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WEIGHT AND HEMATOLOGICAL RESPONSES OF WISTAR RATS ADMINISTERED WITH OCHRATOXIN A AND AFLATOXIN B1 CONTAMINATED HERBAL MEDICINES

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ABSTRACT
This study was carried out to investigate the effect of mycotoxin contamination of herbal medicine preparations on the weight and hematological properties of Wistar rats. Five Wistar rats each weighing about 141 g were separately administered with two powdered herbal medicines, *Agbo typhoid* and *Agbo rashes* contaminated with Aflatoxin B1 (AFB1) and Ochratoxin A (OTA) respectively. Different amounts (1, 2, 3, 4, 5 g) of the herbal medicine powders were administered to the rats by mixing with commercial feeds, while the liquid extracts (0.01, 0.02, 0.03, 0.04, and 0.05 µg/mL) obtained from the contaminated herbal powders were administered orally using oral cannulas. A total of 105 Wistar rats were used. The rats were weighed for a period of two weeks and assessed for hematological effects of the administered toxicants. Results showed that significant weight decline occurred in animals administered with the different mycotoxins, with highest weight loss (23.21 g) obtained in Wistar rats administered with 0.01 µg/mL of AFB1. Both AFB1-contaminated herbal medicines and AFB1 extracts from contaminated herbal medicines caused significant decreases in PCV and Hb levels, while OTA-contaminated herbal medicines and OTA extracts from contaminated herbal medicines also caused significant decreases in PCV, RBC and Hb levels when compared to the normal rats ($P \leq 0.05$). The findings of this study suggest the need to improve processing, handling, and storage of herbal medicines in order to prevent contamination with mycotoxigenic fungi which produce OTA and AFB1 capable of causing significant health challenges to consumers.

Keywords: Aflatoxin, ochratoxin, herbal medicines, weight loss, hematology

INTRODUCTION
Herbal mixtures are herbs, herbal materials, herbal preparations, and finished herbal products containing whole plants, parts of plants, or other plant materials, including leaves, bark, berries, flowers, roots, and/or their extracts as active ingredients which provide therapeutic and other benefits to humans (WHO, 2002). Although herbal medicine is the oldest form of medicine known to man, and is practiced all over the world, herbal mixtures are integral parts and most often synonymous with African traditionalmedicine (Ezekwesili-Ofili and Okaka, 2019). In Nigeria, traditional medicine practice permeates every tribe and ethnic group.Plant species such as *Nauclea latifolia*, *Pilliostigmathonningii*, *Ageratum conyzoides*, *Newlboldialaevis*, *Phyllanthus muererianus*, *Cochlospermumplanchonii*, *Ocimumgratissimum*, and *Parkia biglobosa* are amongst the most prescribed (Ackerman *et al*., 2001; Falodun and Imieje, 2013).

The contamination of herbal medicines with mycotoxins is an emerging concern in the field of public health and safety. Herbal medicines, widely used for their therapeutic benefits, are susceptible to contamination by mycotoxins, which are toxic compounds produced by certain fungi. These contaminants can enter herbal products during various stages, including cultivation, harvesting, storage, and processing, posing significant health risks to consumers (Chen *et al*., 2020; Wang *et al*., 2024). Mycotoxins such as aflatoxins and ochratoxin A are frequently detected in herbal products, often exceeding regulatory safety levels set by authorities like the European Union (Halt, 1998; Ałtyn & Twarużek, 2020; Caldeirão *et al*., 2021).

The presence of mycotoxins in herbal medicines is not only a safety concern but also a challenge for quality control. Various studies have highlighted the prevalence of mycotoxins in herbal products across Africa, Asia, and other continents with significant contamination levels (Ezekwesili-Ofili, 2014; Mannani *et al.*, 2020; Yusuf *et al.*, 2022; Wang *et al*., 2024; Hu *et al*., 2024). The detection and quantification of these mycotoxins require sophisticated analytical methods, including liquid chromatography-tandem mass spectrometry, which are crucial for ensuring the safety and efficacy of herbal medicines (Hitokoto *et al*., 1978; Zhang *et al*., 2018; Caldeirão *et al*., 2021).

Given the widespread use of herbal medicines and the potential health risks associated with mycotoxin contamination, there is an urgent need for stringent quality control measures and improved detection methods. This study was at investigating the effect of herbal medicines contaminated by AFB1 and OTA on the weight and hematological characteristics of Wistar rats. The findings of this study provided evidence-based insights that can guide policymakers, healthcare providers, and manufacturers in ensuring the safety, quality, and efficacy of herbal medicines.

MATERIALS AND METHODS

Study area

The study was conducted in Lafia, the capital of Nasarawa State. Lafia is located at Latitude 8° 29' 38.04" N and longitude 8° 30' 55.15" E in North-Central Nigeria (Latitude, 2023). The city has an average population of 330,712, and the major occupation of the inhabitants is farming (Abah, 2016).

