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Impact of Imarsil™ Adsorption on Aflatoxin M1 (AFM1) Levels in Cow's Milk: Analyzing Hematological Parameters and Histopathological Alterations

Dampak Adsorpsi Imarsil™ terhadap Kadar Aflatoksin M1 (AFM1) pada Susu Sapi: Analisis Parameter Hematologi dan Perubahan Histopatologis

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Abstract

The efficacy of Imarsil™ in mitigating the effects of aflatoxin M1 (AFM1) in cow's milk on hematological and histopathological parameters was investigated in this study. Seventy-two albino rats were randomly allocated to four treatment groups A - D in a six-week study. Rats in all groups were fed standard ration. In addition, 2 mL of clean distilled water, 2 mL of milk, 2 mL of AFM_1 contaminated milk (456 ng/L), and 2 mL of AFM_1 contaminated milk (456 ng/L) treated with Imarsil™ at 2% dosage rate were added to the ration of animals in groups A, B, C, and D respectively. The results of the investigation showed that rats in Group C developed a significant ($p<0.05$) lower weight. Packed Cell Volume (%), Hemoglobin (g/dL), Red Blood Cell ($10^6/mm^3$), Mean Corpuscular Volume (fL), Mean Corpuscular Hemoglobin (pg), and Mean Corpuscular Hemoglobin Concentration (g/dL) were not significantly different ($p>0.05$) among the different groups. In group C, a significant reduction ($p<0.05$) occurred in the white blood cell ($10^3/mm^3$) (12.90 - 8.63), and lymphocytes (87.00 - 74.33%) counts while the neutrophils (%) increased from 13.00 to 25.67. In contrast to those in Group C, tissue sections from Group D showed no histological lesions. Therefore, Imarsil™ represents an effective and safe adsorbent for the remediation of AFM_1-contaminated milk.
***Keywords*:** adsorbents, aflatoxin M_1, cow's milk, hematological parameters and histopathological changes, Imarsil™

Abstrak

Tingkat keberhasilan Imarsil™ dalam memitigasi efek aflatoksin M1 (AFM1) pada susu sapi terhadap parameter hematologi dan histopatologi diidentifikasi dalam penelitian ini. Tikus albino 72 ekor secara acak dibagi menjadi 4 kelompok perlakuan A, B, C, dan D dalam penelitian 6 minggu. Tikus pada semua kelompok diberi ransum standar. Imarsil™ dengan dosis 2%. dicampurkan pada 2 mL air suling bersih, 2 mL susu, 2 mL susu terkontaminasi AFM1 (456 ng/L), dan 2 mL susu terkontaminasi AFM1 (456 ng/L) kemudian ditambahkan pada ransum masing-masing hewan di kelompok A, B, C, dan D. Hasil penelitian menunjukkan bahwa tikus di Kelompok C mengalami penurunan berat badan yang signifikan ($p<0,05$). *Packed Cell Volume* (%), hemoglobin (g/dL), sel darah merah ($10^6/mm^3$), *Mean Corpuscular Volume* (fL), *Mean Corpuscular Hemoglobin* (pg), dan *Mean Corpuscular Hemoglobin Concentration* (g/dL) tidak berbeda signifikan ($p>0,05$) antar kelompok yang berbeda. Kelompok C juga menunjukkan penurunan yang signifikan ($p<0,05$) pada jumlah sel darah putih (103/mm3) (12,90 - 8,63) dan limfosit (87,00 - 74,33%), sedangkan neutrofil (%) meningkat dari 13,00 menjadi 25,67. Berbeda dengan kelompok C, potongan jaringan pada kelompok D tidak menunjukkan lesi histologis. Oleh karena itu, Imarsil™ merupakan adsorben yang efektif dan aman untuk remediasi susu yang terkontaminasi AFM1.
Kata kunci: adsorben, aflatoksin M_1, Imarsil™, parameter hematologi dan perubahan histopatologi, susu sapi

INTRODUCTION

Cow's milk is a naturally nutrient-dense foodstuff and a significant source of many essential nutrients. It has been long recommended as a necessary inclusion component of a healthy balanced diet. Rumbold et al. (2022) stated that beyond milk's nutritional value, an increasing body of evidence illustrates cow's milk may confer numerous benefits related to health as evidence from adult populations suggests that cow's milk may have a role in overall dietary quality, appetite control, hydration, and cognitive function. Studies have also linked milk and dairy products to a lower risk of osteoporosis and fractures, especially in older adults (Yusuf & Obaghwarhievwo, 2021). However, several nations, including Nigeria, Kenya, Pakistan, India, and several sub-Saharan African nations, had aflatoxin M_1 (AFM_1) levels in milk that substantially exceeded the United States and European Union regulatory limits for AFM1, indicating potential risk to individuals in those nations with high milk consumption (Turna & Wu, 2021).

