Biochemical profiling of albino wistar rats (*Rattus norvegicus*) fed imarsil™ - treated aflatoxin M_1 (AFM_1) contaminated milk

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Abstract

The potential of imarsil™ to decontaminate aflatoxin M_1 (AFM_1) from cow's milk has been documented in previous study. The present study focused on biochemical profiling of albino wistar rats (*Rattus norvegicus*) fed Imarsil™ - treated AFM_1 contaminated milk. Seventy-two male Wister albino rats were randomly allocated to four treatment groups (A – D) in a completely randomized design with three replicates of six rats each in a six weeks study. The treatments given to the four groups were as follows: Group A-Rats fed with standard ration and 2 ml of clean distilled water; Group B- Rats fed with standard ration and 2 ml of milk; Group C-Rats fed with standard ration and 2 ml of AFM_1 contaminated milk (456 ng/L); Group D- Rats fed with standard ration and 2 ml of AFM_1 contaminated milk (456 ng/L) treated with imarsil™ at 2% dosage rate. Feeding AFM_1 contaminated milk (456 ng/L) to male albino rats elicited abnormal levels of alanine aminotransferase, serum albumin, creatinine, and blood calcium. However, addition of imarsil at 2% dosage rate to the contaminated milk provoked protective efficacy against the hepatotoxicity of AFM_1 and normalization of blood calcium level. Therefore, imarsil represents an effective and safe adsorbent for the remediation of AFM_1 contaminated milk in the tropical developing world.

Keywords: aflatoxin M_1; cow's milk; imarsil™; kidney status parameters; liver status parameters

1. Introduction

Milks of animal origin are concentrated dietary sources of macro- and micronutrients. There has been a global increase in milk production and consumption over the past decades as, in many cultures; humans continue to consume milk beyond infancy, using the animal milk especially from cattle, goats and sheep as food products. In this regard and for many decades, cow milk has been processed into dairy products such as condensed milk, skimmed milk, ice cream, butter, yoghurt and the more durable cheese products to fight malnutrition (Kumar et al., 2016; Abah et al., 2020). The unbridled global growth in milk consumption is hinged on its many nutritional and health benefits. Rumbold et al. (2022) stated that beyond milk's nutritional value, an increasing body of evidence 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 lower risk of osteoporosis and fractures, especially in older adults (Musa

et al. 2021). Consumption of milk by undernourished children has been reported to improve anthropometric indices and cognitive function and reduces the prevalence of biochemical and functional nutritional deficiencies, and thereby reducing morbidity and mortality (Dror and Allen, 2011).

A large portion of dairy cattle in many developing countries are raised under free-ranging conditions (Oluwafemi et al., 2014a). For instance, more than ninety percent of the ruminant livestock in Nigeria lies in the hands of herders who keep them under extensive and semi-intensive management systems, whereby the animals rely only on natural pasture and crop residues for survival (Oluwafemi et al., 2014a; Lawal-Adebowale, 2012). Herds of free-ranging and free-grazing dairy cattle are a difficult task in aspects of feed monitoring as there is understanding that hay, which contains a large complement of cereal grain infested in the field, could be a significant source of aflatoxins (Lizárraga-Paulín et al., 2011). Consequent upon the usual carry-over of aflatoxin B1 from cows' feed as aflatoxin M1 (AFM1) into milk, evidences have suggested that milk and milk products may present a risk for the consumer, especially, if there is no efficient aflatoxin control in feeds [Galvano et al., 2001; Henry et al., 2001; Creppy, 2002; Gürbay et al., 2006; Oliveira and Farraz, 2007). Britzi et al. (2013) have reported a carry-over rate of 1–2 % and 6 % of the ingested aflatoxin B1 (AFB1 in Israeli dairy cows milked two times daily for low-yielding cows and high-yielding cows respectively.

Turna and Wu (2021) reported that several nations including Pakistan, India, Nigeria, Kenya, and many sub-Saharan African nations, had aflatoxin M1 (AFM1) levels in milk that substantially exceeded United States and European Union regulatory limits for AFM1. This is indicating potential risk to individuals in those nations with high milk consumption. In Post-2015 Development Agenda, the World promised to end hunger, achieve food security and promote agriculture through Sustainable Development Goal 2 (SDG2) by 2030. Therefore, it is imperative for these developing nations to evolve sustainable approaches to control AFM1 in milk of animal origin

In a review of novel approaches to reduce the incidence of AFM1 in milk, Nguyen et al. (2019) submitted that UV at narrow wavelength and high intensity can improve AFM$_1$ reduction efficacy. They also posited that microbial extract and cold plasma are promising method to decrease AFM1 in milk. These authors further stated that the mechanism of the treatment methods needs to be explored and milk quality after treatment should be assessed in order to ascertain safety and efficiency. Meanwhile, Aringbangba et al. (2021) had opined that given that the impact of the global aflatoxin burden is huge in low-income countries due to the lack or insufficient resources needed to tackle the menace; it is therefore necessary that cost-effectiveness, rapidity, safety, simplicity, and technical feasibility will determine the suitability of any aflatoxin decontamination approach in the developing nations.