Source of herbal medicine samples

Powdered herbal medicine samples contaminated with AFB1 and OTA, respectively, *Agbo typhoid* used in the treatment of typhoid fever, and *Agbo rashes* used in the treatment of skin rashes were obtained from previous studies conducted in the Department of Microbiology, Federal University of Lafia (Terna, 2023).

Source of wistar rats

Wistar rats used in the study were obtained from the School of Veterinary Medicine, Joseph Sarwuan Taka University, Makurdi.

Toxicity tests

The method of Shamaki *et al.* (2017) was adopted for evaluating the toxic effects of mycotoxin-contaminated herbal medicine preparations on Wistar rats. Four sets of experiments were carried out to evaluate the toxicity of: (i) powdered herbal mixture (*Agbo typhoid*) showing highest AFB1 activity, (ii) AFB1 extracts from the AFB1-contaminated herbal mixture. (iii) powdered herbal mixture (*Agbo rashes*) showing highest OTA activity, and iv) OTA extracts from the OTA-contaminated herbal mixture.

In each experiment, A total of 25 Wistar rats weighing an average of 141 g were shared into 5 cages and administered with five different concentrations of mycotoxin composition at the rate of five rats (replicates) per concentration. Consequently, five different amounts of mycotoxin-contaminated herbal medicine powder, 1, 2, 3, 4, and 5 g, and toxin extract concentrations, 0.01, 0.02, 0.03, 0.04 µg/mL, and 0.05 µg/mL were used. Mycotoxin-contaminated herbal medicines were administered by mixing with rat feeds, while the extracts were administered orally using an oral cannula. Toxin administration was performed once every 48 h for four weeks. The control experiment comprised five rats fed daily with the commercial rat feed without administration of mycotoxins. A total of 105 Wistar rats were used.

Following the administration of toxins, the Wistar rats were weighed at weekly intervals to determine the effect of the administered mycotoxins on the weight of the evaluated animals. At the end of the four-week experimental period, percentage weight loss was determined using the formula:

$$\%\, Weight\ Loss = \frac{Initial\ \ weig\,ht - Final}{Initial\ \ weig\,ht}\, x\ 100$$

Blood samples of Wistar rats administered with various doses of mycotoxin-contaminated herbal medicines and toxin extracts for four weeks, were taken from the rats' orbital sinuses, placed in sterile test tubes containing EDTA, and used for the determination of haematological parameters in accordance with the methods of Guyton and Hall (2006). The packed cell volume (PCV) and hemoglobin (Hb) of several heparinized microhematocrit capillary tubes were measured using drops of whole blood. Three air-dried blood smears were made using whole blood, stained with Wright's stain, and examined for the presence of red blood cells (RBC), white blood cells (WBC), differential leucocytes count (DLC), and platelet estimates. Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), and Mean corpuscular volume (MCV) were also calculated.

Blood plasma of Wistar rats administered with various doses of mycotoxin-contaminated herbal medicines was collected from To determine the effect of the evaluated mycotoxins on the haematological properties of investigated Wistar rats, parameters measured were, Packed Cell Volume (PCV), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Haemoglobin (Hb), Mean Corpuscular Volume (MCV); Mean Corpuscular Haemoglobin(MCH); Mean Corpuscular Haemoglobin Concentration (MCHC); and Differential Leucocyte Count (DLC).

Data analysis

Data obtained from the study was subjected to Analysis of Variance at 5% level of probability using the Minitab statistical software version 19. Means were separated using Tukey's Honestly Significant Difference.

RESULTS AND DISCUSSION

The effect of different amounts of aflatoxin-contaminated herbal medicine (*Agbo rashes*) on the weight of Wistar rats are presented in Figure 1 and Table 1. All animals fed with different amounts of the aflatoxin-contaminated herbal medicine showed loss of weight after one week and continued for four weeks, with no clear signs of recovery. However, weight loss was not observed in the control experiment, as the animals continued to gain weight throughout the four weeks period. Although percentage weight loss was highest in rats fed with 5 g of the contaminated herbal medicine, differences in weight loss among Wistar rats fed with different amounts of aflatoxin-contaminated herbal medicine were not significant ($p>0.05$), but differed significantly from the control ($p\leq0.05$) (Table 1).

