hoc analysis. Statistical significance level was set at $P<0.05$. Results showed that compared to group 1 (control), group 3 (200 µg/Kg/day AFB_1) had increased levels of ALT; (44.0±6.83 IU/L vs. 61.0±8.19 IU/L; p=0.054), AST (176.75±44.34 IU/L vs. 256±115.99 IU/L; p=0.0195) and ALP (51.75±11.89 IU/L vs. 59.40±6.91 IU/L; p =0.049). Mice that were co-treated with 200 µg/Kg/day of AFB_1 and 200 mg/Kg/day of *S. platensis* extract exhibited lower levels compared to mice treated with only 200 mg/Kg/day of AFB_1; ALT (49.8±7.9 IU/L vs. 61.5±8.19 IU/L; p=0.039), AST (229.8±95 IU/L vs. 256±11.15 IU/L; p=0.04819) and ALP (26.5±13.48 IU/L vs. 49.75±4.1 IU/L; p=0.0444). In conclusion, our study findings suggest that supplementation of *S. platensis* extract at a level of 100 mg/Kg/day and 200 mg/Kg/day can reverse elevation of ALT, AST and ALP serum levels caused by 200 µg/Kg/day of AFB_1 in male Swiss albino mice.

Keywords: Spirulina platensis, aflatoxin B_1, biochemical changes, protective effects.

INTRODUCTION

Aflatoxins are formed by strains of Aspergillums species primarily *A. flavus and A. parasiticus*; as secondary metabolites. Aflatoxin B_1 (AFB_1) is formed by the fungi strain *A. flavus*. It is the most biologically active of the known aflatoxins [1, 2, 3, 4, 5, 6]. About 25% of the global animal food and food crops have been estimated to be aflatoxins contaminated [7, 8, 9, 10, 11]. Estimates suggest that about 4 billion people in the resource limited countries are vulnerable to aflatoxins toxicity [10, 12]. It is well documented that consumption of aflatoxin contaminated food causes hepatotoxicity, immunosuppression, growth retardation, mutagenic, teratogenic and hepatic cancers [1, 5, 7, 8, 9, 13, 14].

Spirulina platensis thrives *in* fresh water as a blue green alga. It is a unicellular cyanobacteria in the class *Cyanophyceae*, family *Oscillatoraceae* [15]. It contains several nutrients among them amino acids, minerals, fatty acids and vitamins. It has been used as a food supplement for several years. Recent animal studies have revealed potential protective effects of *S. platensis* against cytotoxic agents' toxicity including nephrotoxicity, cardiotoxicity and hepatotoxicity [15]. It has been reported to be a very powerful scavenger of free radicals. It has significant antioxidant properties due to presence of carotenoids [15, 16, 17]. However, there is limited data on potential protective effects of *S. platensis* against AFB_1 induced biochemical changes. Subsequently, preliminary investigations are needed to assess its possible hepatoprotective effects against AFB_1 induced hepatotoxicity. Therefore, current investigation aimed to evaluate protective effects of *S. platensis* extract against AFB_1 induced biochemical changes in male Swiss albino mice.

MATERIALS AND METHODS

Plant material

S. platensis powder (MMUSTMUG SPIRULINA®) was purchased from botanical garden shop of Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya. A calibrated

weighing balance was used to weigh 350g of *S. platensis* powder into a 1litre conical flask. 500 ml of distilled water was added and then cotton wool was used to cover it. A water bath was used to heat and maintain temperature at 60^0C for half an hour. Filter paper was used to filter it into a 500 ml round bottom flask. The filtrate was then coated with dry ice and acetone. It was then freeze dried at a temperature of -30^0C and pressure of 10 mbar for a period of 72 hours. 110g of pure *S. platensis* extract powder was obtained after freeze drying. 925 mg of freeze dried *S. platensis* extract was weighed and 98 ml of distilled water added. The fresh aqueous preparation of *S. platensis* was then stored in amber coloured container at 0 - 8^0C until required for the study [17, 18].

Preparation of AFB_1 stock solution

Analytical grade AFB_1 (AF031) was purchased from Fermentek® Ltd, Jerusalem, Israel. It was used to perform experiments without any further purification. 7% Dimethyl sulfoxide (DMSO) was used to dissolve 10mg of AFB_1. 7% DMSO was made by addition of 70 ml of DMSO to 930 ml of distilled water. It was stored in a dark place to minimise decomposition [17, 18, 19].