In a search for a locally effective adsorbent for AFM1 decontamination of cow's milk in Nigeria, Imarsil^{TM} was found to exhibit maximum decontamination efficacy at 2 % dosage rate at 28°C for 5 hours (Oluwafemi et al., 2014b). Imarsil^{TM}, was developed by Akpan and Kareem (2002), and it is an inexpensive synthetic absorbent obtained from oxidized natural polymer of *Brachystegia nigerica*. *B. nigerica* is a legume used especially in the eastern states of Nigeria as condiment to thicken soup. The previous decontamination study using Imarsil^{TM} indicated that Imarsil^{TM} is a promising adsorbent candidate for reduction of aflatoxin M$_1$ in cow's milk, however no study has evaluated the effect of such decontamination strategy on the liver and kidney function status of both humans and animals. The present study therefore focuses on biochemical profiling of albino wistar rats fed Imarsil^{TM} treated AFM1 contaminated milk.

2. Methodology and methods

2.1. Materials

Male Wister albino rats were obtained from the Animal House of Biochemistry Department, University of Ibadan, Nigeria. Imarsil^{TM} used in this study was prepared as previously described by Akpan and Kareem (2002) while AFM1 standard was purchased from Chromogen (New Delhi, India). Veterinary milk and standard ration (Vital Feed Limited, Abeokuta) were obtained from Trust Veterinary, Ibadan, Nigeria.

2.2. Imarsil preparation and detoxification of AFM1 contaminated milk

Oluwafemi et al. (2014a), in an earlier study reported a range of 9.0 to 456.0 ng/L AFM1 in a survey of AFM1 in cows' milk from free-grazing cows in Nigeria. Therefore, in the present study, milk samples (50mL) were spiked with AFM1 at the concentrations of 456 ng/L AFM1.

In our previous report on decontamination of AFM1 contaminated cow milk, Imarsil™ was found to exhibit maximum decontamination efficacy at 2% dosage rate at 28° C for 5 hours (Oluwafemi et al., 2014b). In the present study, Imarsil™ was prepared as previously described by (Akpan and Kareem, 2002). Spiked cow milks (50mL) containing 1g of Imarsil™ were passed through a separating funnel. The experimental setups were in place for 5 hours to obtain remediated milk samples that was used for animal feeding experiment.

2.3. Experimental Animal and Design

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 the dimension 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 the period of two weeks acclimatization. Thereafter, 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 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 AFM1 contaminated milk (456ng/L);
Group D- Rat fed with standard ration and 2 ml of AFM1 contaminated milk (456ng/L) treated with imarsil at 2% dosage rate.

2.4. Management and Sacrifice of Experimental Animal

Rats in each group were fed with standard ration *ad libitum* and clean drinking water was regularly supplied. The measured liquid portion of the diet for each group was given to each rat with the use of an intubator at the tip of a syringe. This was done in order to prevent the harm a needle might cause to the animal while feeding them. All animals received human care according to standard criteria outlined for Laboratory Animals (Ochei and Kolhatkar, 2007) and the ethics of the Augustine University, Ilara-Epe Animal Welfare and Ethical Committee. The ethical considerations of the use of the rats were in line with the specifications of Nuffield Council on Bioethics. The experiment lasted for six weeks (42 d).

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 weeks interval. On days 21(3 weeks) and 42 (6 weeks), three (3) of the rats in each groups were sacrificed. The rats were sedated with diethyl-ether soaked cotton wool swabs in a desiccator and the blood samples (5.0 mL) were collected into lithium heparinized and serum separation tube- (SST-) gel vacutainer bottles. The samples were centrifuged at 3,000 revolutions per minute (rpm) for five minutes. Plasma and serum were extracted from lithium heparinized and SST-gel vacutainer respectively. The samples were kept frozen at -20°C pending liver and kidney function status analyses.

2.5. Biochemical Profiling

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) in the serum of experimental animals were determined using Cobas C111 Autoanalyzer by Roche. Plasma creatinine, albumin, bilirubin, uric acid, and urea were analyzed using Cobas C111 Autoanalyzer by Roche. Electrolytes (Sodium, Potassium, and Calcium) were analyzed with SFRI 4000 ion selective electrode (ISE).