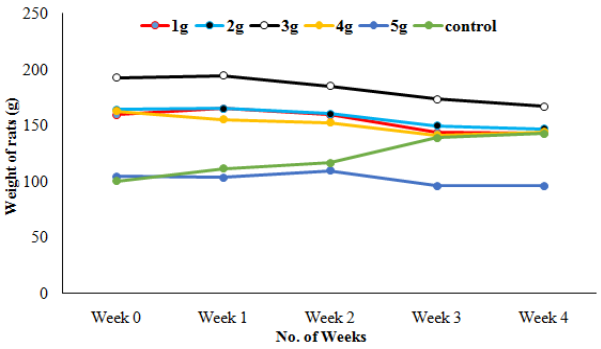

Figure 1: Effect of different amounts of aflatoxin-contaminated powdered herbal medicine (*Agbo typhoid*) on weight of wistar rats

Table 1: Percentage weight loss of wistar rats fed for four weeks with different amounts of aflatoxin-contaminated powdered herbal medicine

Herbal medicine quantity (g)	% Weight loss
1.00	13.43[a]
2.00	12.34[a]
3.00	13.53[a]
4.00	11.56[a]
5.00	16.25[a]
Control	0.00[b]

Means followed by the same superscripts are not significantly different (p>0.05); Means followed by different superscripts are significantly different (p≤0.05)

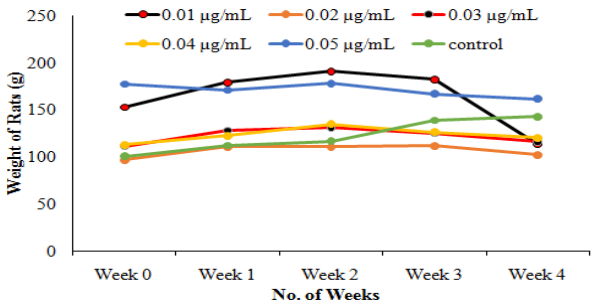

Figure 2: Effect of different concentrations of AFB1 extracts from powdered herbal medicine (*Agbo typhoid*) on weight of wistar rats

Table 2: Percentage weight loss of wistar rats fed for four weeks with different concentrations of aflatoxin extracts from contaminated powdered herbal medicine

Toxin concentration (µg/mL)	% Weight loss
0.01	23.21[a]
0.02	0.00[b]
0.03	0.00[b]
0.04	0.00[b]
0.05	9.12[b]
Control	0.00[b]

Means followed by the same superscripts are not significantly different (p>0.05); Means followed by different superscripts are significantly different (p≤0.05)

The effect of different concentrations of aflatoxin extracts obtained from aflatoxin-contaminated powdered herbal medicine on the weight of Wistar rats is presented in Figure 2 and Table 2. All animals administered with different concentrations of aflatoxin extracts showed different levels of weight loss (Figure 2). Animals administered with 0.01, 0.03, and 0.04 µg/mL concentrations of the aflatoxin extracts showed weight decline after two weeks, while those administered with 0.02 and 0.05 µg/mL showed the onset of weight loss after three weeks and one week respectively. Intermittent weight gains and losses were recorded in all administered animals, however, actual weight loss after four weeks of administration (difference between initial and final weight) was observed in animals administered with 0.01 µg/mL (23.21%) and 0.05 µg/mL (9.23%) concentrations of the aflatoxin extracts (Table 2). Differences in percentage loss of body weight were significant between animals administered with 0.01 µg/mL of aflatoxin extracts (23.21%) and the control (0.00%) (p≤0.05) (Table 2).

The effect of different amounts of powdered herbal medicine contaminated with OTA on the weight of Wistar rats is presented in Figure 3 and Table 3. Wistar rats fed with 2 to 5 g of OTA-contaminated herbal medicine powder showed initial weight gain from week zero to week two, but began to decline afterwards, while decline in weight of rats fed with 1 g of the contaminated herbal medicine powder was observed after three weeks. After four weeks, only rats fed with 3 and 5 g of the contaminated herbal medicine powder showed weight losses i.e. 3.96 and 5.21% respectively that were below the starting weight (Table 3). Differences in percentage weight losses between the rats fed with OTA-contaminated herbal medicine powder and the control experiment were not significant (p≤0.05) (Table 3).

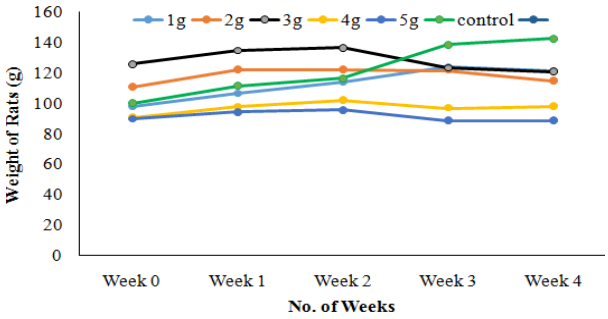

Figure 3: Effect of different amounts of OTA-contaminated herbal medicine (*Agbo rashes*) on weight of wistar rats

Table 3: Percentage Weight loss of wistar rats fed for four weeks with different amounts of OTA-contaminated powdered herbal medicine

Herbal medicine quantity (g)	% Weight loss
1.00	0.00[a]
2.00	0.00[a]
3.00	3.96[a]
4.00	0.00[a]
5.00	5.21[a]
Control	0.00[a]

Means followed by the same superscripts are not significantly different (p>0.05); Means followed by different superscripts are significantly different (p≤0.05)

Wistar rats administered with different concentrations of OTA extracts from OTA-contaminated powdered herbal medicine showed varying weight responses (Figure 4 and Table 4). Weight gains were observed from the first week after toxins administration, but began to decline two weeks after, for rats administered with 0.01, 0.03, and 0.04 µg/mL, and after three weeks for rats administered with 0.02 µg/mL of the toxin extract. Weight loss in Wistar rats administered with 0.05 µg/mL was the earliest to occur, one week after toxin administration, and rats administered with 0.05

µg/mL showed weight loss (3.87%) that was below the starting weight of the animals. Differences in weight loss between Wistar rats administered with 0.05 µg/mL and the control experiment were significant (p≤0.05).