AFM_1 is a hydroxylated metabolite of aflatoxin B_1 (AFB_1) in human food and animal feed and is excreted in urine and secreted in milk in mammalian species. Aflatoxin B1, as well as aflatoxin B2, G1, and G2, are mycotoxins (fungal toxins) produced by *Aspergillus flavus* and *A. parasiticus* as they grow in food and feed crops such as maize, peanuts, cottonseed, sunflower seeds, and tree (Alshannaq & Yu, 2017; Mmongoyo et al., 2017; Wu et al., 2014). Aflatoxin B1 (AFB1) is the most toxic and a strong carcinogenic toxin directly linked to many health problems, including liver cancer, in several animals. The World Health Organization stated that large doses of aflatoxins can result in acute poisoning, known as aflatoxicosis, which can be life-threatening, often through liver damage. In addition, aflatoxins have also been reported to be genotoxic, which means that they can harm DNA and cause cancer in animals (Awuchi et al., 2022). The economic consequences of AFM_1 in milk and dairy products can be severe for dairy producers. Balina et al. (2018) posited that a direct economic impact occurs when products that do not meet the aflatoxin standards are rejected at national or international markets. In Kenya for example, about 10% of the milk samples collected contained aflatoxin levels above 0.5 μg/L, which would cost dairy farmers $113.4 million annually if legislation was enacted (Kemboi et al., 2020; Senerwa et al., 2016). Report of survey of AFM1 in cow's milk from free-grazing cows in Nigeria indicated that toxin levels in positive samples ranged from 9.0 to 456.0 ng/L (Oluwafemi, Badmos, Kareem, et al., 2014).

Given that the impact of the global AF burden is significant in low-income countries due to the lack or insufficient resources needed to tackle the aflatoxin menace, it is therefore imperative that cost-effectiveness, rapidity, safety, simplicity, and technical feasibility will determine the suitability of any AF decontamination approach (Aringbangba et al., 2021). In the last three and half decades, literature has been replete with reports on studies that focused on many approaches to prevent aflatoxins from entering the food chain. Examples of such studies include the use of adsorbents such as activated charcoal and Imarsil™ (Oluwafemi, Badmos, Kolapo, et al., 2014), magnetic chitosan nanoparticles (Aringbangba et al., 2021), sodium and calcium aluminosilicate (Filho et al., 2016), modified rice straw (Mohamed et al., 2016), magnetic carbon nanocomposites (Zahoor & Ali Khan, 2018), activated charcoal, bentonite, and fuller's earth (Mgbeahuruike et al., 2018), and blueberry pomace bio-adsorbent (Rasheed et al., 2020). In an explorative search for a locally effective adsorbent for AFM1 decontamination of cow's milk in Nigeria, Imarsil™ was found to exhibit maximum decontamination efficacy at a 2% dosage rate at 28^0C for 5 hours (Oluwafemi, Badmos, Kolapo, et al., 2014).

Imarsil™, developed by Akpan & Kareem (2002), is an inexpensive synthetic absorbent obtained from oxidized *Brachystegia nigerica*. *B. nigerica* is a legume used especially in the eastern states of Nigeria as a condiment to thicken the soup. Though previous studies indicated that Imarsil demonstrated a potential to reduce aflatoxin M_1 and by extension, ameliorate the risk of aflatoxicosis in the developing tropical world, however no study has evaluated Imarsil's *in vivo* activity, its effect on hematological and histopathological indicators in both humans and animals. The present study, therefore, aimed to evaluate the protective efficacy of Imarsil on AFM_1-induced hematological and histopathological changes in male albino wistar rat models.

METHODS

Materials

Male Wister albino rats were obtained from the Animal House of Biochemistry Department, College of Medicine, University of Ibadan, Nigeria. Imarsil™ was prepared as previously described by Akpan & Kareem (2002). The AFM_1 standard was purchased from Chromogen (New Delhi, India). Veterinary milk was obtained from Trust Veterinary, Ibadan, Nigeria.

Imarsil preparation and detoxification of AFM_1 contaminated milk

Milk samples (50mL) were spiked with AFM_1 (Chromogen, New Delhi, India) at concentrations of 456 ng/L AFM_1, which was reported as the highest level of AFM_1, in positive milk samples from free-grazing cows in Nigeria (Oluwafemi, Badmos, Kareem, et al., 2014). Whereas the maximum decontamination efficacy of Imarsil™ was at a 2% dosage rate at 28°C for 5 hours (Oluwafemi, Badmos, Kolapo, et al., 2014).