2.6. 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. Post hoc multiple comparisons for the ANOVA were done using least significant difference (LSD). Student's t-test was used to test for significant difference between values obtained at 3rd and 6th week of study within the same treatment. Statistical significance was accepted at P value of less than or equal to 0.05.

3. Results and discussion

Figure 1 illustrates the changes in the weight of albino rats in the four treatment groups during the six weeks study. No significant difference ($p>0.05$) was observed among the weight of rats in the different groups at week 0. However, as the study progressed through the 2nd, 4th, and 6th week, there was significant changes ($p<0.05$) in the weight of rats among the four treatments. In this connection, rats fed standard ration and 2 ml of AFM_1 (456 ng/L) contaminated milk had a significant ($p<0.05$) lower weight. In addition, the weight of rats fed imarsil-remediated AFM_1 (456 ng/L) contaminated milk was not significantly different ($p>0.05$) from those fed uncontaminated milk and clean water only.

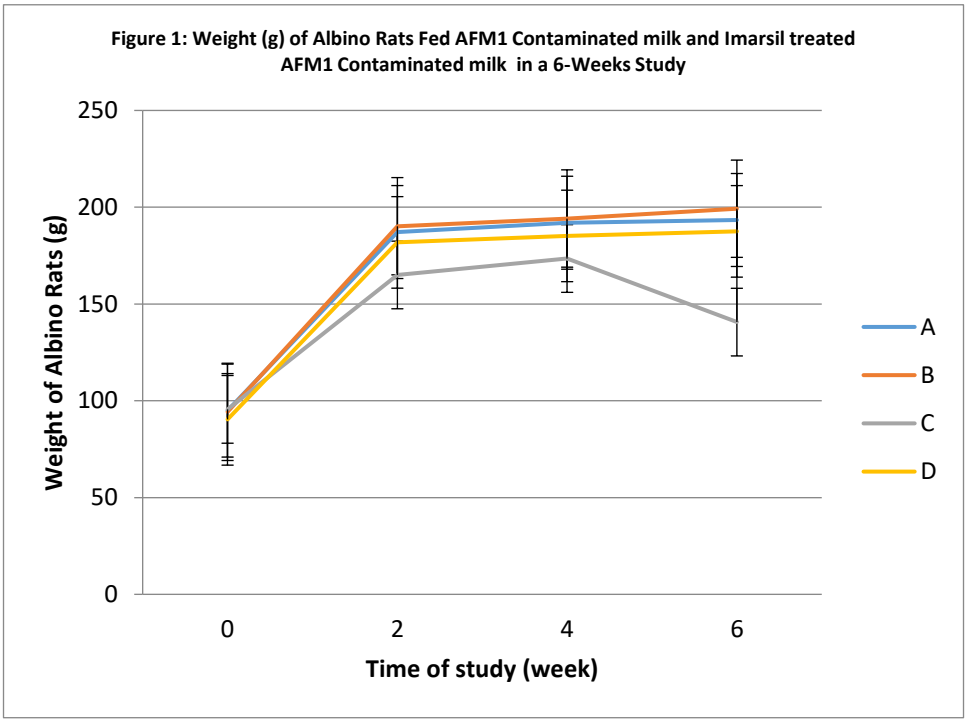
It is well reported that aflatoxicosis causes poor absorption of nutrients from the diet, reduction of biliary acids, and reduction of the activity of lipase, trypsin, amylase, and RNAse in broilers exposed to 1.25 and 2.5 mg AFB1/kg diet thereby leading to adverse effect on intestinal functions (Osborne and Hamilton, 1981; Devegowda and Murthy, 2005; Yunus et al., 2011). Furthermore, aflatoxins have been shown to adversely affect the innate immune system and intestinal intraepithelial cells of intestine leading to disturbance in structural integrity of the intestine (Lakkawar et al., 2017). Kumar and Balachandran (2009) observed catarrhal enteritis with lymphocytic or mononuclear cell infiltrations in the intestine of broilers fed on the toxin contaminated ration (1 mg AF /Kg of feed) for 4 weeks. This influence was believed to have been responsible for a reduction or lack of production of the hormone responsible for the release of digestive enzymes. In a similar vein, Mallmann et al. (2006) reported that in chronic aflatoxicosis outbreaks one of the most remarkable characteristics is the mal-absorption of feed that is manifested by the presence of poorly digested feed particles in the excreta of the birds.

