The Journal of Phytopharmacology 2023; 12(1):27-31
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2023; 12(1): 27-31
January- February
Received: 08-01-2023
Accepted: 22-02-2023
Published: 28-02-2023

doi: 10.31254/phyto.2023.12104

Masese Johnson
School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology, P.O Box 190 – 50100, Kakamega Kenya

Kipkoech Gilbert
1. Department of Biochemistry, Biotechnology, Kenyatta University P.O Box 43844 - 00100, Nairobi, Kenya
2. Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute (KEMRI), P.O. Box 54840 - 00200, Nairobi, Kenya

Kimoloi Sammy
Department of Medical Laboratory Sciences, School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science & Technology P.O Box 190 – 50100, Kakamega.

Mwitari Peter
Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute (KEMRI), P.O. Box 54840 - 00200, Nairobi, Kenya

Nguka Gordon
Department of Nutritions and diatetics, School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science & Technology P.O Box 190 – 50100, Kakamega, Kenya.

Mutai Charles
Department of Medical Laboratory Sciences, School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science & Technology P.O Box 190 – 50100, Kakamega Kenya.

Correspondence:
Dr. Masese Johnson
School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology, P.O Box 190 – 50100, Kakamega Kenya
Email: johnsonmasese2017@gmail.com

Protective effect of *Spirulina platensis* extract on aflatoxin B_1 immunotoxicities in mice

Masese Johnson, Kipkoech Gilbert, Kimoloi Sammy, Mwitari Peter, Nguka Gordon, Mutai Charles

ABSTRACT

Aspergillus flavus and *Aspergillus parasiticus* are the main Aspergillums species that form aflatoxins. Aflatoxins are hepatotoxic, teratogenic, carcinogenic and immunosuppressive. This study aimed to assess *Spirulina platensis (S. platensis)* extract inhibitory effect against aflatoxin B_1 (AFB_1) induced immunotoxicity in male Swiss albino mice. Twenty-five inbred weaned mice were randomly divided into five groups. Group I (Control group), were given routine diet. Treatments administered were: Group II (*S. platensis* extract 100 mg/kg/day), Group III (AFB_1 200 µg/kg/day), Group IV (*S. platensis* extract 100 mg/kg/day and AFB_1 200 µg/kg/day) and Group V (*S. platensis* extract 200 mg/kg/day and AFB_1 200 µg/kg/day) for 28 consecutive days. Blood was aseptically collected and centrifuged to obtain serum. Quantitative determination of IgG, IgM and IgA in blood serum was done using ELIZA kits. One-way ANOVA data analysis was done. Post-hoc analysis was done using Tukey's Honestly Significantly Differenced (HSD). $P<0.05$ statistical significance level was considered significant. Compared to group I (control), treatment with AFB_1 200 µg/Kg/day (group III) led to reduced IgA (0.7147±0.001 vs. 0.7075±0.010); reduced IgM (0.0916±0.003 vs. 0.0866±0.019) and elevated IgG (0.1746±0.001 vs. 0.2808±0.243) mean levels. Supplementation of *S. platensis* extract 200 mg/Kg/day (group V) reversed the AFB_1 (200 µg/kg/day)-induced depression of IgA levels (0.7124±0.005 vs. 0.7075±0.010; P=0.05437); IgM (0.1005±0.004 vs. 0.0866±0.019; P=0.0178); as well as the induced elevation of IgG levels (0.1749±0.001 vs. 0.2808±0.243; P=0.0155). In conclusion, immune changes in IgG and IgM caused by AFB_1 could be reversed by supplementation of *S. platensis* extract.

Keywords: *Spirulina platensis*, Aflatoxin B_1, Immune changes, Protective effects.

INTRODUCTION

A. flavus and A. parasiticus strains of Aspergillums species are the primary sources of aflatoxins. Aflatoxins are produced as secondary metabolites. *A. flavus* is the chief producer of Aflatoxin B_1 (AFB_1). Of all the known aflatoxins, AFB_1 is the most active biologically [1-6]. Aflatoxins have been estimated to affect about one quarter of global animal feed and human food [7-11]. Consumption of food contaminated by aflatoxins causes immunosuppression, hepatotoxicity, mutagenic, growth retardation and liver cancer [1, 5, 7-9, 12, 13].

