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supplements for humans. The diet preparation of air dried leaves of the plant significantly increased red blood cells counts, white blood cells packed cell volume and haemoglobin concentration in rats (Alada, 2000) while the dietary preparation made with sun dried leaves had no significant effect on haematological parameters in broilers (Fasuyi and Nonyerem, 2007), indicating that the potency of the plant depends on the method of preparation of the plant for consumption. Adaramoye et al. (2007) reported that *T. occidentalis* leaves have hypolipidemic effect and may be a useful therapy in hypercholesterolemia. It was found out that aqueous and ethanol extract of *T. occidentalis* could salvage and prevent free radical production and at the same time have antimicrobial properties (Obboh et al., 2010). In spite of the widespread use of *T. occidentalis*, there are scanty information on its various biological effects on cockerels. This study investigated haematological parameters, serum biochemical indices and histological changes of some organs associated with long term administration of *T. occidentalis* Leaves Extract (TOLE) on cockerels.

MATERIALS AND METHODS

Preparation of *Telfairia occidentalis* leaves extract

A kilogram of freshly cut TOLE with leaf stalks were washed, drained, chopped and pounded in mortar and pestle. This was then squeezed and filtered with cheese cloth to obtain a homogenous extract of the *T. occidentalis* leaf extract (Nworgu et al., 2007). The extract was prepared at four days' interval and served to the birds fresh according to the treatments.

Experimental animals and management

A total of one hundred and sixty-two (162) Nera black cockerel chicks were weighed and randomly allotted to six dietary treatments that contains 30, 60, 90, 120 and 150mL TOLE per one liter of water (Nworgu et al., 2007) for B, C, D, E and F respectively. Treatment A served as the control with no extract. Each treatment was replicated three times with nine birds per replicate in a completely randomized design. The experiment lasted for 24 weeks. The birds were fed the same diets for the first eight weeks and later grower diet was given to the birds till the end of the experiment. The TOLE supplement was served at four days interval throughout the period of the experiment according to the treatment per liter of water and later clean water was served. Feed and water were served ad-libitum. Both the feed intake and weight gain were monitored. Other management practices such as routine vaccination,

drug administration and maintenance of cleanliness within and outside the poultry houses were observed.

Collection of blood samples

Blood samples were collected at 24th week of the experiment. Six birds per treatment were randomly selected and bled via wing veins, for haemoglobin and serum biochemical analysis Serum was obtained by centrifugation and the serum samples were stored in deep freezers at minus 10⁰c until analyzed.

Heamatological analysis

The Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and Haemoglobin (Hb) concentration were determined using the Wintrobe Microhematocrit, Improved Neubauer haemocytometer and Cyanmethemoglobin methods, respectively (Coles, 1986). Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) levels were computed using the formula described by Jain (1986).

$$MCH = \frac{\text{Haemoglobin}}{\text{RBC}} \times 10$$

$$MCV = \frac{\text{PCV} \times 10}{\text{RBC}}$$

$$MCHC = \frac{\text{Haemoglobin} \times 100}{\text{PCV}}$$

Serum biochemical analysis

The serum biochemical assays were carried out using the standard chemical procedures. Total serum protein by Goldberg refractometer (Kohn and Allen, 1995), albumen by Bromocresol Green (BCG) method (Peters et al., 1982) while globulin was calculated according to Coles (1986). Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and triglyceride were determined using Randox test kits (Randox Laboratories Crumlin, County Antrim, United Kingdom).

Histological studies

Histological studies of the organs and tissues were done according to procedures described by Disbrey and Rack (1970) and Drury and Wallington (1967). The kidneys, liver, spleen and testes of the cockerel were removed from the surrounding tissues. They were fixed in 10% formal saline, and after 72 hours the organs were dehydrated in graded alcohol (20, 30, 50, 70 and 95%) for five minutes, cleared in xylene and embedded in paraffin. The resulting blocks were completely sectioned and randomized. The selected sections were stained in haemotoxylin and eosin and the slides were examined at magnification of x400 under light or optical microscope.

Ethical approval

The study was conducted after approval of ethic and research committee of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Data analysis

The mean values obtained for the determination of various indices in respect of the six treatments were subjected to one-way analysis of variance (SAS, 2003). Treatment means were also separated by Duncan option of the software at p = 0.05 (5% significant level).

RESULTS

Data on the effects of TOLE on haematological parameters and serum biochemical constituents of cocks

are presented in tables 2 and 3 respectively. The RBC, lymphocytes, heterophils, albumin, and AST values were similar (P>0.05) among the various treatment groups, while the PVC, Hb, WBC, MCV, MCH, MCHC, monocytes and Eosinophils were statistically different (P<0.05) across the treatments. Except for albumin and AST, all other serum parameters investigated were significantly (P<0.05) affected by the administration of the TOLE. For histopathological examination, there were lesion/necrosis in the liver and kidney of cocks on TOLE treatments while the spleen and testes did not show any visible lesion in all the treatments. However, for birds served 120mL and 150mL TOLE/L of water there were reduced germinal epithelia heights and sloughing of the germinal epithelia respectively (Table 4).