Having observed a significantly lower body weight in birds fed aflatoxin contaminated feed, Lakkawar et al. (2017) correlated their observation to 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 experiment. Therefore, in conformity with the earlier reports, the significantly lower body weight in rats fed standard ration and 2 ml of AFM_1 (456 ng/L) contaminated milk in the present study could also be related to reduce ability of the rats to digest the feed in presence of aflatoxin in the intestines of the rats.

Blood serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) of both controlled and experimental albino rats are shown in Table 1. At both 3rd and 6th week of the study, there was no significant difference ($p>0.05$) among the four groups in relation to the examined three liver enzymes. In another development, test for significant difference ($p<0.05$) among rats with the same treatment between the third and sixth week of the study indicates that, there was a significant decrease ($p<0.05$) in the values for ALT and ALP while the changes in the AST values between 3rd and 6th week of the study for all the treatments were not significant.

AST and ALT levels are widely accepted biomarkers for the function and integrity of the liver and heart as they are usually released from damaged liver cells (Mancinelli and Ceccanti, 2007). Their elevation in the serum has been associated with tissue necrosis, cardiovascular damage and non-alcoholic fatty liver disease (Ioannou et al., 2006). As per ALP, it is a membrane bound enzyme and elevation in ALP levels is usually observed in cancer, heart infections and liver damage (Valchev et al. 2014). According to the manufacturer of Cobas C111 Autoanalyzer, the normal ranges for AST, ALT, and ALP are 5 – 18 IU/L, 3 – 15 IU/L, and 25 – 92 IU/L respectively (Odiegwu et al., 2021). In the current study, the AST, ALT, and ALP values for rats in all treatment groups at the 3rd and 6th week of the study,

are within the normal ranges for these enzymes. The exception to this general trend is found in ALT values for rats fed with standard ration and 2 ml of AFM1 (456 ng/L) contaminated milk (group C) at the 3rd week of the study. Of all the three enzymes examined in this study, ALT is the only enzyme that indicated a possible damage of liver cells.



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 AFM1 (456 ng/L) contaminated milk ;
D- Rat fed with standard ration and 2 ml of AFM1 contaminated milk (456 ng/L) treated with imarsil at 2% dosage rate.

Table 1: Blood serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) of Albino Rats Fed AFM1 Contaminated milk and Imarsil treated AFM1 Contaminated milk in a 6-Weeks Study.

Treatment Group	AST (IU)		ALP (IU)		ALT(IU)	
	Time of Study (Week)					
	3	6	3	6	3	6
A	4.50±0.00[a]	4.05±0.06[a]	71.75±1.12[a]	16.04±14.44[a]	15.8±5.20[b]	11.6± 7.60[a]
B	5.55±0.60[a]	4.50±0.10[a]	71.89±2.85[a]	7.89±7.07[a]	15.0±6.60[b]	7.50± 4.30[a]
C	6.00±0.10[a]	4.50±0.00[a]	71.19±1.18[a]	28.44±21.47[a]	26.7±2.08[a]	15.8± 7.20[a]
D	5.55±0.62[a]	4.50±0.17[a]	69.66±2.68[a]	21.88±17.78[a]	16.0±2.50[b]	10.0± 5.00[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 AFM1 (456 ng/L) contaminated milk ; D- Rat fed with standard ration and 2 ml of AFM1 contaminated milk (456 ng/L) treated with imarsil at 2% dosage rate

Aflatoxin M_1 is a toxic metabolite of aflatoxin B_1 (AFB_1). Although AFM_1 is less carcinogenic and mutagenic than AFB_1, it exhibits a high level of genotoxic activity and certainly represents a health risk due to its possible accumulation and linkage to DNA (Shundo And Sabino, 2006). The maximum tolerance limits accepted by the US

and joint FAO/WHO Expert Committee on Food Additives, and European Union are 500 ng/kg and 50 ng/kg respectively. Data from the present study is suggesting that the 500 ng/kg limit set by US and joint FAO/WHO Expert Committee on Food Additives investigations will need to be reviewed downward. This is plausible on the understanding that group C rats that were fed with standard ration and 2 ml of AFM1 (456 ng/L) contaminated milk (group C) still exhibited liver damage. However, Imarsil™ protected the liver cells against the hepatotoxic effect of AFM_1 as shown in the lowering of ALT values for rats in group D.

The serum albumin, bilirubin, creatinine, urea, and uric acid concentrations of both controlled and experimental albino rats are shown in Table 2. At the 3rd week of the study, there was no significant difference ($p>0.05$) among the four groups in relation to albumin, creatinine, urea and uric acid but rats in group C had a significant highest value of 13.06±5.11 µmol/L of bilirubin. However, by the 6th week of the study, there was no significant difference ($p>0.05$) among the four groups in relation to bilirubin, creatinine, urea and uric acid but rats in group C had a significant highest value of 13.63±1.21 µmol/L of albumin. In another development, 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 increase ($p<0.05$) in the values for albumin, bilirubin, creatinine, urea, and uric acid for all treatment groups except group C where there was only a moderate increase was observed.