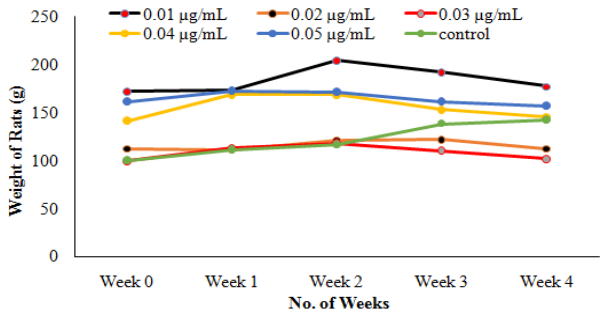

Figure 4: Effect of different concentrations of OTA extracts from powdered herbal medicine (*Agbo rashes*) on weight of wistar rats

Table 4: Percentage weight loss of wistar rats fed for four weeks with different concentrations of OTA extracts from contaminated powdered herbal medicine

Toxin concentration (µg/mL)	% Weight loss
0.01	0.00[a]
0.02	0.00[a]
0.03	0.00[a]
0.04	0.00[a]
0.05	3.87[a]
Control	0.00[a]

Means followed by the same superscripts are not significantly different (p>0.05); Means followed by different superscripts are significantly different (p≤0.05)

The haematological characterisics of Wistar rats fed with OTA-Contaminated herbal medicine powder for four weeks are presented in Table 5. The results showed that Wistar rats fed with 5 g of OTA-contaminated herbal medicine powder had significantly lower Packed Cell Volume (27.50%), Red Blood Cell Count (5.85 g/dL), and Haemoglobin (9.16 g/dL), while White Blood Cell Count (6.10 10^9L) and Basophils (2.50%) were higher, compared to the control, which had 51%, 9.33 g/dL, 17.00 g/dL, 3.75 10^9/L, and 1.00% Packed Cell Volume, Red Blood Cell Count, Haemoglobin, White Blood Cell Count, and percentage Basophils, respectively (p≤0.05). Lymphocytes were significantly lower while Neutrophils were significantly higher in Wistar rats fed with all concentrations of OTA-contaminated herbal medicine powder, compared to the control (p≤0.05). Differences in Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Monocytes, and Eosinophils between Wistar rats fed with OTA-contaminated powdered herbal medicine and the control were not significant (p>0.05).

The haematological effects of AFB1 extracts from powdered herbal medicine are presented in Table 6. Wistar rats administered with 0.02 µg/mL of the extracts showed significantly higher White Blood Cell count (6.80 10^9L), lower Mean Corpuscular Volume (41.00 fl/µm^3) and Mean Corpuscular Haemoglobin (13.66 g/dL), compared to 3.75 10^9/L, 54.69 fl/µm^3, and 18.23 g/dL, White Blood Cell count, Mean Corpuscular Volume, and Mean Corpuscular Haemoglobin, respectively, recorded in the control animals (p≤0.05). Also, Monocytes (5.00%) were significantly higher in Wistar rats administered with 0.03 µg/mL of AFB1 extracts, compared to control (1.50%) (p≤0.05), while administration of 0.04 µg/mL of the AFB1 extracts led to significant reduction in Packed Cell Volume (21.50%), and Haemoglobin (7.16 g/dL) compared to the control which had 51.00% and 17.00 g/dL Packed Cell Volume and Haemoglobin, respectively (p≤0.05). Administration of 0.05 µg/g of AFB1 extracts led to significant reduction in Red Blood Cell count (4.63 g/dL) and Leucocytes (28.00%), while significantly increasing the levels of Neutrophils (62.00%) and Eosinophils (3.50%), compared to the control experiment which recorded 9.33 g/dL Red Blood Cell count, 77.80% Leucocytes, 19.50% Neutrophils, and 3.50% Eosinophils (p≤0.05). Differences in other haematological parameters such as Mean Corpuscular Haemoglobin Concentration and Basophils, between Wistar rats administered with different AFB1 extract concentrations from powdered herbal medicines and the unadministered control were not significant (p≤0.05).

