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CHANGES IN SELECTED HAEMATOLOGICAL PARAMETERS OF WISTAR RATS FED WITH *FICUS EXASPERATA* VAHL LEAF-BASED DIET

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Abstract

In recent years, there has been increase in the use of non-culinary herbs in food products. This technology of incorporating medicinal or non-culinary herbs into diet offers great promise for the management of diseases. The aim of this study was to evaluate the effect of *Ficus exasperata* Vahl leaf-based diet (FELD) on selected haematological parameters of albino rats. Twenty-four rats were randomly selected into 4 groups of 6 animals each namely: C [control rats fed diet without *Ficus exasperata* leaf (FEL)], F1, F2, and F3 [experimental groups fed diets containing 10 %, 30 % and 50 % FEL respectively]. Rats were allowed access to the compounded feed *ad libitum* for 7 and 21 days. They were thereafter sacrificed and their blood samples collected and analysed using automated haematologic analyser. Results showed significant reduction ($p<0.05$) in packed cell volume (PCV) and mean cell volume (MCV) after 7 days of consuming FELD while there was a significant increase ($p < 0.05$) in haemoglobin (HGB), PCV and MCV after 21 days of consuming FELD. The leucocytic parameters showed a significant decrease ($p<0.05$) in white blood cell (WBC) of rats fed with FELD at all the levels of FEL inclusion compared to control group at the end of day 7 while platelets were significantly reduced at the end of 21 days of consuming FELD. Findings from this study revealed that FELD elicited some level of toxicity as revealed by the

observed alterations in the haematological parameters studied. It should therefore be consumed with caution.

***Keywords**:* *Ficus exasperata* leaf-based diet, Percentage inclusion, Haematological parameters

Introduction

In Nigeria, Ficus exasperata Vahl is commonly known as *Igi ewe ipin* in Yoruba, *Kawusa* in Nupe, *Ameme* in Edo and *Anwerenwa* in Igbo (Ijeh and Ukweni, 2007). *Ficus exasperata* is widely distributed in tropical Africa and the Arabian Gulf (Adewole *et al*., 2011). Traditionally, the decoctions and infusions of its leaves are used for the treatment of diabetes mellitus and hypertension (Adewole *et al*., 2011). The aqueous leaf extract has been reported to have antidiabetic and antifungal activities (Sonibare *et al.,* 2006; Yakubu *et al*., 2014). In addition, its ethanolic leaf extract had been reported to possess antibacterial activity against *Escherichia coli* and *Staphylococcus albus* (Odunbaku *et al*., 2008; Buniyamin *et al*., 2007).

In recent years, there has been an increase in the number and extent of use of non-culinary herbs in food products (Jenkins *et al*., 2008; Peng *et al*., 2010; Ansari and Kumar, 2012; Soji-Omoniwa, 2018). For instance, Peng *et al.* (2010) fortified bread with grape seed extract (GSE) which contains catechins and proanthocyanidins, having strong antioxidant and free radical scavenging activity. Similarly, Jenkins *et al*. (2008) extracted glucomannan, a glucose-mannose polysaccharide from the tuber roots of *Amorphophallus konjac* and incorporated it into the dough of biscuits. Consumption of these biscuits reduced glycemic index significantly by 74% in healthy human volunteers and by 63% in participants with diabetes mellitus. This technology of incorporating medicinal or non-culinary herbs into diet offers great promise for the management of diseases.

Previously, *Ficus exasperata* leaves incorporated into locally-sourced food ingredients to produce *Ficus exasperata* leaf-based diet (FELD), was used as a treatment diet for diabetic rats. Results from that study showed that FELD elicited normoglycemic activity and also improved glucose utilization (OGTT) in diabetic rats (Soji-Omoniwa and Oloyede, 2018; Soji-Omoniwa and Oloyede, 2019).