In the present study, Imarsil™ was prepared as described by Akpan & Kareem (2002). Spiked cow milk (50 mL) containing 1g of Imarsil™ were passed through a separating funnel. The experimental setups were in place for 5 hours to obtain remediated milk samples used for animal feeding experiments.

Management and Experimental Animal-Design

All animals received human care according to standard criteria outlined for Laboratory Animals by the National Research Council (2011) and the ethics of the Augustine University, Ilara-Epe Animal Welfare and Ethical Committee. The ethical considerations of using the rats were in line with the Nuffield Council on Bioethics specifications. Rats in each group were fed with standard rations *ad libitum* and clean drinking water was regularly supplied. The measured liquid portion of the diet for each group was given to each rat using an incubator at the tip of a syringe. This was done to prevent the harm a needle might cause the animal while feeding them. The experiment lasted for six weeks (42 days).

Seventy-two male Wister albino rats weighing 40 -75g were obtained from the Animal House of Biochemistry Department, College of Medicine, University of Ibadan, Nigeria. The animals were kept in stainless steel cages of 30×50×25 cm under standard laboratory conditions. Rats were maintained on a 12-hour light/dark cycle at 27 °C and 60-70% humidity during the dry season of 2019. The animals were kept in standard room conditions and fed with standard ration (Vital Feed Limited, Abeokuta) and clean water *ad libitum* throughout two weeks of acclimatization. After that, they were randomly allocated to four treatment groups A - D in a completely randomized design with three replicates of six rats each. The treatments given daily to the four groups are as follows:

Group A-Rat fed with standard ration and 2 mL of clean distilled water;
Group B- Rat fed with standard ration and 2 mL of milk;
Group C-Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L);
Group D- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) and treated with Imarsil™ at a 2% dosage rate.

Rats were observed daily for mortality and adverse clinical signs throughout the experimental period. The weight of rats in each group was obtained using electronic balance at two-week intervals. On days 21(3 weeks) and 42 (6 weeks), three (3) of the rats in each group were sacrificed. The rats were sedated with diethyl-ether-soaked cotton wool swabs in a desiccator, and the blood samples (2.5 mL) were collected in an EDTA bottle through the orbital sinus and processed for hematological assays. The sacrificed rats were immediately transferred to a dissecting board cleaned with ethanol. Their limbs were pinned to the board with thumb pins. The rats were dissected to harvest both the liver and kidney into a sterile plain tube containing normal saline.

Hematological and Histopathological Assays

Blood samples for hematological assays were analyzed using Spincell 3Ref 5006101 automated hematology analyzer (Spinreact, Spain). Representative tissue samples from the liver and kidney were fixed in 10% neutral buffered formalin for 24 hours and washed with 70% ethanol. Samples from tissue were dehydrated in ascending grades of ethanol (70% to 100%), cleared in xylene, and embedded in paraffin.

Sections of 3-5 μm were obtained and stained with hematoxylin/eosin (H&E). Light microscopy was used to evaluate congestion, degeneration, necrosis, fatty changes, and leucocytic infiltration. The histopathological grading was done using a semi-quantitative scale: Normal=0, Mild=<25%, Moderate=25-50% and severe =>50%, of the affected area.

Statistical Analysis

Data obtained from this study were analyzed using SPSS 20.0 for windows. One-way analysis of variance (ANOVA) was used to compare means, and values were considered significant at $p<0.05$. Post hoc multiple comparisons for the ANOVA were done using least significant difference (LSD).

RESULTS AND DISCUSSION

The changes in the weight of albino rats in the four treatment groups during the six-week study are shown in Table 1. At the beginning of the experiment (Week 0), no significant difference ($p>0.05$) was observed among the weight of rats in the different groups. However, there were significant changes ($p<0.05$) in the weight of rats among the four treatments at weeks 2, 4, and 6. In this regard, rats fed standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk (Group C) had a significant ($p<0.05$) lower weight. Furthermore, it is worth noting that the weight of rats fed Imarsil^{TM}-remediated AFM_1 (456 ng/L) contaminated milk (Group D) was not significantly different ($p>0.05$) from those fed uncontaminated milk (Group B) and clean water only alongside the standard ration.