Experimental animals

Randomly 25 healthy inbred male Swiss albino mice aged 8 weeks were obtained from the experimental animal breeding house of Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. Sample size was determined using resource equation approach; Group comparison one-way ANOVA [20]. Previous investigators have used similar sample size in their investigation [21].

Their weights ranged between 30-35g. They were kept in clean labelled polypropylene cages. They were transferred from the breeding room to the experimental room. KEMRI animal facility is well-designed, well-ventilated building that provides ideal experimental conditions; away from excess noise, excess heat and pollution. This minimised possible stress factors to the mice before and during the study. The mice had uncontrolled access to water and standard laboratory animal pellet diet purchased from Unga® Feeds Limited, Nairobi, Kenya. Acclimatization period of 2 weeks was allowed before the start of the experiments. A 12 hour light and dark cycle was maintained. Humidity was at 70%. Body weights were measured at day 0, 7, 14 and 28.

Experimental protocol

One control group and four treatment groups were used in the study. Randomly twenty five (25) mice were allocated into 5 groups; each had 5 mice. Group I (Control group) mice received normal diet. Group II mice received 100 mg/kg/day of *S. platensis* extract. Group III mice received 200 µg/kg/day of AFB_1. Group IV mice received *S. platensis* extract 100 mg/kg/day and 200 µg/kg/day of AFB_1. Group V mice received *S. platensis* extract 200 mg/kg/day and 200 µg/kg/day of AFB_1. The treatments were daily administered orally using a curved dosing cannula with 1ml syringe. After 4 weeks the mice were euthanized using carbon dioxide (CO_2) in an enclosed chamber. Blood collection was aseptically done by heart puncture technique into plain glass tubes having EDTA as anticoagulant. The blood samples were then subjected to biochemical analysis of alkaline Phosphatase (ALP), aspartate aminotransferase (AST), globulin, alanine aminotransferase (ALT), albumin and plasma total protein. All these parameters were analysed at Pathologists Lancet® Kenya Ltd Laboratories, Nairobi, Kenya using an automated biochemistry analyser.

Statistical analysis

Comparison of the means of the groups was using one way ANOVA statistical test. Statistical significance of data was set at ($P<0.05$). Post-hoc comparisons between multiple groups using Tukey's Honestly Significantly Differenced (HSD) was performed for data that had statistically significant differences. Python® 3.0 with statistical libraries data analysis software was used to perform data analysis.

Ethical Considerations

Ethical clearance was obtained from Institutional Ethics Review Committee (IERC) of MMUST; reference number: *MMU/COR: 403012 vol. 2(15)*. Study clearance was obtained from National Commission for Science, Technology and Innovation (NACOSTI), Nairobi, Kenya, reference number: *NACOSTI/P/18/70580/24532*. NACOSTI study licence number: *A20847* was obtained. Lastly, study approval was also obtained from KEMRI Animal use and Care committee, reference No: *KEMRI/ACUC/02.06.19*. During the study, standard laboratory animal handling procedures were adhered to.

RESULTS

***S. platensis* inhibits AFB_1 induced weight loss**

A decrease in mean weight of mice in group 3 (200 µg/kg/day of AFB_1) was noted. A progressive rise in weight was observed for mice in the control group. There was intermediate increase in weight for mice in group 2 (100 mg/kg/day of *S. Platensis* extract), group 4 (*S. platensis* extract 100 mg/kg/day and 200 µg/kg/day of AFB_1) and group 5 (*S. platensis* extract 200 mg/kg/day and 200 µg/kg/day of AFB_1) as shown in figure 1. One way ANOVA analysis revealed the weight changes were statistically significant among the 5 groups ($P< 0.05$). Subsequently, Post-hoc comparison of mean values of weight changes using Tukey's Honestly Significantly Differenced (HSD) was performed as shown in table 1. Statistically significant differences for all group to group comparisons were noted except for Group 2 (100 mg/kg/day of *S. platensis* extract) when compared with group 5 (*S. platensis* extract 200 mg/kg/day and 200 µg/kg/day of AFB_1) and group 3 (AFB_1 200 µg/Kg/day) when compared with group 4 (*S. platensis* extract 100 mg/kg/day and 200 µg/kg/day of AFB_1); which both had a P value of 0.900.