Ficus exasperata leaf-based diet (FELD)'s efficacy as an antidiabetic feed will not be complete without its toxicological evaluation in healthy rats. Study on extracts of *F. exasperata* leaves by some researchers revealed its potential toxic effects, while others reported that the extracts is relatively safe (Sowemimo *et al*., 2007; Bafor and Igbinuwen, 2009; Ikpeme *et al*., 2010). For instance, in an acute toxicity study, oral administration of single dose of the aqueous leaf extract (2.5, 5, 10, and 20 g kg^{-1}) did not affect the body temperature, body weights, blood cell counts and hemoglobin concentration in mice over 24 hours. However by day 14, there was a significant rise in body temperature and a significant decrease in the red blood cell count, hemoglobin and hematocrit values (Bafor and Igbinuwen, 2009). In another study, hydroethanol extract (20:80 v/v) of leaves of *F. exasperata* showed no toxic effects in brine shrimp lethality test, no inhibition of telomerase activity, and no induction of chromosomal aberrations in rat lymphocytes, hence, rendering it relatively safe for possible human consumption (Sowemimo *et al*., 2007).

Assessment of haematological parameters can be used to determine the extent of deleterious effect of a substance on blood constituents of an animal (Muhammad *et al.*, 2004; Stockham and Scott, 2008). These parameters, which are of utmost clinical importance, include the red blood (RBC) counts, packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular values, white blood cells and differential cell counts (Thrall and Weiser, 2002).

The aim of this study therefore was to assess the effect of FELD on selected haematological parameters of albino rats as part of evaluation of its overall safety.

Materials and Methods

Plant materials and authentication

Fresh leaves of *Ficus exasperata* were collected in September, 2019, from Odo Eri in Kogi State, Nigeria. The specimen sample was prepared and deposited for identification and authentication at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria and was issued a voucher number UILH/001/883.

Experimental animals

Wistar rats of *norvegicus* strain of average weight 125.35 ± 25 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in well ventilated cages and allowed to acclimatize to animal house conditions for 7 days. They were fed with normal rat pellet and tap water prior to the commencement of this experiment.

Feed ingredients

Yellow corn, maize husk, soy bean oil, soy bean grain and sucrose (sugar) were purchased from local markets in Ilorin, Kwara State, Nigeria. The corn and soy bean were stored in air tight containers prior to the commencement of the experiment. The soy bean oil was a product of Kewalram Nigeria Limited, Nigeria. Vitamin and mineral mix, D-methionine and L- lysine were purchased from Rofat Feed Nigeria Limited, Ilorin, Nigeria.

Ethical Clearance

Ethical clearance for this study was obtained from the University of Ilorin Ethical Committee with protocol identification code UERC/LSC 067.

Plant preparation

Fresh leaves of *F. exasperata* were washed and air dried to a constant weight. They were then pulverized into fine powder using an electronic blender. The pulverized leaves were oven treated using laboratory oven at 110 ^{o}C for 6 mins and then stored in an air tight container prior to analyses.

Feed preparation

Corn starch was prepared by rinsing and soaking 10 kg of yellow corn in 20 L of distilled water for 72 h, followed by grinding and sieving. The filtrate was drained for 6 h and oven dried at 40 ^{o}C to constant weight. Ten kilograms (10 kg) of maize husk was sun-dried for 3 days and pulverised using commercial grinder. Soy beans (7 kg) were soaked in 15 L of distilled water for 6 h and the seed coats were removed. Thereafter, they were sun-dried for 3 days and ground to smooth texture.

Corn starch, maize husk (cellulose source), sucrose, soybean powder, vitamin/mineral mix, D- methionine and L- lysine were thoroughly mixed together in the various proportions indicated in Table 1 for the control diet, while the test diets in addition to the aforementioned ingredients were added 10 %, 30 % and 50 % w/w *Ficus exasperata* leaves powder. Soybean oil (40 ml) and distilled water (1000 ml) were added slowly to the mixed ingredients until the mixture formed a slurry paste. The paste was then grated on a wire mesh to form pellets which were then oven-dried at 40 ^{o}C to a constant weight. Formulated feed was administered to experimental rats *ad libitum* for a period of 7 and 21 days.