Table 1. Weight (g) of albino rats fed AFM_1 contaminated milk and Imarsil^{TM} treated AFM_1 contaminated milk in a 6-weeks study. (No need to show data of week 0, instead please show data on week 3 if available)

Treatment Group	Time of Study (Week)			
	0	2	4	6
A	94.95±7.79^{a}	187.17±9.68^{a}	192.00±11.18^{a}	193.40±20.89^{a}
B	94.28±7.69^{a}	190.17±10.93^{a}	194.17±12.40^{a}	199.23±13.40^{a}
C	95.57±8.22^{a}	165.00±8.53^{b}	173.50±15.08^{ab}	140.63±12.36^{b}
D	90.40±7.02^{a}	181.83±6.44^{a}	185.17±11.39^{a}	187.53±24.36^{a}

Data of week 0 serve as baseline data for each of the treatment. Weight measurement was done at 2 weeks interval.
Values are means ± standard deviation of triplicate readings. Within a column, values with different superscript differ significantly ($p<0.05$)
A-Rat fed with standard ration and 2 mL of distilled water; B- Rat fed with standard ration and 2 mL of milk; C-Rat fed with standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk; D- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) treated with imarsil^{TM} at 2% dosage rate.

It has been reported that aflatoxicosis caused poor absorption of nutrients from the diet, steatorrhea, reduction of biliary acids, and reduction of the activity of lipase, trypsin, amylase, and RNAse in animals leading to adverse effects on intestinal functions (Awuchi et al., 2022; Dhakal et al., 2023; Kumar et al., 2017; Sarma et al., 2017) Furthermore, aflatoxin adversely affects the innate immune system and intestinal intraepithelial cells of intestine leading to disturbance in structural integrity of the intestine (Sarker et al., 2023), as Mgbeahuruike et al. (2023) stated that contaminated poultry feed adversely affects feed intake, feed conversion ratio, weight gain, organ function and alters blood and clinical chemistry parameters of birds. In this connection, Lakkawar et al. (2017) reported a significantly lower body weight in birds fed aflatoxin-contaminated feed. The authors correlated their observation to the reduced ability of the birds to digest the feed in the presence of aflatoxin, as intestines of birds fed with 0.5mg/Kg of AF showed severe villus degeneration, sub-epithelial infiltration of cells (heterophils and lymphocytes) on 35th day of the experiment. Therefore, the significantly lower body weight in rats fed standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk (Group C) in the present study could be related to the reduced ability of the rats to digest the feed in the presence of aflatoxin in the intestines of the rats.

The hematological parameters of albino rats in the four treatment groups at three and six weeks of the study are shown in Table 2. The results obtained indicate that, at both third and sixth week of the study, there was no significant difference ($p>0.05$) among rats in the different groups in relation to Packed Cell Volume (%), Hemoglobin (g/dL), Red Blood Cell ($10^6/mm^3$), Mean Corpuscular Volume (*f*L), Mean Corpuscular Hemoglobin (pg), and Mean Corpuscular Hemoglobin Concentration (g/dL). In addition, for each of the measured hematological indices, except Packed Cell Volume of rats fed standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk (Group C), there was no significant difference ($p>0.05$) among rats in the same treatment between the third and sixth week of the study.

The normal ranges reported for Packed Cell Volume (%), Hemoglobin (g/dL), Red Blood Cell ($X10^6/mm^3$), Mean Corpuscular Volume (*f*L), Mean Corpuscular Hemoglobin (pg), and Mean Corpuscular Hemoglobin Concentration (g/dL) are 36 – 54, 11 – 19.2, 6 – 8, 29.4 – 123.1, 18.4 – 36.9, and 25.4 – 80.1 respectively (Delwatta et al., 2018; Wikivet, 2012). The results for the aforementioned hematological parameters obtained from the present study compare favorably with the values reported by Augustine et al.(2020) and are within the normal ranges reported in the literature. Therefore, it implies that feeding AFM_1-contaminated milk (456 ng/L) and Imarsil™-remediated AFM_1 (456 ng/L) contaminated milk to male albino rats had no adverse effects on these measured hematological parameters. The maximum tolerance limit accepted by the US and joint FAO/WHO Expert Committee on Food Additives and the European Union is 500 ng/kg and 50 ng/kg, respectively (Omar, 2016). Data on hematological parameters from the present study justifies the relaxed limit of 500 ng/kg adopted by the US and JEFCA. However, several nations, including Nigeria, Pakistan, India, and several sub-Saharan African nations, had AFM_1 levels in milk that substantially exceeded the United States and European Union regulatory limits for AFM_1, indicating a potential risk to individuals in those nations with high milk consumption (Turna & Wu, 2021).

Table 3 shows the blood platelet, neutrophil, lymphocyte, and white blood cell counts of albino rats in the four treatment groups recorded at three and six weeks of the study. The results revealed that in the third week of the study, there was no significant difference ($p>0.05$) among rats in the different groups in relation to neutrophil and lymphocyte counts (%), while platelet and white blood cell counts (%), exhibited a significant ($p<0.05$) change. However, in the sixth week of the study, there was no significant difference ($p>0.05$) among rats in the different groups in relation to neutrophil, lymphocyte, and platelet counts. However, the rats fed standard ration, and 2 mL of AFM_1 (456 ng/L) contaminated milk (Group C) had a significant ($p<0.05$) lowest value of 8.63 ± 2.61 ($10^3/mm^3$) of white blood cells. In another development, a test for significant difference ($p<0.05$) among rats in the same treatment between the third and sixth week of the study indicates that there was a significant reduction ($p<0.05$) in the white blood cell ($10^3/mm^3$) (12.90 - 8.63) and lymphocytes (87.00% - 74.33%) counts from third to sixth week of the study respectively while the neutrophils (%) increased from 13.00 to 25.67 for the albino rats fed standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk (Group C).