S. platensis belongs to the class *Cyanophyceae* and the family *Oscillatoraceae* [14]. It is a unicellular cyanobacteria blue green alga. It is rich in minerals, fatty acids, amino acids and vitamins. Powerful antioxidant properties in *S. platensis* have been attributed to carotenoids presence [14-16]. In addition, *S. platensis* has been reported to have nephroprotective, cardioprotective and hepatoprotective effects against toxicants, including naturally substances and synthetic substances like BHA, oltipraz, coumarin and ethoxyquine [14]. However, there is inadequate data on *S. platensis* extract inhibitory effect against AFB_1- induced immune changes. Therefore, this study aimed to assess *S. platensis* extract inhibitory effects against immune changes caused by AFB_1 in mice.

MATERIALS AND METHODS

Spirulina platensis

Powder of *S. platensis* (MMUSTMUG SPIRULINA®) was bought from herbal outlet of Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya. Then 350 g of *S. platensis* powder was weighed into a 1 litre flask. Addition of 500 ml of distilled water into the conical flask and stoppered using cotton wool was done. The mixture was heated in a water bath and the temperature kept at 60^0C for half an hour. Filtration was done using Whatman® qualitative filter paper grade 1 (WHA 1001125) into round bottom flask of 500ml. Coating of the filtrate using acetone and dry ice was then done. The temperature was maintained at -30^0C and at 10 mbar of pressure during freeze drying for a period of 72 hours. After freeze drying, 110 g of pure *S. platensis* powder was obtained. From the *S.*

S. platensis extract freeze-dried powder obtained, 925 mg was weighed into a 200 ml flask and addition of 98 ml of distilled water was done. Amber coloured bottle was used to store the mixture at 0 – 8 ^{0}C until when needed for the experimental work [16, 17].

AFB_1 stock solution preparation

Importation of analytical standard AFB_1 (AF031) from Fermentek® Ltd, Jerusalem, Israel was done. It did not undergo any additional purification. Ten (10) mg of AFB_1 was dissolved in 7% Dimethyl sulfoxide (DMSO). This gave a final concentration of 100 µg/ml. To minimise decomposition, it was stored in a dark place [16, 17].

Experimental animals

Twenty-five (25) inbred healthy male Swiss albino mice, 8 weeks of age, were randomly selected from experimental animal rearing unit of Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. Their weight ranged between 30-35 g. One-way ANOVA group comparison [18] using resource equation was used to determine sample size, as previously described [19, 20].

They were housed in labelled clean polypropylene cages in the experimental room of the animal facility. The animal facility was inspected before initiation of the study. This ensured conservation of ideal experimental environment: good ventilation, cleanliness, minimal noise, pollution and heat. The animals had unrestrained access to water. They also had unrestricted access to animal pellet feed bought from Unga® Feeds Limited, Nairobi, Kenya. Two weeks were allowed for acclimatization. There was a 12-hour dark and light cycle. Humidity was 70%.

Experimental protocol

The study had one control group and four treatment groups. Twenty-five mice were randomly divided into five groups. Group I (Control group) were given routine feed. Group II were given *S. platensis* extract 100 mg/kg/day. Group III were given AFB_1 200 µg/kg/day. Group IV were given *S. platensis* extract 100 mg/kg/day and AFB_1 200 µg/kg/day. Group V were given *S. platensis* extract 200 mg/kg/day and AFB_1 200 µg/kg/day. A curved dosing cannula attached to 1ml syringe was used to administer treatments orally daily. After 4 weeks, the mice were kept in an enclosed chamber and sacrificed using carbon dioxide (CO_2).

Serum separator tubes were aseptically used in collection of blood samples. The blood was kept at room temperature (25^{0}C ± 2^{0}C) for 2 hours. It was then centrifuged at 4000Xg (Himac centrifuge Hitachi®, Equipment model No: SCTABD; Serial NO: 00676; Kemri No: JTJ0016) for 15 minutes. A calibrated micropipette was used to measure 0.3 ml of serum from the centrifuged sample. The serum obtained was then stored at -20^{0}C until needed for analysis. During the assay procedures the stored serum sample was allowed to thaw at room temperature (25^{0}C ± 2^{0}C) before being used to perform the assay procedures.