Animal grouping

Twenty-four (24) albino rats were randomly selected into 4 groups of 6 animals each as indicated below:

C - Control rats fed diet without *Ficus exasperata* leaves (FEL)

F1 - Rats fed diet containing 10 % w/w *Ficus exasperata* leaves

F2 - Rats fed diet containing 30 % w/w *Ficus exasperata* leaves

F3 - Rats fed diet containing 50 % w/w *Ficus exasperata* leaves

Table 1: Feed Formulation of *Ficus exasperata* Leaf-based Diet

Ingredients g/kg	**Control**	**10%**	**30%**	**50%**
Corn starch	512	412	212	12
Cellulose	40	40	40	40
Sucrose	100	100	100	100
Soybean	250	250	250	250
Soybean Oil	40	40	40	40
Vitamin/mineral mix	50	50	50	50
D- Methionine	4	4	4	4
L-lysine	4	4	4	4
F. exasperata leaves	-	100	300	500

Animal sacrifice

Rats were sacrificed 24 h after days 7 and 21 respectively. Anaesthetization with diethyl ether followed by jugular puncturing of their veins using a scapel. Blood samples were thereafter collected into EDTA sample bottles for haematological analyses.

Haematological parameters analysis

Haematological parameters (white blood cells (WBC), lymphocytes (LYM), platelets (PLT), red blood cells (RBC), haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)) were determined using automated haematologic analyser [sysmex KX-21 (Japan)].

Procedure:

Blood sample (50 µl) was aspirated into 80 mm tubes. The analyser automated the addition of appropriate volumes of stromatolyser and cell pack reagents. The sample was then partitioned into different haematological parameters and results generated automatically and displayed on a monitor. Results were thereafter printed out.

Statistical analysis

Data were expressed as mean of 6 determinations ± SEM. The data were subjected to statistical analysis using the IBM® statistical package for social sciences (SPSS) software version 20. All significant differences were determined by one way analysis of variance (ANOVA). Post hoc multiple comparisons was done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95 %).

Results

The findings from this study revealed that consumption of FELD for 7 days resulted in significant decrease ($p < 0.05$) in PCV and MCV of the rats (Table 2). All other erythrocytic parameters investigated did not show any significant alteration ($p > 0.05$) (Table 2). The intake of FELD for 21 days led to a significant increase ($p < 0.05$) in HGB, PCV and MCV of rats fed with all the levels of inclusion of FELD compared to the control

(Table 3). All other erythrocytic parameters were not significantly altered ($p > 0.05$) compared to the control (Table 3).

The leucocytic parameters investigated showed that the WBC of rats fed with FELD at all the levels of inclusion at the end of day 7 were significantly reduced ($p < 0.05$) compared to the control (Table 4). The PLT and LYM on the other hand were not significantly ($p > 0.05$) altered compared to the control (Table 4). At the end of day 21 of the experiment, PLT was significantly reduced ($p < 0.05$) for rats fed with 10, 30 and 50 % inclusion level of FELD compared to the control, while WBC and LYM were not significantly altered ($p > 0.05$) (Table 5).

Table 2: Erythrocytic Parameters of Wistar Rats Fed with *Ficus exasperata* Leaf-based Diet for 7 days

Group	RBC x 10^6/µl	HGB (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
C	9.63 ± 0.26^a	16.20 ± 0.50^a	57.23 ± 1.71^a	59.40 ± 0.20^a	16.85 ± 0.25^a	29.07 ± 0.28^a
F1	8.91 ± 0.28^b	15.25 ± 0.18^b	52.23 ± 1.04^b	58.65 ± 0.25^b	16.76 ± 0.24^a	29.50 ± 0.15^a
F2	9.69 ± 0.25^a	16.20 ± 0.24^a	55.23 ± 1.07^{ac}	57.00 ± 0.37^b	16.73 ± 0.17^a	29.37 ± 0.19^a
F3	9.62 ± 0.29^a	15.57 ± 0.09^a	54.28 ± 0.39^c	56.55 ± 1.59^b	16.23 ± 0.43^a	28.70 ± 0.14^a