The normal ranges reported for blood platelet ($X10^5/\mu L$), neutrophil (%), lymphocyte (%), and white blood cell ($/mm^3$) counts are 1.70 – 8.03, 13 – 16, 61– 86, 4,400 – 14,800 (Delwatta et al., 2018). The results for these additional hematological parameters, as obtained from the present study, were within the normal ranges reported in the literature. This observation holds for all the treatment groups and at both the third and sixth week of the study. Therefore, it implies that feeding AFM_1-contaminated milk at the dosage of 456 ng/L and Imarsil™-remediated AFM_1 (456 ng/L) contaminated milk to male albino rats had no adverse effects on all the measured hematological parameters.

Many disorders can decrease the number of lymphocytes in the blood, but viral infections and undernutrition are the most common causes (Dale, 2023). Other risk factors include exposure to chemicals such as cladribine tablets (Comi et al., 2019; Spiezia et al., 2022), chemotherapy, and radiotherapy (Kanegasaki et al., 2019). The significant reduction ($p<0.05$) in the white blood cell and lymphocyte counts at the end of the sixth week was observed for the albino rats fed standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk (Group C) in this study, though the final values are still within the normal range, is a pointer to the fact that regular consumption of AFM1 contaminated milk even at 456 ng/L concentration is

Table 2. Hematological indices of albino rats fed AFM_1 contaminated milk and Imarsil™ treated AFM_1 contaminated milk in a 6-weeks study.

Treatment Group	Packed Cell Volume (%)		Hemoglobin (g/dL)		Red Blood Cell (10^6/mm^3)		Mean Corpuscular Volume (*f*L)		Mean Corpuscular Hemoglobin (pg)		Mean Corpuscular Hemoglobin Concentration (g/dL)	
	Time of Study (Week)											
	3	6	3	6	3	6	3	6	3	6	3	6
A	52.33±4.93[a]	49.33±5.77[a]	14.70±1.00[a]	15.43±0.42[a]	8.01±0.88[a]	8.23±1.16[a]	68.40±2.17[a]	61.53±3.10[a]	18.40±0.82[a]	18.97±2.37[a]	26.93±0.75[a]	31.53±2.55[a]
B	41.33±21.08[a]	47.00±6.56[a]	12.13±4.90[a]	15.93±0.85[a]	6.53±2.86[a]	7.48±1.34[a]	62.10±7.45[a]	65.93±2.73[a]	18.90±1.04[a]	21.97±5.53[a]	30.80±4.80[a]	34.77±6.95[a]
C	53.00±3.61[a]	44.67±0.58[a]	15.17±0.91[a]	14.67±0.23[a]	8.22±0.45[a]	7.62±0.23[a]	64.50±1.66[a]	65.87±2.66[a]	18.43±0.45[a]	19.27±0.29[a]	28.60±0.27[a]	32.80±0.10[a]
D	51.00±3.46[a]	41.67±8.74[a]	14.73±038[a]	15.77±1.66[a]	7.87±0.23[a]	6.84±1.65[a]	64.93±2.54[a]	62.70±2.10[a]	18.73±0.21[a]	23.97±6.35[a]	28.90±1.25[a]	38.96±8.92[a]

Values are means ± standard deviation of triplicate readings. Within a column, values with different superscript differ significantly ($p<0.05$)
A-Rat fed with standard ration and 2 mL of distilled water; B- Rat fed with standard ration and 2 mL of milk; C-Rat fed with standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk; D- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) treated with Imarsil™ at 2% dosage rate.

Table 3. Blood platelet, neutrophil, lymphocyte and white blood cell counts of albino rats fed AFM_1 contaminated milk and Imarsil™ treated AFM_1 contaminated milk in a 6-weeks study.