Table 5: Effect of OTA-contaminated herbal medicine powder on the haematological characteristics of wistar rats after four weeks

Quantity of herbal medicine (g)	Haematological Parameters											
	PCV (%)	RBC (g/dL)	WBC (10^9/L)	HB (g/dL)	MCV (fl/µm^3)	MCH (g/dL)	MCHC (g/dL)	DLC (%)				
								M	L	N	B	E
1.00	35.00[b]	6.48[b]	5.75[b]	11.67[bc]	54.04[a]	18.02[a]	33.33[a]	1.00[b]	65.00[b]	29.50[c]	2.50[a]	2.00[a]
2.00	36.00[b]	6.33[b]	5.35[c]	12.00[b]	56.85[a]	18.95[a]	3.33[a]	2.50[ab]	57.50[bc]	37.50[abc]	2.00[ab]	0.50[a]
3.00	35.00[b]	6.25[b]	5.60[bc]	11.66[bc]	54.73[a]	18.23[a]	33.31[bc]	3.00[ab]	52.00[c]	43.50[a]	0.50[c]	1.00[a]
4.00	31.00[bc]	6.28[b]	5.55[bc]	11.16[bc]	53.46[a]	17.82[a]	33.33[a]	4.00[a]	55.50[c]	34.50[bc]	2.50[a]	1.00[a]
5.00	27.50[c]	5.85[c]	6.10[a]	9.16[c]	49.59[a]	15.65[a]	33.31[bc]	3.00[ab]	53.50[c]	38.50[ab]	2.50[a]	2.50[a]
Control	51.00[a]	9.33[a]	3.75[d]	17.00[a]	54.69[a]	18.23[a]	33.32[ab]	1.5[ab]	77.50[a]	19.50[d]	1.00[bc]	0.50[a]

Means followed by the same superscripts are not significantly different (p>0.05), while Means followed by different superscripts are significantly different (p≤0.05)

DLC = Differential Leukocyte Count; PCV = Packed Cell Volume; RBC = Red Blood Cell Count; WBC = White Blood Cell Count; HB = Haemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; M = Monocytes; L = Lymphocytes; N = Neutrophils; B = Basophils; E = Eosinophils

Table 6: Effects of AFB1 extracts from powdered herbal medicine on the haematological characteristics of wistar rats after four weeks

Concentration of toxin extract (µg/mL)	Haematological Parameters											
	PCV (%)	RBC (g/dL)	WBC (10^9/L)	HB (g/dL)	MCV (fl/µm^3)	MCH (g/dL)	MCHC (g/dL)	DLC (%)				
								M	L	N	B	E
0.01	31.00^{b}	6.05^{b}	5.85^{cd}	10.30^{b}	51.23^{ab}	17.08^{ab}	33.32^{a}	2.50^{ab}	52.00^{b}	42.00^{cd}	2.50^{a}	1.00^{b}
0.02	25.00^{c}	6.10^{b}	6.80^{a}	8.33^{c}	41.00^{c}	13.66^{c}	33.32^{a}	2.00^{b}	53.00^{b}	39.50^{d}	3.00^{a}	2.50^{ab}
0.03	26.00^{bc}	4.90^{c}	6.25^{b}	8.67^{bc}	53.10^{ab}	17.70^{ab}	33.33^{a}	5.00^{a}	47.50^{b}	45.50^{c}	1.00^{a}	1.00^{b}
0.04	21.50^{c}	4.83^{c}	5.70^{d}	7.16^{c}	44.52^{bc}	14.83^{bc}	33.30^{a}	3.00^{ab}	37.00^{c}	56.00^{b}	2.50^{a}	1.50^{ab}
0.05	24.00^{c}	4.63^{d}	6.10^{bc}	8.00^{c}	51.84^{ab}	17.27^{ab}	33.31^{a}	4.00^{ab}	28.00^{d}	62.00^{a}	2.50^{a}	3.50^{a}
Control	51.00^{a}	9.33^{a}	3.75^{e}	17.00^{a}	54.69^{a}	18.23^{a}	33.32^{a}	1.50^{b}	77.50^{a}	19.50^{e}	1.00^{a}	0.50^{b}

Means followed by the same superscripts are not significantly different ($p>0.05$), while Means followed by different superscripts are significantly different ($p\leq0.05$)

DLC = Differential Leukocyte Count; PCV = Packed Cell Volume; RBC = Red Blood Cell Count; WBC = White Blood Cell Count; HB = Haemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; M = Monocytes; L = Lymphocytes; N = Neutrophils; B = Basophils; E = Eosinophils

Table 7: Effects of OTA extracts from powdered herbal medicine on the haematological characteristics of wistar rats after four weeks