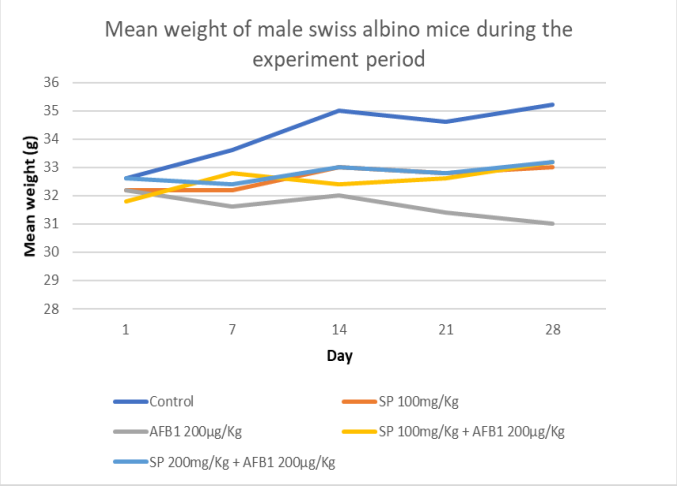

Figure 1: showing time-dependent mean body weight changes (grams, g) of the controls and mice treated with *S. platensis* extract 100 mg/kg/day, AFB_1 200 µg/Kg/day, *S. platensis* extract 100 mg/kg/day + 200 µg/Kg/day and 200 mg/kg/day of *S. platensis* extract + AFB_1 200 µg/Kg/day

Table 1: Showing Post-hoc comparison of weight changes mean values using Tukey's Honestly Significantly Differenced (HSD) for the 5 groups

	Group 1	Group 2	Diff	Lower	Upper	q-value	p-value
0	Group 1	Group 2	3.60	2.61	4.59	15.43	0.0010[a]
1	Group 1	Group 3	4.52	3.53	5.51	19.38	0.0010 [a]
2	Group 1	Group 4	4.72	3.73	5.71	20.24	0.0010 [a]
3	Group 1	Group 5	3.44	2.45	4.43	14.75	0.0010 [a]
4	Group 2	Group 3	0.92	-0.07	1.91	3.94	0.0755
5	Group 2	Group 4	1.12	0.13	2.11	4.80	0.0214 [a]
6	Group 2	Group 5	0.16	-0.83	1.15	0.69	0.9000
7	Group 3	Group 4	0.20	-0.79	1.19	0.86	0.9000
8	Group 3	Group 5	1.08	0.09	2.07	4.63	0.0278 [a]
9	Group 4	Group 5	1.28	0.29	2.27	5.49	0.0074 [a]

Mean values ± SD [a] statistically significant (P≤ 0.05)

Biochemical changes

Overall, one way ANOVA analysis revealed statistically significant differences among the 5 groups for ALT, AST and ALP (P= 0.0491, 0.0519 and 0.004) respectively. Subsequently, Post-hoc comparison of mean values for these 3 parameters was determined using Tukey's Honestly Significantly Differenced (HSD) as shown in tables 3, 4 and 5.

Table 2: Showing biochemical changes in serum of male Swiss albino mice after twenty eight days exposure period of the controls and mice treated with *S. platensis* extract 100 mg/kg/day, AFB_1 200 µg/Kg/day, *S. platensis* extract 100 mg/kg/day + 200 µg/Kg/day and 200 mg/kg/day of *S. platensis* extract + AFB_1 200 µg/Kg/day