Quantitative determination of immunoglobulin biomarkers IgG, IgM and IgA was performed using Mouse IgG, IgM and IgA ELISA Kits (Beijing Solabio® Science & Technology co. Ltd; China (Cat#SEKM-0098; Lot No. 20191212; Lot. No. 0604K9121, and Lot No. 0604K9122 respectively). The assay procedures were as per the manufacturer's user manual specifications (catalog number SEKM-0098). Reading of absorbance was done at 450 nm using a multiscan Go® equipment. A 96 microplate well (12 strips of 8 wells) template was used to tabulate the results. The software used during reading of absorbance was Skanit Software 4.1 for Microplate Readers RE, ver. 4.1.0.43.

Statistical analysis

Group means were compared applying One-way ANOVA statistical test. P<0.05 statistical significance level was considered significant. Multiple groups post-hoc analysis was then performed applying Tukey's Honestly Significantly Differenced (HSD). The data analysis software used was Python® 3.0 with statistical libraries.

Ethical Considerations

Ethics Review Committee (IERC) of Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya, gave ethical clearance; reference No: *MMU/COR: 403012 vol. 2(15)*. Secondly, National Commission for Science, Technology and Innovation (NACOSTI), Nairobi, Kenya, gave study approval; reference No: *NACOSTI/P/18/70580/24532*. A study licence No: *A20847* from NACOSTI was obtained. In addition to these, study clearance was obtained from KEMRI Scientific and Ethics Review Unit (IRB), approval No: KEMRI/SERU/CTMDR/074/3815. Lastly, approval was obtained from KEMRI Animal Use and Care committee, reference No: KEMRI/ACUC/02.06.19. Experimental animal handling guidelines and procedures were followed throughout the study.

RESULTS

Table 1: Mean values for IgA, IgM and IgG for the 5 groups

	IgA (ng/ml)	IgM (ng/ml)	IgG (pg/ml)
Control	0.7147±0.001	0.0916±0.003	0.1746±0.001
Spirulina 100 mg/Kg/day	0.7133±0.004	0.0975±0.003	0.1739±0.002
AFB_1 200 µg/Kg/day	0.7075±0.010	0.0866±0.019	0.2808±0.243
Spirulina 100 mg/Kg + AFB_1 200µg/Kg/day	0.7123±0.0102	0.0899±0.003	0.1746±0.001
Spirulina 200 mg/Kg + AFB_1 200 µg/Kg/day	0.7124±0.005	0.1005±0.004	0.1749±0.001

Mean values ± standard deviation

With regard to IgG, group III (AFB_1 200 µg/Kg/day) mice, an elevated mean value of 0.2808 pg/ml compared to the other four groups whose mean values ranged from 0.1739 to 0.1749 pg/ml (Table 1) was noted. In contrast to IgG, group III (AFB_1 200 µg/Kg/day) had a decreased IgM levels (0.0866 ng/ml) compared to the other four groups whose mean values ranged from 0.0899 to 0.1005 ng/ml (Table 1). Similarly, the IgA levels of group III (AFB_1 200 µg/Kg/day) mice had significantly lower levels (0.7075 ng/ml) of IgA compared to the other four groups whose mean values ranged from 0.7123 to 0.7147 ng/ml (Table1).

Predominantly, statistically significant differences were found after one-way ANOVA analysis (p=0.04512, 0.01448 and 0.0541) in IgG, IgM and IgA respectively. Consequently, IgG, IgM and IgA mean values post-hoc comparison by application of Tukey's Honestly Significantly Differenced (HSD) was done. Group V (Spirulina 200 mg/Kg + AFB_1 200 µg/Kg/day) mice had a significantly lower mean

IgA levels in comparison to group I (control) mice, (p=0.019), (table 2). Group V (Spirulina 200 mg/Kg + AFB1 200 µg/Kg/day) had significantly higher IgM levels in comparison to groups III (p= 0.0178) and IV mice, (p=0.019) (Table 3). Group III mice (AFB1 200 µg/Kg/day) had significantly higher levels of IgG in comparison to groups I (p=0.009), II (p=0.0136) and V (p=0.0155). Altogether, study findings demonstrate that four-week treatment of mice with AFB_1 200 µg/Kg/day induces a decrease in IgA and IgM mean levels, but elevates IgG mean levels. These changes were effectively reversed by co-administration of Spirulina 200 mg/Kg/day.