Table 2: Blood plasma albumin concentration, bilirubin concentration, creatinine concentration, and uric acid concentration of Albino Rats Fed AFM1 Contaminated milk and Imarsil treated AFM1 Contaminated milk in a 6-Weeks Study.

Treatment Group	Albumin(g/dL)		Bilirubin (µmol/L)		Creatinine (mg/dL)		Urea (mg/dL)		Uric acid (mg/dL)	
	Time of Study (Week)									
	3	6	3	6	3	6	3	6	3	6
A	2.80±0.96[a]	7.85±0.96[b]	4.78±3.59[b]	13..47±3.66[a]	0.23±0.19[a]	1.04±0.88[a]	3.51±0.92[a]	19.84±0.037[a]	3.21±0.70[a]	7.28±2.36[a]
B	4.94±02.88[a]	7.51±0.32[b]	9.58±0.90[ab]	17.71±6.45[a]	0.26±0.17[a]	0.92±0.11[a]	1.91±0.88[a]	19.25±0.021[a]	4.46±2.19[a]	7.12±1.69[a]
C	12.11±8.95[a]	13.63±1.21[a]	13.06±5.11[a]	14.74±1.86[a]	0.15±0.12[a]	2.05±1.68[a]	2.42±0.89[a]	16.43±0.326[a]	5.00±0.67[a]	6.07±2.75[a]
D	4.34±1.96[a]	8.19±0..32[b]	7.17±4.91[ab]	13.62±7.23[a]	0.17±0.12[a]	1.50±1.10[a]	2.32±0.73[a]	19.56±0.122[a]	3.16±0.38[a]	3.86±1.45[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 AFM1 (456 ng/L) contaminated milk ; D- Rat fed with standard ration and 2 ml of AFM1 contaminated milk (456 ng/L) treated with imarsil at 2% dosage rate.

Serum creatinine and urea are both widely accepted and used markers to evaluate the renal function. Urea is a waste product of protein metabolism and its serum levels rise in patients with kidney failure, leading to uremia (Entedhar and Nawal, 2016). Creatinine is produced by the breakdown of creatine phosphate in muscles (Zuo et al., 2008). Its values depend on muscle composition and function, activity, diet and health status (Banfi and Del Fabbro, 2006). Creatinine is a commonly used biomarker of kidney function, allowing for the monitoring of renal failure progression (Borisova et al., 2019). The increased serum albumin levels normally indicate impairment in the normal function of the liver (Imafidon and Okunrobo, 2012). Bilirubin accumulates from the breakup of haemoglobin present in red blood cells. During normal function, the liver removes bilirubin from the blood and excretes it through bile. A spike in total bilirubin usually indicates a compromise in the normal function of the liver (Imafidon and Okunrobo, 2012). The normal ranges for serum albumin, bilirubin, creatinine, urea, and uric acid concentrations are 3.4 – 5.4 g/dL, 1.71 – 20.5 µmol/L, 0.5 – 1.3 mg/dL, 5 – 20 mg/dL, 3.5 – 7.2 mg/dL (Sumathy, 2014; Webpath, 2023). In the current study, data obtained in relation to serum albumin for rats fed with standard ration and 2 ml of AFM1 (456 ng/L) contaminated milk (group C) at both 3rd and 6th week of the study were outside the normal range thus indicating a possible liver damage. However, data obtained from rats in group D showed that Imarsil exhibited protective efficacy against the hepatotoxicity of AFM1. In a similar vein, the values obtained for creatinine for rats in group C at the 6th week of the study were clearly outside the normal range while the corresponding data for group D suggests the hepato-protective efficacy of imarsil against AFM1. In a trend that was dissimilar to the liver function parameters described above, data collected on the kidney function parameters vis a viz bilirubin, urea and uric acid in all treatment groups are within the normal ranges for these parameters thus suggesting a healthy kidney status of both control and experimental rats.