Values are expressed as mean of 6 determinations ± S.E.M. and those with different superscripts down the column are statistically different ($p < 0.05$)

RBC – red blood cell; HGB – haemoglobin; PCV – packed cell volume; MCV – mean cell volume; MCH – mean corpuscular haemoglobin ; MCHC - mean corpuscular haemoglobin concentration;

C- Control Rats fed with feed diet without Ficus exasperata leaf; F1- Rats fed diet with 10 % inclusion level of Ficus exasperata leaf; F2- Rats fed diet with 30 % inclusion level of Ficus exasperata leaf; F3- Rats fed diet with 50 % inclusion level of Ficus exasperata leaf

Table 3: Erythrocytic Parameters of Wistar Rats Fed with *Ficus exasperata* Leaf-based Diet for 21 days

Group	RBC x 10^6/µl	HGB (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
C	7.23± 0.10^a	9.23 ± 0.36^a	38.00 ± 0.85^a	52.60 ± 0.49^a	12.97± 0.51^a	24.28± 0.71^a
F1	8.17± 0.17^a	10.80± 0.12^b	43.38± 0.48^b	53.13±0.74^{ab}	13.25± 0.26^a	24.90± 0.12^a
F2	8.02 ± 0.33^a	10.93± 0.36^b	44.68± 1.35^b	55.80±1.38^{ab}	13.63± 0.24^a	24.43± 0.20^a
F3	8.22± 0.58^a	11.32 ± 0.38^b	46.63 ± 1.96^b	57.17±2.17^b	13.88±0.61^a	24.30±0.37^a

Values are expressed as mean of 6 determinations ± S.E.M. and those with different superscripts down the column are statistically different ($p < 0.05$)

RBC – red blood cell; HGB – haemoglobin; PCV – packed cell volume; MCV – mean cell volume; MCH – mean corpuscular haemoglobin ; MCHC - mean corpuscular haemoglobin concentration; C- Control Rats fed with feed diet without Ficus exasperata leaf; F1- Rats fed diet with 10 % inclusion level of Ficus exasperata leaf; F2- Rats fed diet with 30 % inclusion level of Ficus exasperata leaf; F3- Rats fed diet with 50 % inclusion level of Ficus exasperata leaf

Table 4: Leucocytic Parameters of Wistar Rats Fed with *Ficus exasperata* Leaf-based Diet for 7 days

Group	WBC x 10^3/µl	PLT x 10^3/µl	LYM (%)
C	16.95 ± 0.57^a	730.50 ± 13.89^a	69.90 ± 6.64^a
F1	15.73 ± 1.08^{ab}	708.00 ± 12.69^a	76.15 ± 9.42^a
F2	13.55 ± 0.65^b	798.75 ± 17.01^a	76.15 ± 1.23^a
F3	13.05 ± 1.08^b	831.00 ± 15.87^a	75.35 ± 3.80^a

Values are expressed as mean of 6 determinations ± S.E.M. and those with different superscripts down the column are statistically different ($p < 0.05$)

RBC – red blood cell; HGB – haemoglobin; PCV – packed cell volume; MCV – mean cell volume; MCH – mean corpuscular haemoglobin ; MCHC - mean corpuscular haemoglobin concentration;
C- Control Rats fed with feed diet without Ficus exasperata leaf; F1- Rats fed diet with 10 % inclusion level of Ficus exasperata leaf; F2- Rats fed diet with 30 % inclusion level of Ficus exasperata leaf; F3- Rats fed diet with 50 % inclusion level of Ficus exasperata leaf

Table 5: Leucocytic Parameters of Wistar Rats Fed with *Ficus exasperata* Leaf-based Diet for 21 days

Group	WBC x 10^3/µl	PLT x 10^3/µl	LYM (%)
C	16.13 ± 3.07^{ab}	811.50 ± 5.48^{a}	80.28 ± 2.91^{ab}
F1	15.02 ± 0.48^{a}	566.00 ± 14.07^{b}	76.25 ± 2.21^{ab}
F2	20.45 ± 0.16^{b}	584.50 ± 4.97^{b}	84.75 ± 1.56^{b}
F3	18.70 ± 0.75^{ab}	552.00 ± 13.02^{b}	73.60 ± 4.55^{a}