Table 2: IgM mean values post-hoc comparison of the 5 groups using Tukey's Honestly Significantly Differenced (HSD)

	Group	Group	Diff	Lower	Upper	q-value	p-value
0	Group I	Group II	0.0014	-0.0121	0.0149	0.4387	0.9000
1	Group I	Group III	0.0072	-0.0063	0.0208	2.2686	0.05105
2	Group I	Group IV	0.0025	-0.0111	0.0160	0.7708	0.9000
3	Group I	Group V	0.0023	-0.0112	0.0158	0.7207	0.0523
4	Group II	Group III	0.0058	-0.0077	0.0194	1.8299	0.6778
5	Group II	Group IV	0.0011	-0.0124	0.0146	0.3321	0.9000
6	Group II	Group V	0.0009	-0.0126	0.0144	0.2820	0.9000
7	Group III	Group IV	0.0048	-0.0087	0.0183	1.4978	0.8044
8	Group III	Group V	0.0049	-0.0086	0.0185	1.5479	0.0178 [a]
9	Group IV	Group V	0.0002	-0.0133	0.0137	0.0501	0.0190 [a]

Note: [a] Statistically significant

Table 3: IgA mean values post-hoc comparison of the 5 groups using Tukey's Honestly Significantly Differenced (HSD)

	Group	Group	Diff	Lower	Upper	q-value	p-value
0	Group I	Group II	0.0007	-0.2047	0.2060	0.014015	0.9000
1	Group I	Group III	0.1062	-0.0992	0.3115	2.188400	0.5411
2	Group I	Group IV	0.0000	-0.20530	0.2054	0.000824	0.9000
3	Group I	Group V	0.0003	-0.20500	0.2057	0.007007	0.0190 [a]
4	Group II	Group III	0.1069	-0.09848	0.3122	2.202415	0.5357
5	Group II	Group IV	0.0007	-0.20462	0.2061	0.014839	0.9000
6	Group II	Group V	0.0010	-0.20432	0.2064	0.021022	0.9000
7	Group III	Group IV	0.1061	-0.0992	0.3115	2.187575	0.5414
8	Group III	Group V	0.1058	-0.0995	0.3112	2.181392	0.05437
9	Group IV	Group V	0.0003	-0.2050	0.2056	0.006183	0.0560

Note: [a] Statistically significant

Table 4: IgG mean values post-hoc comparison of the 5 groups using Tukey's Honestly Significantly Differenced (HSD)

	Group	Group	Diff	Lower	Upper	q-value	p-value
0	Group 1	Group 2	0.0059	-0.0114	0.023088	1.4396	0.82658
1	Group 1	Group 3	0.0050	-0.0122	0.022208	1.2234	0.0090 [a]
2	Group 1	Group 4	0.0017	-0.0155	0.018968	0.4274	0.90000
3	Group 1	Group 5	0.0087	-0.0084	0.026088	2.1766	0.5458
4	Group 2	Group 3	0.0108	-0.0064	0.028068	2.6630	0.0136 [a]
5	Group 2	Group 4	0.0076	-0.0096	0.024828	1.8670	0.66360
6	Group 2	Group 5	0.0030	-0.0142	0.020228	0.7370	0.90000
7	Group 3	Group 4	0.0032	-0.0140	0.020468	0.7960	0.06710
8	Group 3	Group 5	0.0138	-0.0034	0.031068	3.3999	0.0155 [a]
9	Group 4	Group 5	0.0106	-0.0066	0.027828	2.60470	0.38002

Note: [a] statistically significant

DISCUSSION

It is well established that chronic intake of aflatoxin contaminated animal feeds and human foods causes immune suppression in both animals and man, respectively [5, 21, 22, 23, 24, 25]. AFB_1 is the most toxic and frequent of the aflatoxins known [26, 27]. AFB_1 is widely spread in Sub-Saharan Africa [28, 29, 30].