The blood calcium, sodium and potassium concentrations of albino rats in different treatment groups are shown in Table 3. At the 3rd week of the study, there was no significant difference ($p>0.05$) among the four groups in relation to calcium while significant difference ($p<0.05$) existed for sodium and potassium with animals in group D and C having significant highest value of sodium (108.6±10.7 mmol/L) and potassium (1.74±0.02 mmol/L) respectively. However, at the 6th week of the study, there was no significant difference ($p>0.05$) among the four groups in relation to for sodium and potassium while significant difference ($p<0.05$) existed for calcium with animals in group C having significant highest value of 11.13±6.21 mg/dL. In another development, 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 increase ($p<0.05$) in the values for blood sodium and potassium levels for all treatment groups. There was also a significant decrease ($p<0.05$) in the values for calcium for all treatment groups except group C where non-significant ($p>0.05$) increase was observed.

Table 3: Blood Serum Calcium Concentration, Sodium Concentration, and Potassium Concentration, of Albino Rats Fed AFM1 Contaminated milk and Imarsil treated AFM1 Contaminated milk in a 6-Weeks Study.

Treatment Group	Calcium Concentration (mg/dL)		Sodium Concentration (mmol/L)		Potassium Concentration (mmol/L)	
	Time of Study (Week)					
	3	6	3	6	3	6
A	8.31±0.15^{a}	2.23±0.58^{b}	68.0±22.9^{b}	147.5±34.0^{a}	1.09±0.27^{b}	4.12±0.29^{a}
B	8.38±3.50^{a}	3.33±0.200^{b}	107.5±10.0^{a}	133.5±23.3^{a}	1.26±0.39^{ab}	4.07±0.64^{a}
C	8.37±1.04^{a}	11.13±6.21^{a}	84.1±16.6^{ab}	127.9±30.3^{a}	1.74±0.02^{a}	4.07±0.52^{a}
D	8.13±0.24^{a}	5.89±0.07^{ab}	108.6±10.7^{a}	163.8±31.3^{a}	1.62±0.20^{a}	4.03±0.87^{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 AFM1 (456 ng/L) contaminated milk ; D- Rat fed with standard ration and 2 ml of AFM1 contaminated milk (456 ng/L) treated with imarsil at 2% dosage rate.

Calcium is a mineral that makes the bones, as well as a salt that dissolves in the blood and regulates bodily function. The parathyroid glands are the "calcium thermostat" of the body. If the calcium level is too low, the parathyroid glands will release parathyroid hormone (PTH) that will raise blood calcium to the appropriate levels. However, if the calcium levels are too high, the parathyroid glands will stop releasing PTH so as to bring the calcium back down to normal (UCLA, 2023). Potassium and sodium are electrolytes that help the body function normally by maintaining fluid and blood volume. Electrolytes are particles that carry an electric charge when they are dissolved in blood. The kidneys help to maintain electrolyte concentrations by regulating its concentrations in the body. Any disturbance in this process often leads to an electrolytes imbalance (UCLA, 2023).

The normal ranges for blood calcium, sodium, and potassium concentrations are 8.6 – 10.3 mg/dL, 136 – 145 mmol/L, 3.6 – 5.2 mmol/L (Goldstein, 1990; UCLA, 2023). In all treatment groups, the blood calcium levels are within the normal range, except at the 6th week for the rats in group C. This is a possible indication that the thermostatic influence of parathyroid gland on blood calcium level might have been compromised. However, data collected from rats in group D is suggesting the protective efficacy of Imarsil against this deleterious effect of AFM1 on blood calcium level. The data reported for blood sodium and potassium for all treatment groups are within the normal ranges thus suggesting the absence of renal failure in both control and experimental rats.

4. Conclusion

Feeding AFM_1 contaminated milk (456 ng/L) to male albino rats elicited abnormal levels of alanine aminotransferase, serum albumin and creatinine, and blood calcium. However, addition of imarsil at 2% dosage rate to the contaminated milk provoked protective efficacy against the hepatotoxicity of AFM_1 and normalization of blood calcium level. Therefore, imarsil represents an effective and safe adsorbent for the remediation of AFM_1 contaminated milk in the tropical developing world. The maximum tolerance limit accepted by the US and joint

FAO/WHO Expert Committee on Food Additives, and European Union are 500 ng/kg and 50 ng/kg respectively. Results obtained on some biochemical parameters in the present study are indicating a need for downward review of the limit set by the US and joint FAO/WHO Expert Committee on Food Additives.

References

Abah, C. R., Ishiwu, C. N., Obiegbuna, J. E. and Oladejo, A. A. (2020) Sorghum Grains: Nutritional Composition, Functional Properties and Its Food Applications. *European Journal of Nutrition and Food Safety* 12(5): 101-111.

Akpan, I., S.O. Kareem (2002) Imarsil and Process of Preparation. Nigerian Patent No RP14784.