	Control	SP 100mg/Kg	AFB_1 200µg/Kg	SP 100mg/Kg + AFB1 200µg/Kg	SP 200mg/Kg + AFB1 200µg/Kg
AST (IU/L)	176.75±44.34	131.60±19.32	256.0±115.99	230.5±96.03	229.80±95.01[a]
ALT (IU/L)	44.00±6.83	43.20±12.38	61.50±8.19	47.25±10.81	49.80±7.92 [a]
Bilirubin (IU/L)	2.40±0.55	2.20±0.45	2.25±0.50	2.0±0.01	2.0±0.01
ALP (IU/L)	51.75±11.89	41.80±20.57	59.40 ±6.91	49.75±4.11	26.50±13.48 [a]
Total protein (g/L)	66.00±3.39	71.40±10.06	69.25±4.79	76.25±11.38	77.25±12.12
Albumin (g/L)	34.2±16.4	34.2±1.30	34.0±0.82	34.25±1.89	34.00±1.41
Globulin (g/L)	31.8±3.35	37.20±9.20	35.25±4.99	42±9.83	43.25±11.17

Mean values ± SD [a] statistically significant (P≤ 0.05)

Table 3: Showing Post-hoc comparison of ALT mean values using Tukey's Honestly Significantly Differenced (HSD) for the 5 groups

	Group	Group	Diff	Lower	Upper	q-value	p-value
0	Group 1	Group 2	6.60	-9.66	22.86	1.72	0.7205
1	Group 1	Group 3	0.95	-15.31	17.21	0.25	0.0540 [a]
2	Group 1	Group 4	1.70	-14.56	17.96	0.44	0.9000
3	Group 1	Group 5	2.55	-13.71	18.81	0.66	0.9000
4	Group 2	Group 3	7.55	-8.71	23.81	1.97	0.05262 [a]
5	Group 2	Group 4	8.30	-7.96	24.56	2.16	0.5518
6	Group 2	Group 5	4.05	-12.21	20.31	1.05	0.49000 [a]
7	Group 3	Group 4	0.75	-15.51	17.01	0.20	0.9000
8	Group 3	Group 5	3.50	-12.76	19.76	0.91	0.039000 [a]
9	Group 4	Group 5	4.25	-12.01	20.51	1.11	0.9000

Mean values ± SD [a] statistically significant (P≤ 0.05)

Table 4: Showing Post-hoc comparison of AST mean values using Tukey's Honestly Significantly Differenced (HSD) for the 5 groups

	Group	Group	Diff	Lower	Upper	q-value	p-value
0	Group 1	Group 2	58.2	-66.86	183.26	1.97	0.6245
1	Group 1	Group 3	94.9	-30.16	219.96	3.21	0.01954 [a]
2	Group 1	Group 4	124.4	-0.66	249.46	4.21	0.0517 [a]
3	Group 1	Group 5	48.9	-76.16	173.96	1.65	0.7445
4	Group 2	Group 3	36.7	-88.36	161.76	1.24	0.9000
5	Group 2	Group 4	66.2	-58.86	191.26	2.24	0.5213
6	Group 2	Group 5	9.3	-115.76	134.36	0.31	0.9000
7	Group 3	Group 4	29.5	-95.56	154.56	0.10	0.9000
8	Group 3	Group 5	46.0	-79.06	171.06	1.56	0.04819 [a]
9	Group 4	Group 5	75.5	-49.56	200.56	2.56	0.3986

Mean values ± SD [a] statistically significant (P≤ 0.05)

Table 5: Showing Post-hoc comparison of ALP mean values using Tukey's Honestly Significantly Differenced (HSD) for the 5 groups

	Group 1	Group 2	Diff	Lower	Upper	q-value	p-value
0	Group 1	Group 2	17.60	-5.21	40.41	3.27	0.1830
1	Group 1	Group 3	9.65	-13.16	32.46	1.79	0.04928 [a]
2	Group 1	Group 4	7.65	-15.16	30.46	1.42	0.834
3	Group 1	Group 5	32.90	10.10	55.71	6.10	0.0028 [a]
4	Group 2	Group 3	7.95	-14.86	30.76	1.46	0.8130
5	Group 2	Group 4	9.95	-12.86	32.76	1.85	0.6715
6	Group 2	Group 5	15.30	-7.51	38.11	2.84	0.2984
7	Group 3	Group 4	2.00	-20.81	24.81	0.37	0.9000
8	Group 3	Group 5	23.25	0.44	46.06	4.31	0.0444 [a]
9	Group 4	Group 5	25.25	2.44	48.06	4.69	0.0256 [a]

Mean values ± SD [a] statistically significant (P≤ 0.05)

DISCUSSION

In the present study, investigations were performed using AFB_1. It is the most frequent and harmful of the known aflatoxins [22, 23]. AFB_1 has been shown to be prevalent in Sub-Saharan Africa, including Kenya [24, 25, 26]. The AFB_1 dose used in this study was 200 µg/Kg/day. This was an intermediate dose when compared to doses used by other investigators [8, 27, 28].