In the current study, administration of 200 µg/Kg/day of AFB_1 (group III) reduced IgM mean level in comparison to group I (control); (0.0866±0.019 vs. 0.0916±0.003; Table 1). This was in agreement with earlier investigators [5,31]. Administration of AFB_1 200 µg/Kg/day preceded by *S. platensis* extract 100 mg/Kg/day (group IV) reversed the depression of IgM when compared with group III (AFB_1 200 µg/Kg/day); (0.0899±0.003 vs. 0.0866±0.019; Table 1). Similarly, administration of *S. platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day (group V) reversed the depression of IgM when compared with group III (AFB_1 200 µg/Kg/day); (0.1005±0.004 vs. 0.0916±0.003; Table 1). One-way ANOVA analysis revealed p value was 0.01448 for comparison of IgM mean values. Post-hoc IgM mean values comparison by using Tukey's Honestly Significantly Differenced (HSD) (Table 2); demonstrated that group III (AFB_1 200 µg/Kg/day) compared to group V (*S. platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day (group V), (p=0.0178) and group IV (*S. platensis* extract 100 mg/Kg/day + AFB_1 200 µg/Kg/day (group V) compared to group V (*S. platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day (group V); (p=0.0190) were statistically significant.

With regard to IgA, AFB_1 administered at 200 µg/Kg/day (group III) reduced IgA mean levels in comparison to group I (control); (0.7075±0.010 vs. 0.7147±0.001, Tables 1). This was in line with previous study findings [5,31]. Administration of AFB_1 200 µg/Kg/day preceded by supplementation of 100 and 200 mg/Kg/day of *S. platensis* extract as seen in group IV and V respectively; reversed depression of IgA mean levels when compared with group III (AFB_1 200 µg/Kg/day); (0.7123±0.0102 and 0.7124±0.005 vs. 0.7075±0.010 respectively; Table 1). One-way ANOVA analysis revealed p value was 0.01448 for comparison of IgA mean values. IgA post-hoc mean values comparison by using Tukey's Honestly Significantly Differenced (HSD) (Table 3); demonstrated that only group I (control) compared to group V (*S. platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day), (p=0.0190) was statistically significant.

In contrast to IgM and IgA, AFB_1 administered at 200 µg/Kg/day (group III), elevated mean level of IgG in male Swiss albino mice in comparison to group I (control); (0.2808±0.243 vs. 0.1746±0.001; Table 1). This was in line with an earlier study; that reported elevated mean levels of immunoglobulins [32]. Administration of AFB_1 200 µg/Kg/day preceded by supplementation of *S. platensis* extract at the dose of 100 and 200 mg/Kg/day as seen in group IV and V respectively; reversed elevation of IgG levels compared to group III (AFB_1 200 µg/Kg/day); (0.1746±0.001 and 0.1749±0.001 vs. 0.2808±0.243; Table 1). One-way ANOVA analysis revealed p value was 0.04512 for comparison of IgG mean values. IgG post-hoc mean values comparison by using Tukey's Honestly Significantly Differenced (HSD) was done as presented in Table 4. Results revealed that group I (control) compared to group III (AFB_1 200 µg/Kg/day), (p=0.009); group II (*S. platensis* extract 200 mg/Kg/day) compared group III (AFB_1 200 µg/Kg/day), (p=0.0136); group III (AFB_1 200 µg/Kg/day) compared to group V (*S. platensis* extract 200 mg/Kg/day + AFB_1 200 µg/Kg/day), (p=0.0155); were statistically significant.

CONCLUSION

These study findings suggest that immune changes in IgG and IgM caused by AFB_1 could be reversed by supplementation of *S. platensis* extract.

Acknowledgements

Authors of the manuscript are grateful to the following institutions for their support during the study.

1. School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya.
2. Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya.

Conflict of interest

We declare none.

Financial support

1. National Research Fund, Nairobi, Kenya; Ref: NRF 1st call PhD projects 2016/2017.
2. Kenya Medical Research Institute (KEMRI), Nairobi, Kenya; KEMRI IRG Grant REF: KEMRI/IRG/INNOV/029/2018.