Aringbangba, O.E., Oluwafemi, F., Kolapo, A. L., Adeogun, A. I. and Popoola, T .O. S. (2021) Elimination of Aflatoxins from Two Selected Nigerian Vegetable oils using MagneticChitosan nanoparticles. *Industria: Jurnal. Teknologi. Dan. Manajemen. Agroindustri*. 10:1-11.

Banfi, G. and Del Fabbro, M. (2006) Serum creatinine values in elite athletes competing in 8 different sports: comparison with sedentary people. *Clinical Chemistry* 52:330-331.

Borisova, V. A., Eftimov, M. T. and Valcheva-Kuzmanova, S. V. (2019) Biochemical evaluationof liver and kidney function after subchronic administration of *Chaenomeles maulei* fruit juice to rats. *Bulgarian Chemical Communication* 51 (Special Issue A), 29 - 34.

Britzi, M., Friedman, S., Miron, J. , Solomon, R., Cuneah, O. , Shimshoni, J. A., Soback, S., Ashkenazi, R., Armer, S.and Shlosberg, A. (2013) Carryover of aflatoxin B1 to aflatoxinM1 in high yielding Israeli cows in mid and late lactation. *Toxins* 5:173–183.

Creppy, E. E. (2002): Update of survey, regulation and toxic effects of mycotoxins in Europe.*Toxicology Letter* 127: 19–28.

Devegowda, G. and Murthy, T. N. K. (2005) Mycotoxins: Their effects in poultry and some practical solutions. In The Mycotoxin Blue Book; Diaz, D.E., Ed.; NottinghamUniversity Press: Nottingham, UK. pp. 25–56.

Dror, D. K. and Allen, L. H. (2011) The Importance of Milk and other Animal-Source Foods for Children in Low-Income Countries. *Food and Nutrition Bullettin* 32, 227-243.

Entedhar, R. S. and Nawal, A. M. (2016) Biochemical Changes in Chronic Renal Failure Pre and Post Hemodialysis *Journal of Environmental Science and Engineering Technology* 5:190-195.

Galvano, F., Galofaro, V., Ritieni, A., Bognanno, M., De Angelis, A. and Gabano, G. (2001) Survey of the occurrence of aflatoxin M1 in dairy products marketed in Italy: second year of observation. *Food Additive and Contaminant* 18: 644–646.

Goldstein, D. A. (1990) Serum Calcium. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3^{r} edition. Boston: Butterworths.

Gürbay, A. S., Aydin, G., Girgin, A. B. and Sahih, G. (2006) Assessment of aflatoxin M1 levels in milk in Ankara, Turkey. *Food Control* 17: 1–4.

Henry, S. H., Whitaker, T. B., Rabbani, I., Bowers, J., Verger, P., Yoshizawa, T., Van Egmond, T, H., Jonker, M. A. and Coker, R. (2001) Aflatoxin M1, chemical safety information from inter-governmental organization. Joint FAO/WHO Expert Committee on Food Additives (JFCFA). *WHO Food Additive Series* 47: 45–80.

Imafidon,K. E. and Okunrobo, L.O. (2012) Study on Biochemical Indices of Liver Function Tests of Albino Rats Supplemented with Three Sources of Vegetable Oils. Nigerian Journal of Basic Applied Science 19: 105-110.

Ioannou, G.N., Weiss, N. S., Boyko, E. J., Mozaffarian, D. and Lee, S. P. (2006) Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States *Hepatology* 43:1145-1151. .

Kumar, R. and Balachandran, C. (2009) Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid. *Veterinarski. Arhiv* 79, 31-40.

Kumar, A., Metwal, M., Kaur, S., Gupta, A. K., Puranik, S., Singh, S., Singh, M., Gupta, S., Babu, B. K., Sood, S. and Yadav, R. (2016) Nutraceutical Value of Finger Millet [Eleusine coracana (L.) Gaertn.], and Their Improvement Using Omics Approaches.*Frontier in Plant Science* 29:7:934.

Lakkawar, A., Narayanaswamy, H. and Satyanarayana, M. (2017) Study on Efficacy of Diatomaceous Earth to Ameliorate Aflatoxin Induced Patho-Morphological Changes in Liver and Intestines of Broiler Chicken. International Journal of Livestock Research 7:71-84.

Lawal-Adebowale, O. A. (2012): Dynamics of ruminant livestock management in the context of the Nigerian Agricultural system. In: Livestock Production. Edited by Khalid Javed. pp 61–80.