Treatment Group	Platelet (10^3/µL)		Neutrophil (%)		Lymphocyte (%)		White Blood Cell (10^3/mm^3)	
	Time of Study (Week)							
	3	6	3	6	3	6	3	6
A	689.67±77.09[ab]	786.67±88.95[a]	16.33±3.06[a]	15.67±4.04[a]	83.67±3.06[a]	84.33±4.04[a]	16.93±6.25[a]	13.60±2.33[a]
B	305.67±333.89[b]	798.00±139.82[a]	16.33±11.59[a]	22.67±7.02[a]	83.76±11.59[a]	77.33±7.02[a]	7.40±5.31[b]	15.33±2.90[a]
C	769.000±118.19[a]	782.67±64.31[a]	13.00±11.14[a]	25.67±8.14[a]	87.00±11.14[a]	74.33±8.14[a]	12.90±1.66[ab]	8.63±2.61[b]
D	637.00±179.10[ab]	763.00±108.35[a]	17.67±4.62[a]	18.33±5.13[a]	82.33±4.62[a]	81.67±5.13[a]	14.27±3.73[ab]	15.13±1.33[a]

Values are means ± standard deviation of triplicate readings. Within a column, values with different superscript differ significantly ($p<0.05$)
A-Rat fed with standard ration and 2 mL of distilled water; B- Rat fed with standard ration and 2 mL of milk; C-Rat fed with standard ration and 2 mL of AFM_1 (456 ng/L) contaminated milk; D- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) treated with Imarsil™ at 2% dosage rate

still a potential risk, and this could be surmounted by detoxification of AFM1 contaminated milk using Imarsil™. Neutrophils are a type of white blood cell that helps the body fight infections and heal injuries. They may increase in response to several conditions or disorders, including infections, injuries, inflammatory disorders, and some drugs such as corticosteroids. In many instances, the increased number of neutrophils is a necessary reaction by the body as it tries to heal or ward off an invading microorganism or foreign substance (Dale, 2023). A significant increase ($p<0.05$) in the neutrophils counts at the end of the sixth week, observed for the albino rats in Group C in the present study, though the final values are still within the normal range, is a possible indication of infections, injuries or inflammatory disorders in the rat models fed AFM_1 (456 ng/L) contaminated milk.

The rat kidney and liver sections from each group at the third and sixth weeks of the study are shown in Figure 1 and Figure 2, respectively. There was no visible histopathological kidney cell lesion in rats fed with a standard ration and 2 mL of distilled water (A1, A2) and rats fed with a standard

ration, and 2 mL of milk (BI, B2). However, sections obtained from rats fed standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) had severe and generalized interstitial congestions (C1, C2). In kidney sections of rats fed standard ration and 2 mL of Imarsil™-remediated AFM_1 (456 ng/L) contaminated milk, there was no evidence of histopathological lesions (D1, D2). In a similar trend to the histopathological changes observed in the kidney sections, the liver sections from rats fed standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) had severe portal congestion and periportal cellular infiltration by mononuclear cells (C3, C4) while sections from other three treatments showed no histopathological evidence of liver parenchyma lesions (A3, A4, B3, B4, D3, D4).

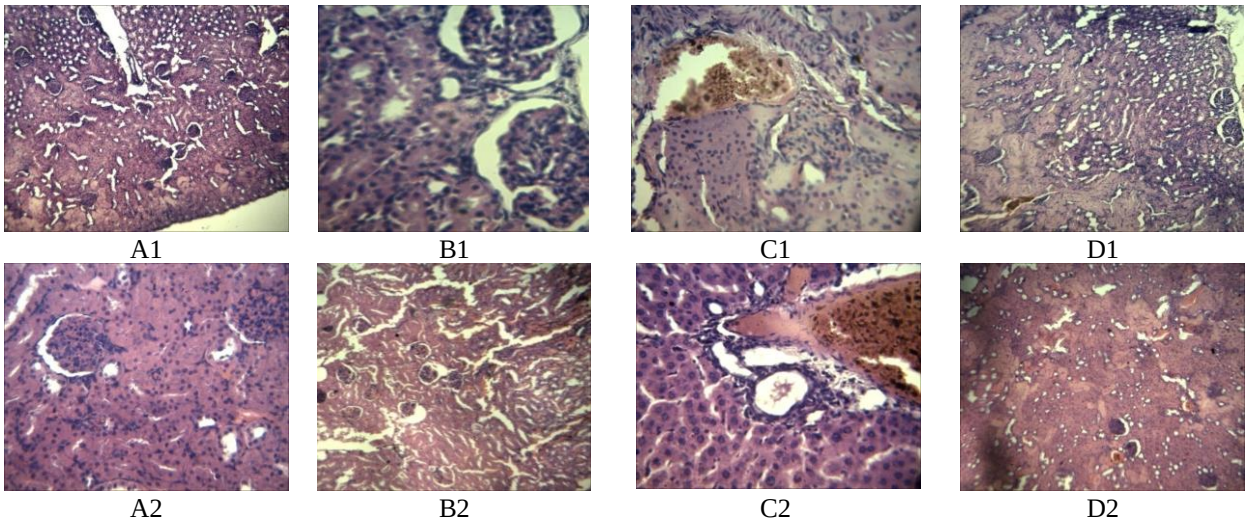

Figure 1. Photomicrograph of rat kidney sections (original magnification X 200) from each group after 3 and 6 weeks of study. KEY:

A1-Rat fed with standard ration and 2 mL of distilled water at 3 weeks of study; A2-Rat fed with standard ration and 2 mL of distilled water at 6 weeks of study;

B1- Rat fed with standard ration and 2 mL of milk at 3 weeks of study; B2- Rat fed with standard ration and 2 mL of milk at 6 weeks of study;

C1-Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) at 3 weeks of study; C2-Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) at 6 weeks of study:

D1- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) treated with Imarsil™ at 2% dosage rate. for 3 weeks; D2- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) treated with Imarsil™ at 2% dosage rate for 6 weeks.

A1, A2, B1, B2, and D1, D2 = No visible lesion; C1, C2= severe and generalized interstitial congestion after 3 and 6 weeks of study

Sequel to the consumption of aflatoxin-contaminated food/feed, aflatoxins are absorbed through the intestinal mucosa and enter the blood circulation to produce changes in multiple body organ systems. The primary organ for detoxification in vertebrates is the liver. It filters blood coming directly from the intestines and prepares toxins for excretion from the body. The liver is a target organ for aflatoxin toxicity as it is the site where aflatoxins undergo bioactivation to reactive 8,9-epoxide, which then binds to DNA and proteins (Benkerroum, 2020), causing widespread damage to the hepatic tissue. The hepatotoxicity of AF includes disturbances in lipid, carbohydrate, and protein metabolism (James et al., 2022), as well as hematopoiesis (Hamada et al., 2019). Aflatoxicosis-induced pathomorphological liver changes consist of dystrophic and necrotic changes in hepatocytes and bile duct epithelial hyperplasia vascular congestion, occasional areas of hemorrhages, varying degrees of diffused fatty change, leukocytic infiltration, mild to moderate degree of biliary hyperplasia and periportal fibrosis (Dhakal et al., 2023; Lakkawar et al., 2017; Valchev, Kanakov, et al., 2014; Yaman et al., 2016).

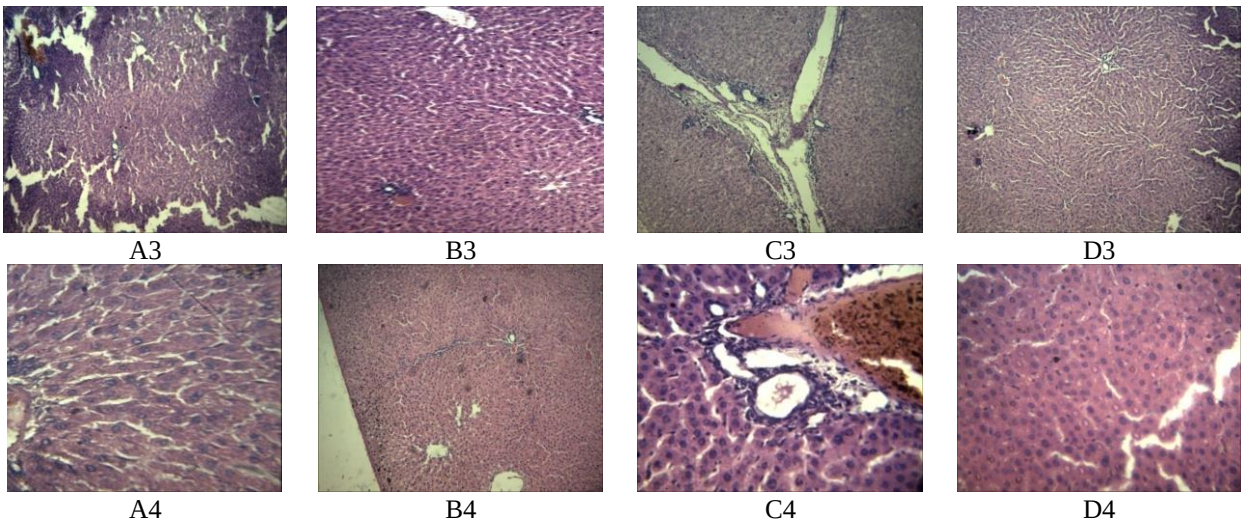

Figure 2: Photomicrograph of rat liver sections (original magnification X 200) from each group after at 3 and 6 weeks of study. KEY:

A3-Rat fed with standard ration and 2 mL of distilled water at 3 weeks of study; A4-Rat fed with standard ration and 2 mL of distilled water at 6 weeks of study;

B3- Rat fed with standard ration and 2 mL of milk at 3 weeks of study; B4- Rat fed with standard ration and 2 mL of milk at 6 weeks of study;

C3-Rat fed with standard ration and 2 mL of AFM_1 contaminated milk at 3 weeks of study; C4-Rat fed with standard ration and 2 mL of AFM_1 contaminated milk at 6 weeks of study:

D3- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk treated with Imarsil™ at 3 weeks of study; D4- Rat fed with standard ration and 2 mL of AFM_1 contaminated milk treated with Imarsil™ at 6 weeks of study.