ORCID ID

Johnson Masese: http://orcid.org/0000-0001-8128-003X

Sammy Kimoloi: http://orcid.org/0000-0003-3364-4980

Gordon Nguka: http://orcid.org/0000-0002-6749-4602

REFERENCES

1. Abeer M.E Protective effect of curcumin against experimentally induced aflatoxicosis on the renal cortex of adult male albino rats: a histological and immunohistochemical study. *Int J Clin Exp Pathol*, 2015;8(6):6019-6030
2. Ali S.K and Mohammed A.S Review article: Spirulina – an overview, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2012;4(3).
3. Arpita M, Misra N, Srivastav D, Umapathy D, Kumar S Spirulina-The Nature's wonder: A review. Scholars Journal of Applied Medical Sciences (SJAMS) 2014;2(4C):1334-1339.
4. Asieh A, Mohammad F, Ali M.L, Hossain S and Ali C. A Review on antioxidant properties of Spirulina. *Journal of Applied Biotechnology Reports*, Winter 2016;3(1):345-351
5. Bbosa, Kitya D, Lubega A, Ogwal-O, William W, Kyegombe D, Review of the biological and health effects of aflatoxins on body organs and body systems. 2013. http://dx.doi.org/10.5772/51201
6. Brahmi D, Bouaziz C, Ayed Y, Mansour H B, Zourgui L and Hassen Bacha. Chemopreventive effect of cactus *Opuntia ficus indica* on oxidative stress and genotoxicity of aflatoxin B1. Nutrition and metabolism 2011;8:73
7. Carvajal MM. Metabolic changes of aflatoxin B1 to become an active carcinogen and the control of this toxin. Immunome Res 2015;11:104.