Concentration of toxin extract (µg/mL)	Haematological Parameters											
	PCV (%)	RBC (g/dL)	WBC (10^9/L)	HB (g/dL)	MCV (fl/µm^3)	MCH (g/dL)	MCHC (g/dL)	DLC (%)				
								M	L	N	B	E
0.01	25.00^{cd}	6.00^{b}	6.35^{ab}	8.33^{cd}	41.23^{b}	13.87^{c}	33.32^{a}	3.00^{a}	52.50^{b}	40.50^{b}	2.50^{a}	1.50^{a}
0.02	28.00^{bc}	6.13^{b}	6.35^{ab}	9.33^{bc}	44.20^{b}	15.23^{bc}	33.32^{a}	2.00^{a}	51.00^{b}	42.50^{b}	3.00^{a}	1.50^{a}
0.03	22.00^{d}	5.80^{c}	6.60^{a}	7.33^{d}	37.92^{b}	12.63^{c}	33.32^{a}	3.50^{a}	41.50^{c}	51.50^{a}	2.50^{a}	1.00^{a}
0.04	23.00^{cd}	5.73^{cd}	5.85^{c}	7.67^{cd}	40.19^{b}	13.40^{c}	33.32^{a}	4.00^{a}	42.50^{c}	52.00^{a}	0.50^{a}	1.00^{a}
0.05	32.00^{b}	5.58^{d}	6.15^{bc}	10.67^{b}	57.44^{a}	19.14^{a}	33.33^{a}	3.00^{a}	38.00^{c}	54.00^{a}	3.00^{a}	2.50^{a}
Control	51.00^{a}	9.33^{a}	3.75^{d}	17.00^{a}	54.69^{a}	18.23^{ab}	33.32^{a}	1.50^{a}	77.50^{a}	19.50^{c}	1.00^{a}	0.50^{a}

Means followed by the same superscripts are not significantly different ($p>0.05$), while Means followed by different superscripts are significantly different ($p\leq0.05$)

DLC = Differential Leukocyte Count; PCV = Packed Cell Volume; RBC = Red Blood Cell Count; WBC = White Blood Cell Count; HB = Haemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; M = Monocytes; L = Lymphocytes; N = Neutrophils; B = Basophils; E = Eosinophils

Table 8: Effect of AFB1-contaminated herbal medicine powder on the haematological characteristics of wistar rats after four weeks

Quantity of herbal medicine (g)	Haematological Parameters											
	PCV (%)	RBC (g/dL)	WBC (10^9/L)	HB (g/dL)	MCV (fl/µm^3)	MCH (g/dL)	MCHC (g/dL)	DLC (%)				
								M	L	N	B	E
1.00	33.00^{c}	6.20^{b}	5.60^{b}	10.50^{c}	51.64^{b}	17.07^{ab}	33.33^{a}	2.00^{a}	62.50^{b}	32.50^{c}	2.00^{a}	1.00^{a}
2.00	31.00^{c}	6.12^{b}	5.80^{b}	10.50^{c}	51.45^{b}	14.64^{b}	33.32^{a}	3.00^{a}	60.50^{b}	33.50^{c}	2.00^{a}	1.00^{a}
3.00	35.00^{b}	6.08^{b}	5.35^{b}	11.67^{b}	57.61^{a}	19.21^{a}	33.33^{a}	4.00^{a}	51.00^{c}	42.00^{b}	1.50^{a}	1.50^{a}
4.00	31.50^{c}	5.88^{c}	6.30^{a}	10.50^{c}	53.62^{ab}	17.87^{a}	33.32^{a}	2.50^{a}	47.50^{cd}	47.00^{a}	2.00^{a}	1.00^{a}
5.00	29.50^{c}	5.60^{d}	6.45^{a}	9.83^{c}	52.68^{b}	17.56^{ab}	33.32^{a}	2.50^{a}	43.50^{d}	49.00^{a}	3.00^{a}	2.00^{a}
Control	51.00^{a}	9.33^{a}	3.75^{c}	17.00^{a}	54.69^{ab}	18.23^{a}	33.32^{a}	1.50^{a}	77.50^{a}	19.50^{d}	1.00^{a}	0.50^{a}

Means followed by the same superscripts are not significantly different ($p>0.05$), while Means followed by different superscripts are significantly different ($p\leq0.05$)

DLC = Differential Leukocyte Count; PCV = Packed Cell Volume; RBC = Red Blood Cell Count; WBC = White Blood Cell Count; HB = Haemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; M = Monocytes; L = Lymphocytes; N = Neutrophils; B = Basophils; E = Eosinophils

The haematological characteristics of Wistar rats administered with OTA extracts from powdered herbal medicine samples are presented in Table 7. Wistar rats administered with 0.03 µg/mL concentration of the OTA extracts showed significantly lower Packed Cell Volume (22.00%), and Haemoglobin (7.33 g/dL), and higher White Blood Cell count (6.60 10^9/L), compared to the control which had 51.00% Packed Cell Volume, 17.00 g/dL Haemoglobin, and 3.75 10^9/L White Blood Cell count ($p\leq0.05$). Significantly lower concentrations of Red Blood Cells (5.58 g/dL) and Lymphocytes (38.00%), were also accompanied by higher Mean Corpuscular Volume (57.44 fl/µm^3), Neutrophils (54.00%), Basophils (3.00%), and Eosinophils (2.50%) in Wistar rats administered with 0.05 µg/mL concentration of the AFB1 extracts, compared to the unadministered control, which had 9.33 g/dL Red Blood Cell count, 77.50% Lymphocytes, 54.69 fl/µm^3, 19.50% Neutrophils, 1.00% Basophils, and 0.50% Eosinophils ($p\leq0.05$). The differences in Mean

Corpuscular Haemoglobin Concentration and Monocytes between Wistar rats administered with AFB1 extracts and the unadministered controls were not significant ($p>0.05$).