A3, A4, B3, B4, D3, and D4= No visible lesion; C3 severe portal congestion and periportal cellular infiltration by mononuclear cells; C4 very severe portal congestion

In the present study, severe portal congestion and periportal cellular infiltration by mononuclear cells were observed in the liver sections, and severe and generalized interstitial congestions in kidney sections of rat fed standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) is similar to the observations recorded by many workers (Lakkawar et al., 2017; Valchev, Kanakov, et al., 2014; Valchev, Zarkov, et al., 2014) As stated earlier, neutrophils may increase in response to a several number of conditions or disorders, including infections, injuries, inflammatory disorders, and in many instances, the increased number of neutrophils is a necessary reaction by the body, as it tries to heal or ward off an invading microorganism or foreign substance (Dale, 2023). In the present study, the significant increase in the neutrophils counts at the end of the sixth week observed for the albino rats fed standard ration and 2 mL of AFM_1 contaminated milk (456 ng/L) might be an immunologic response geared toward the healing tissue injury occasioned by AFM_1 in the contaminated milk.

Managerial Implication for Dairy Industry

The results of this study emphasize the critical nature of efficiently managing aflatoxin M1 (AFM1) in the dairy sector to protect consumer health and comply with rigorous quality and safety regulations. Diary producers have been presented with an innovative method to reduce the concentrations of AFM1 in their products with the introduction of Imarsil™ as a mitigation strategy. This development may also bolster consumers' confidence in such products to enhancing the compliance and safety of dairy products.

A comprehensive understanding of its application and the subsequent impact on product quality is imperative for the successful integration of Imarsil™ into the dairy production workflow (Gonçalves et al., 2019). Dairy administrators are responsible for assessing and potentially adjusting their current operational

frameworks to facilitate the Imarsil™ treatment protocol. Potential prerequisites for this adaptation include initial capital outlays for apparatus acquisition and personnel training. These expenditures are considered appropriate in light of the increased product safety they facilitate which subsequently aids in mitigating public health risks and the financial consequences of product recalls.

A unique opportunity exists for brands to position themselves as leaders in terms of product safety and innovation through the strategic implementation of Imarsil™ in the dairy industry. Furthermore, dairy companies can expand their consumer allure by differentiating their products in a market that is placing a greater emphasis on health (Bublitz & Peracchio, 2015). This strategic positioning plays a crucial role in cultivating intensified brand loyalty by emphasizing a company's commitment to upholding the utmost levels of quality and safety.

CONCLUSIONS

In this study, milk contaminated with aflatoxin M1 (AFM1) at a concentration of 456 ng/L did not adversely affect the hematological parameters when administered to male albino rats over six weeks. However, daily feeding of the contaminated milk provoked severe portal congestion and periportal cellular infiltration by mononuclear cells in the liver and severe and generalized interstitial congestions in the kidneys of rats. The addition of imarsil at a 2% dosage rate to the contaminated milk reliably obviated the histological lesions resulting from aflatoxicosis without negatively affecting the monitored blood parameters. Therefore, imarsil represents an effective and safe adsorbent for the remediation of AFM1-contaminated milk, especially in the tropical developing world, and the limit set by the US and joint FAO/WHO Expert Committee on Food Additives will need to be reviewed downward.

Future studies could investigate alternate adsorbents to Imarsil™ for AFM1 decontamination in milk, especially those available locally in different regions. Studying the long-term impact of Imarsil™ therapy on milk nutritional quality could benefit dairy producers. Expanding studies to include Imarsil™'s efficacy in mitigating other mycotoxins in food matrices could increase its applicability and impact on food safety.

SOURCE OF ANIMALS AND ETHICAL CLEARANCE

The male Wister albino rats used in this study were obtained from the Animal House of Biochemistry Department, College of Medicine, University of Ibadan, Nigeria. Human care, according to standard criteria outlined for Laboratory Animals by National Research Council (2011), was given to the rats used in this study. The ethical considerations of the use of the rats were in line with the specifications of Nuffield Council on Bioethics. Augustine University, Ilara-Epe Animal Welfare and Ethical Committee approved the conduct of the study.

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