8. Chebon S, Wanyoike W, Bii C, Gathumbi J, Ogoyi D. Incidence of aflatoxigenic fungi and aflatoxins in maize cultivated in highland and mid-altitude agro-ecological zones in Kenya, Journal of Scientific Research and Reports 2016;9(6):1-12. Article no. JSRR.17836 ISSN: 2320-0227
9. Colla L.M, Bertol C.D, Ferreira D.J, Bavaresco J. and Costa J.A.V Thermal and photo-stability of the antioxidant potential of *Spirulina platensis* powder. *Braz. J. Biol.*, 2017;77(2):332-339.
10. Craig P.T *Review Article* The molecular epidemiology of chronic aflatoxin driven impaired child growth. *Hindawi Publishing Corporation Scientifica* 2013, Article ID 152879, 21 pages
11. DiNicolantonio J.J, Bhat AG, OKeefe J. Effects of spirulina on weight loss and blood lipids: a review. *Open Heart*; 7: e001003. 2020.
12. Ellen E. Y, Johnni H. D, Lauren S. L, Michael E. R, Ekaterina M. P, *et al*., Human aflatoxin exposure in Kenya, 2007: a cross-sectional study, Food Additives & Contaminants: Part A, 2013;30(7):1322-1331.
13. Eva G. Lizárraga-Paulín, Ernesto Moreno-Martínez and Susana P. Miranda-Castro Aflatoxins and their impact on human and animal health: an emerging problem, aflatoxins - *Biochemistry and Molecular Biology*, Dr. Ramon G. Guevara-Gonzalez (Ed.), ISBN: 978-953-307-395-8, 2011.
14. Farombi E.O, Adepoju1 B F, Ola-Davies and Emerole G O (2005). Chemoprevention of aflatoxin B_1-induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural biflavonoid of Garcinia kola seeds. European Journal of Cancer Prevention 2005;14(3):207-214.
15. Ghada Z.A. Soliman, Alia M. Hashem, and Arafa M Protective effect of *curcuma longa or nigella sativa* on aflatoxin B1 induced hepatotoxicity in rats in relation to food safety on public health. *Medical Journal of Cairo University* 2012;80(2):191-203.
16. Hua-Lian W., Guang-Hua W., Wen-Zhou X., Tao L. and Hui H. Stability and antioxidant activity of food-grade phycocyanin isolated from *Spirulina platensis*. International Journal of Food Properties, 2016;19(10)2349-2362,
17. Colla L.M, Bertol C.D, Ferreira D.J, Bavaresco J. and Costa J.A.V. Thermal and photo-stability of the antioxidant potential of *Spirulina platensis* powder. Braz. J. Biol., 2017;77(2):332-339.
18. Lijuan X, Min C & Yibin Y. Development of methods for determination of aflatoxins. Critical Reviews in Food Science and Nutrition, 2016;56(16):2642-2664.
19. Arifin W.N, Zahiruddin W.M. Sample size calculation in animal studies using resource equation approach. Malays J Med ci. 2017;24(5):101-105. https://doi.org/10.21315/mjms2017.24.5.11
20. Yassein and Zghair Protective role of honey in rats with experimental aflatoxicosis, BMC Complementary and alternative study of toxicity and pathogenicity of aflatoxin B_1 and G_1 in Rats. Al-anbar Journal of veterinary science, 2012;5(1):23-31.
21. Cardwell KF. Mycotoxin contamination of foods in Africa: Antinutritional factors. Food and Nutrition Bulletin, The United Nations University. 2000;21(4):488-92.
22. Rania J, Samir A, Jalila B.S, Ridha B.Y, Zohra H *et al*., Ability of *Lactobacillus plantarum* MON03 to mitigate aflatoxins (B_1 and M_1) immunotoxicities in mice. Journal of Immunotoxicology 2015;12(3):290-299.
23. Jalila B.S, Rania J., Hakimeh S., Kambiz A.N, Ridha O., *et al*., Immuno-physiological alterations from AFB1 in rats counteracted by treatments with *Lactobacillus paracasei* BEJ01 and montmorillonite clay mixture. Journal of Immunotoxicology, 2016;13(5):628-637, DOI: 10.3109/1547691X.2016.1145157
24. Rushing BR and Selim MI (2019). Review: Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food and Chemical Toxicology. 2019;124:81-100.
25. Oladeji EA, Gondwe T, Akello J, Maziya-Dixon B and Mukanga M Relationship between serum aflatoxin concentrations and the nutritional status of children aged 6–24 months from Zambia, International Journal of Food Sciences and Nutrition, 2020;71(5):593-603, DOI: 10.1080/09637486.2019.1689547.
26. Sahar T.A, Abeer S.H, Manal A.H and Mogda K.M. Influence of Rosemary extract on immune responses and oxidative stress in mice intoxicated by aflatoxins. Nature and Science 2011;9(10):54-63]. (ISSN: 1545-0740).
27. Craig P.T. Review Article The molecular epidemiology of chronic aflatoxin driven impaired child growth. Hindawi Publishing Corporation Scientifica, 2013. Article ID 152879, 21 pages
28. Eva G. Lizárraga-Paulín, Ernesto Moreno-Martínez and Susana P. Miranda-Castro Aflatoxins and their impact on human and animal health: an emerging problem, aflatoxins - Biochemistry and Molecular Biology, Dr. Ramon G. Guevara-Gonzalez (Ed.) ISBN: 978-953-307-395-8, 2011.
29. Gong Y, Shona W, Mwatha J, Routledge M, Castelino J *Et al*., Aflatoxin exposure may contribute to chronic hepatomegaly in Kenyan school children. Environmental Health Perspectives 2012;120(6):893 -896
30. Hamid A.S, Tesfamariam I.G, Zhang Y. and Zhang Z.G. Aflatoxin B_1-induced hepatocellular carcinoma in developing countries: Geographical distribution, mechanism of action and prevention (Review). Oncology letters 2013;5:1087-1092,
31. Williams J.H, Phillips T.D, Jolly P.E, Stiles J.K, Jolly C.M *et al.* Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr 2004;80:1106-22.
32. Samir A., Jalila B.S., Mosaad A.A., and Ridha Ouslati Immunotoxicological and biochemical effects of Aflatoxins in rats prevented by Tunisian Montmorillonite with reference to HSCAS. Immunopharmacology and Immunotoxicology. 2010;32(3):514-522.

HOW TO CITE THIS ARTICLE

Johnson M, Gilbert K, Sammy K, Peter M, Gordon N, Charles M. Protective effect of *Spirulina platensis* extract on aflatoxin B_1 immunotoxicities in mice. J Phytopharmacol 2023; 12(1):27-31. doi: 10.31254/phyto.2023.12104