Values are expressed as mean of 6 determinations ± S.E.M. and those with different superscripts down the column are statistically different ($p < 0.05$)
RBC – red blood cell; HGB – haemoglobin; PCV – packed cell volume; MCV – mean cell volume; MCH – mean corpuscular haemoglobin ; MCHC - mean corpuscular haemoglobin concentration;
C- Control Rats fed with feed diet without Ficus exasperata leaf; F1- Rats fed diet with 10 % inclusion level of Ficus exasperata leaf; F2- Rats fed diet with 30 % inclusion level of Ficus exasperata leaf; F3- Rats fed diet with 50 % inclusion level of Ficus exasperata leaf

Discussion

The significant decrease in the packed cell volume (PCV) and mean corpuscular volume (MCV) of rats fed diets containing 10 %, 30 % and 50 % w/w *Ficus exasperata* leaves for 7 days recorded in this study suggests anaemia in the experimental rats. According to Wintrobe and Greer (2009), a lower than normal PCV is representative of anemia, while MCV is a measure of the average volume or size of a red blood cell and its low level is consistent with microcytic anemia. This depletion might be due to the presence of certain toxic constituents of *Ficus exasperata* leaf,

such as saponins (Ijeh Ukweni, 2007; Soji-Omoniwa and Oloyede, 2018). Previous studies had reported the hemolytic ability of saponins (Okwu, 2004; Savage, 2016). Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties. They are characterized by their hemolytic activity. The release of hemoglobin from red blood cells is the direct result of the interaction of saponins with membrane-bound sterols, which causes an increase in the permeability of the plasma membrane, bringing about the destruction of the cell (Savage, 2016). The red blood cell count, haemoglobin concentration and its differentials at the end of day 7 of feeding on the test diets however remained unaltered.

The recorded reduction in WBC of rats fed diets containing 10 %, 30 % and 50 % w/w *Ficus exasperata* leaves for 7 days in this study suggests that the plant might possess the ability to inhibit leukopoiesis by altering colony stimulating factors and interleukins which increases the number of WBC precursors (Li *et al*., 2001). The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Soetan *et al*., 2013).

The increase in the concentrations of haemoglobin, PCV and MCV recorded after 21 days of feeding the rats diets containing 10 %, 30 % and 50 % w/w *Ficus exasperata* leaves suggests improved erythropoiesis and oxygen-carrying capacity of the blood. PCV is the percentage of red blood cells in blood (Purves *et al*., 2003). Haemoglobin is the intracellular protein of the RBCs which binds oxygen in the pulmonary artery for transport to tissues and binds tissue carbon dioxide for transport back to lungs for exhalation (Wintrobe and Greer, 2009). Yakubu *et al*. (2014), in their study on the evaluation of the toxicity effect of aqueous leaf extract of FEL, reported lack of effect on the RBC and factors relating to it. Bafor and Igbinuwen (2009) however reported a decrease in RBC and haemoglobin following the administration of aqueous leaf extract of *F.*

exasperata for 14 days in mice. Their submission is however contrary to the findings of this study.

The decrease in platelets after 21 days of consuming diets containing 10 %, 30 % and 50 % w/w *Ficus exasperata* leaves suggests that the plant might possess the ability to inhibit thrombopoiesis thus decreasing the number of platelet precursors. Platelets are responsible for coagulation (Li *et al*., 2001). When bleeding occurs, the platelets swell, clump together, and form a sticky plug that helps stop the bleeding. If there are too few platelets, uncontrolled bleeding may occur.

Conclusion

The consumption of FELD has been reported to possess normoglycemic ability amongst other benefits, nothwitstanding, the formulated diet (FELD) should be consumed with caution, because it elicited some level of toxicity as revealed by the studied rat haematological indices.

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