Body weight change was identified to be a very sensitive indicator of toxic effect caused by xenobiotics and chemical toxins [29]. Weight loss was identified to be among the earliest markers of onset of toxic effects [30]. In the current study a decrease in mean weight of mice in group 3 (AFB_1 200 µg/Kg/day) was noted in comparison to the control group. This was in agreement with earlier study findings [31]. An increase in mean weight was progressively noted in both group 2 (*S. Platensis* extract 100 mg/Kg/day) and group 5 (*S. platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day). One way ANOVA analysis revealed that the weight changes among the 5 groups were statistically significant at P< 0.05. Post-hoc pairwise comparison using Tukey's Honestly Significantly Differenced (HSD) revealed there was significant statistical differences upon pairwise comparison for all the groups (P< 0.05) except Group 2 (*S. platensis* extract 100 mg/Kg/day) compared with group 5 (*S. Platensis* extract 100 mg/Kg/day + AFB_1 200 µg/Kg/day) and Group 3 (AFB_1 200 µg/Kg/day) in comparison to group 4 (*S. platensis* extract 100 mg/kg + 200 µg/kg/day of AFB_1) that had P>0.05. Consequently, our study findings suggest that supplementation of 200 mg/kg/day of *S. platensis* extract followed by administration AFB_1 200 µg/Kg/day reverses weight loss in male Swiss albino mice (P<0.001).

ALT and AST have been used routinely as major diagnostic biochemical indicators of liver injury. In our study, group 3 (AFB_1 200 µg/Kg/day) had increased mean level of ALT compared to group 1 (control group) (44.0±6.83 IU/L vs. 61.0±8.19 IU/L; p=0.054). Statistically insignificant differences were noted in the mean ALT levels of group 1 (control group) and Group 2 (*S. platensis* extract 100 mg/kg/day) P (0.6245). In group 5 (*S. Platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day) lowered ALT levels were noted; (49.8±7.9 IU/L vs. 61.5±8.19 IU/L; p=0.039). Similarly in the current study, AFB_1 at a dose of 200 µg/Kg/day increased the mean level of AST compared with the control group; (176.75±44.34 IU/L vs. 256±115.99 IU/L; p=0.0195). These results were in agreement with previous studies that have documented elevated levels of ALT and AST [4, 30, 32, 33]. Supplementation of *S. Platensis* extract at level of 200 mg/Kg/day followed by administration of AFB_1 200 µg/Kg/day as seen in group 5; reversed the elevation of AST levels (229.8±95 IU/L vs. 256±115.99 IU/L; p=0.04819).

In this study, there was a statistically significant reversal of ALP levels in group 4 (*S. Platensis* extract 100 mg/Kg/day + AFB_1 200 µg/Kg/day) and Group 5 (*S. Platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day) P (0.0444).

Consequently, our study findings suggests that supplementation of *S. Platensis* extract at doses ranging from 100 mg/Kg/day to 200 mg/Kg/day can reverse elevation of ALT, AST and ALP levels caused by 200 µg/Kg/day of AFB_1 in male Swiss albino mice.

These findings suggest statistically insignificant changes in the levels of albumin, globulin, total protein and bilirubin across the 5 groups (p= 0.960, 0.320, 0.30 and 0.3202) respectively.

CONCLUSION

The findings of this study suggest that supplementation of *S. Platensis* extract at doses ranging from 100 mg/Kg/day to 200 mg/Kg/day can inhibit elevation of ALT, AST and ALP levels induced by 200 µg/Kg/day of AFB_1 in male Swiss albino mice. This study findings can form the preliminary basis for further investigation on possible supplementation of *S. Platensis* to reverse AFB_1 induced hepatotoxicity. Therefore, further studies are recommended to identify the specific phytochemical responsible for inhibition of elevation of biochemical parameters by *S. platensis* extract.

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Conflict of interest

None declared.

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