Lizárraga-Paulín, E. G., Moreno-Martínez, E.and Miranda-Castro, S. P. (2011) Aflatoxins andtheir impact on human and animal health: an emerging problem. In:Aflatoxins—biochemistry and molecular biology (Guevara-Gonzalez, R. G. Ed.).Intech

Mallmann, C. A., Dilkin, P. , Giacomini, L. Z. and Ranber, R. H. (2006) Criteria for selection of a good paramycotoxin scavenger. APINCO Poultry Science and Technology Conference. Annals. (Critériosparaseleção de um bomsequestrante paramicotoxinas. Conferência APINCO de Ciência e Tecnologia Avícolas. Anais.) pp 213-224.

Mancinelli, R. and Ceccanti, M. (2009) Biomarkers in Alcohol Misuse: Their Role in the Prevention and Detection of Thiamine Deficiency. *Alcohol and Alcoholism* 44:177-182.

Musa, Y. and Adjene, J. O. (2021) Fura Da Nono Drink and Its Implications in the Treatment of Malnutrition in Bauchi Metropolis of Bauchi State. *Biomedical Journal of Science and Technology Research* 37:29047- 29052

Nguyen, T., Flint, S. and Palmer, J. (2019) Control of aflatoxin M1 in milk by novel methods: A review. *Food Chemistry* 311: 125984

Ochei, J. and Kolhatkar, A. (2007) Medical laboratory science theory and practice. 6thed. Tata McGraw-Hill Publishing Company Limited, New Delhi, 1232 pp.

Odiegwu, C.N.C., Chianella, I., Azubike, N. C., Odiegwu, U. O. and Ogbuowelu, O.S. (2021) Liver function tests values in Albino Wistar rats administered with isolated Nigeria *Achatina achatina* snail lectin. *GSC Biological and Pharmaceutical Science* 15: 92–102.

Oliveira, C. A. J. and Farraz, C. O. (2007) Occurrence of aflatoxin M1 in pasteurized UHT milk and milk powder from goat origin. *Food Control* 18: 375–378.

Oluwafemi, F., Badmos, A., Kareem, S. O., Ademuyiwa, O. and Kolapo, A. L. (2014) Survey of aflatoxin M1 in cows' milk from free-grazing cows in Abeokuta, Nigeria. *Mycotoxin Research* 30: 207–211.

Oluwafemi, F., Badmos, A., Kolapo, A., Kareem, S. and Ademuyiwa, O. (2014) Comparative efficacies of imarsil and activated charcoal in reducing aflatoxin M1 in cows' milk. *Global Journal of Science Frontier Research* 14(10): 1–9.

Osborne, D. J. and Hamilton, P. B. (1981) Decreased pancreatic digestive enzymes duringaflatoxicosis. *Poultry Science* 60:1818-1821.

Rumbold, P., Mccullogh, N., Boldon, R., Haskell-Ramsay, C., James, L., Stevenson, E. and Green, B. (2022) The potential nutrition-, physical- and health-related benefits of cow's milk for primary-school-aged children. *Nutrition Research and Review* 35:50-69. .

Shundo, L. S. and Sabino, M. (2006) Aflatoxin M1 in milk by immunoaffinity column clean-up with TLC/HPLC determination. *Brazilian Journal of Microbiology* 37:164-167.

Sumathy, J. H. (2014) Clinical, Histological, Hematological and Biochemical Study of Human Polycystic Kidney. *Universal Journal of Pharmacy* 3:86-114.

Turna, N. S. and Wu, F. (2021) Aflatoxin M1 in milk: A global occurrence, intake, and exposure assessment. *Trends in Food Science and Technology* 110:183-192.

UCLA Health (2023). Acid-Base Electrolytes. https://www.uclahealth.org/programs/core-kidney/conditions-treated/acid-base-electrolytes#:~:text=Electrolytes%20are%20particles%20that%20carry,leads%20to%20a n%20electrolyte%20imbalance. Accessed 22/03/2023.

Valchev, I., Kanakov, D., Hristov, T. S., Lazarov, L., Binev, R., Grozeva, N. and Nikolov, Y. (2014) Investigations on the liver function of broiler chickens with experimental aflatoxicosis. *Bulgarian Journal of Veterinary Medicine* 17:302- 313.

Webpath. (2023): Laboratory Reference. https://webpath.med.utah.edu/EXAM/LABREF.html. Accessed 22/03/2023.

Yunus, A. W., Razzazi-Fazeli, E. and Abohm, J. (2011) Aflatoxin B1 in Affecting Broiler's Performance, Immunity, and Gastrointestinal Tract: A Review of History and Contemporary Issues. *Toxins* 3:566-590.

Zuo, C., Wang, J., Zhou, V. and Ruelos, C. (2008) Simultaneous determination of creatinineand uric acid in human urine by high-performance liquid chromatography *Analytical Science* 24:1589-1592.