Table 8 presents the Effects of different amounts of AFB1-contaminated herbal medicine powder on the haematological profile of Wistar rats after four weeks. Wistar rats fed with 5 g of AFB1-contaminated powdered herbal medicine had significantly lower Packed Cell Volume (29.50%), Red Blood Cell count (5.60 g/dL), Haemoglobin (9.83 g/dL), and Lymphocytes (43.50%), and significantly higher White Blood Cell count (6.45 10^9/L), and Neutrophils (49.00%) compared to the unadministered control which had 51.00%, 9.33g g/dL, 17.00 g/dL, 77.50%, 3.75 10^9/L, and 19.50%, Packed Cell Volume; RBC = Red Blood Cell Count, Haemoglobin, Lymphocytes, White Blood Cell count, and Neutrophils, respectively ($p\leq0.05$). Mean Corpuscular Haemoglobin was significantly lower in Wistar rats fed with 2 g of AFB1-contaminated herbal medicine powder (14.64 g/dL) compared to the control (18.23 g/dL). Differences in Mean Corpuscular Haemoglobin Concentration, Monocytes, Basophils, and Eosinophils between Wistar rats fed with different concentrations of AFB1-contaminated powdered herbal medicines and unadministered controls were not significant ($p>0.05$).

In the present study, significant weight losses were observed in Wistar rats fed with AFB1-contaminated powered herbal medicines and liquid AFB1 extracts from the contaminated herbal medicine samples, however, weight losses induced in the test animals by OTA extracts and OTA-contaminated herbal medicine powder were not significant. Although weight loss of Wistar rats as a result of the intake of AFB1 and OTA-contaminated herbal medicines is yet to be reported, significant weight losses of Wistar rats in response to intake of various doses of AFB1 and OTA have been reported (Alvarez *et al.*, 2004; Supriya *et al.*, 2014; Yaman *et al.*, 2016). Weight loss is an important indicator of animal toxicity (Campra*et al.*, 2020), hence, the loss of weight in animals fed with mycotoxin extracts and mycotoxin-contaminated herbal medicine samples in the present study could be as a result of immunological responses in the tissues of the affected animals. This is corroborated by the report of Gbore*et al.* (2010) that mycotoxins in diets typically decrease nutrient utilization by negatively affecting Wistar rat growth performance and adequate nutrient digestion, absorption, or metabolism. Intake of mycotoxins also results in oxidative damage to the gastro intestinal tract's cell lining, causing inflammation and irritation that hinders nutritional digestion and absorption, which either slows or stops the process of rat body weight increase (Saki *et al.*, 2018).

In this study, both AFB1-contaminated herbal medicines and AFB1 extracts from contaminated herbal medicines caused significant decreases in PCV and Hb levels when compared to the normal rats. This suggests that these rats developed anemia as a result of the administration of this toxin even at the loweset concentration of 1.0 g. Similarly, Abdel-Wahhab *et al.* (2002) reported that treatment of rats with aflatoxin for 30 days caused a significant decrease in Hb, PCV, total RBC counts, WBC, neutrophils, basophils and monocytes. In another study, broiler chicks treated with AFB developed anemia due to significant reduction of the values of Hb and PCV (Rathod *et al.*, 2017). Dönmez *et al.* (2012) also reported significant decreases in RBC count, leukocyte count and Hb levels in rams fed with food containing 250 µg/day aflatoxin for 12 weeks. Reductions in PCV was also recorded. The authors also observed significant increase in neutrophile, accompanied by significant decrease in lymphocytes and monocytes. Another study reported significant decreases in Hb, PCV, MCHC and MCV in broilers fed basal diet containing 3 mg/kg feed of aflatoxin for 40 days. They also reported significant decreases in total WBC count, lymphocyte, monocyes, basophils, and a relative decrease was recorded for eosinophils (Umar *et al.*, 2012). The changes in the haematologic parameters may be due to impaired iron absorption, decrease of total iron binding capacity, inhibition of protein synthesis as evidenced by lower serum albumin, combined with suppression of hematopoiesis (Rathod *et al.*, 2017; Dönmez *et al.*, 2012). Aflatoxins are also able to metabolize intermediates that bind to macromolecules and disrupt transcription and translation processes (Rathod *et al*, 2017).

Both OTA-contaminated herbal medicines and OTA extracts from contaminated herbal medicines also caused significant decreases in PCV, RBC and Hb levels when compared to the normal rats. This also suggests that these rats developed anemia as a result of the administration of the toxin. In a similar study, significant reductions in Hb, PCV, MCHC and MCV in broilers fed basal diet containing 3 mg/kg feed ochratoxin were recorded. The study also reported significant decreases in total WBC count, lymphocyte, monocytes and basophils. A relative decrease was also recorded for eosinophils (Umar *et al*., 2012). Abidin *et al*. (2013) recorded significant reductions in Hb, PCV, RBC count, and WBC count in cockerels administered ochratoxin for 42 days. Ramasamy *et al*. (2014) also observed significant decrease in PCV and RBC count in broiler chicken fed with ochratoxin.

The effect of a toxic compound can be measured by assessing its effect on the liver and the kidney. This is because the liver and kidney are responsible for drug metabolism/detoxification and excretion respectively after gastrointestinal absorption (Kulkarni *et al*., 2021). In the present study, there was relative increase in concentration of total protein in the administered groups when compared with the normal group, with the highest increase seen in the group administered 0.05 µg/mL of aflatoxin extract. This implies that aflatoxin extract has effect on the total protein and this effect increases with an increase in the concentration of the toxin. Total protein refers to all different proteins in the

plasma excluding fibrinogen and others involved in blood clotting (Smith *et al*., 2013). These proteins leak into the ultrafiltrate as a result of damaged glomerular filtration barrier. Excess leakage impairs reabsorption and proteinuria occurs. An increase in total protein concentration signifies an injury to the kidney, therefore from the results obtained, AFB1 can be said to have demonstrated capacity to cause appreciable damage to the kidney at high concentrations.

Bilirubin is the end product of haem breakdown. The primary source is haemoglobin due to the destruction of erythrocyte, this account for 80-85% of bilirubin production (Ruiz *et al.,* 2021). The remaining percentage comes from other haem containing molecules such as catalase, peroxidase, myoglobin and cytochrome P450 (York, 2017). Haem is converted to biliverdi which is in turn reduced to bilirubin by *Bilirubin reductase* (Sakoh*et al.,* 2015). Bilirubin is water insoluble; it is therefore transported in association with albumin to the liver for conjugation. In the endoplasmic reticulumn, a UDP-glucoronyltransferase catalyzes the conjugation of bilirubin with gkucoronide to make it water soluble (Kulkarni *et al*., 2021). Conjugated bilirubin is either excreted as bile or recirculated back to the bloodstream where it is filtered by the kidney and excreted through urine (Ruiz *et al.*, 2021). Overproduction of bilirubin is an indication of excessive red blood cell destruction or hemolysis (York, 2017). Hyperbilirubinemia can result due to liver lesions which induce a decrease in hepatocyte cell count (Ruiz *et al.,* 2021). This elevated serum concentration of bilirubin is thus used as a marker of liver dysfunction (Targher*et al.,* 2009). Elevation can also result from excess production, impaired liver uptake, conjugation defect or biliary excretion defect, therefore it is not a specific marker (Ruiz *et al.*, 2021). In the present study, both ochratoxin-contaminated powdered herbal medicine and aflatoxin-contaminated powdered herbal medicines caused significant rise in serum level of bilirubin at different administered concentrations, implying that there must have been an excessive destruction of red blood cells or introduction of a liver lesion.

Creatinine is a non-protein nitrogenous (NPN) end-product formed from the non-enzymatic breakdown of creatine and phosphocreatine (Salazar, 2014). Creatine is an energy storage for muscle, synthesised in the liver and converted to phosphocreatine in the skeletal muscle and brain (Salazar, 2014). Creatinine has long been used as a marker of glomerular filtration rate (GFR) which is used to characterize kidney function (Delanaye and Rule, 2015). An increase in serum creatinine is associated with a decrease in GFR although it can also increase after ingestion of cooked animal protein (Delanaye and Rule, 2015). However, it is less affected by diet compared to urea, thus is more suitable for indicating renal failure (Washington and Hoosier, 2012). Salazar stated that if GFR is decreased, as seen in renal disease, creatinine clearance via the renal system is compromised and creatinine remains in the plasma at high concentration (Salazar, 2014). In the present study, Aflatoxin-contaminated powdered herbal medicine caused a significant rise in serum creatinine at concentration of 3.0 g, which is suggestive of the possible reduction in GRF, which is an indication of renal impairment.

CONCLUSION

The study showed significant weight decline in Wistar rats fed with herbal medicines contaminated with AFB1. Both AFB1-contaminated herbal medicines and AFB1 extracts from contaminated herbal medicines caused significant decreases in PCV and Hb levels, while OTA-contaminated herbal medicines and OTA extracts from contaminated herbal medicines also caused significant decreases in PCV, RBC and Hb levels when compared to the normal rats ($P \leq 0.05$). The findings of this study suggest the need to improve processing, handling, and storage of herbal medicines in order to prevent contamination with mycotoxigenic fungi which produce OTA and AFB1 capable of causing significant health challenges to consumers.

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