Table 2. Haematological response of Nera black cockerels served *Telfairia occidentalis* leaves extract at 4 weeks interval for 24 weeks

Treatments Level of TOLE	A 0 ml	B 30 ml/l	C 60 ml/l	D 90 ml/l	E 120 ml/l	F 150 ml/l	SEM
PCV (%)	35.20 ^d	37.22 ^{ab}	38.39 ^a	35.53 ^{dc}	36.58 ^{bcd}	37.11 ^{abc}	0.14
RBC (× 10 ⁶ /mm ³)	2.72	2.71	2.70	2.68	2.73	2.53	0.01
Hb (g/dL)	11.73 ^c	12.41 ^{bc}	12.79 ^a	11.84 ^{bc}	12.28 ^{abc}	12.63 ^a	0.05
WBC (×10 ⁶ /mm ³)	13.24 ^{ab}	12.45 ^{bc}	13.34 ^{ab}	13.92 ^a	12.16 ^c	13.55 ^a	0.06
MCV (μ ³)	131.32 ^b	137.38 ^{ab}	142.48 ^a	132.60 ^b	134.02 ^b	135.10 ^b	0.05
MCH (μg)	42.87 ^b	45.78 ^{ab}	48.50 ^a	44.25 ^b	44.99 ^{ab}	45.03 ^{ab}	0.31
MCHC (%)	33.33 ^a	33.19 ^{ab}	32.97 ^b	33.33 ^a	33.33 ^a	33.33 ^a	0.21
Lymphocytes (%)	65.50	60.55	63.19	65.43	64.83	67.80	0.89
Heterophils (%)	29.00	35.36	30.36	28.33	29.52	31.33	0.87
Monocytes (%)	2.66 ^{abc}	2.86 ^{abc}	2.14 ^c	3.88 ^a	3.51 ^{ab}	2.28 ^{bc}	0.10
Eosinophils (%)	4.83 ^a	2.55 ^b	3.25 ^{ab}	3.25 ^{ab}	2.22 ^b	2.22 ^b	0.01

^{abcd} Means in the same row with different superscript are significantly (P<0.05) different. PCV =packed cell volume, RBC=red blood cell, Hb= haemoglobin, WBC= white blood cell, MCV= mean cell volume, MCH=mean cell haemoglobin, MCHC= mean cell haemoglobin concentration, SEM=standard error of means

Table 3. Serum biochemical response of Nera black cockerels served *Telfairia occidentalis* leaves extract at 4 weeks interval for 24 weeks

Treatments/ Level of TOLE	A 0 ml/l	B 30 ml/l	C 60 ml/l	D 90 ml/l	E 120 ml/l	F 150 ml/l	SEM
Total protein (g/dL)	3.88 ^b	4.24 ^{ab}	4.84 ^{ab}	4.97 ^a	4.98 ^a	4.26	0.08
Albumen (g/dL)	2.14	2.18	2.37	2.30	2.08	2.04	0.03
Globulin (g/dL)	1.61 ^b	2.06 ^{ab}	2.48 ^{ab}	2.67 ^a	2.91 ^a	2.08 ^{ab}	0.07
Albumin/globulin ratio	1.44 ^a	1.01 ^b	0.98 ^b	0.93 ^b	0.75 ^b	1.02 ^b	0.02
Aspartate amino transferase (IU/L)	91.60	102.40	101.86	97.28	94.86	96.10	1.28
Alanine amino transferase (IU/L)	7.96 ^{bc}	7.74 ^c	8.41 ^{ab}	8.00 ^{abc}	7.84 ^{bc}	8.06 ^a	0.05
Triglyceride (mg/dL)	202.84 ^a	163.57 ^b	160.52 ^b	149.42 ^b	158.62 ^b	154.92 ^b	1.54

^{abc} Means in the same row with different superscript are significantly (P<0.05) different. SEM=Standard error of means

Table 4. Histopathological effects of *Telfairia occidentalis* leaves extract served at 4 weeks interval for 24 weeks to Nera black cockerels on internal organs

Treatments	A	B	C	D	E	F
Organs	0 TOLE (mL/L)	30 TOLE (mL/L)	60 TOLE (mL/L)	90 TOLE (mL/L)	120 TOLE (mL/L)	150 TOLE (mL/L)
Kidney	No visible lesion	Wide spread coagulative necrosis of renal tubules	Severe generalized interstitial congestion.	Mild moderate loss and sloughing of renal tubular epithelium	Severe generalized interstitial congestion	Widespread coagulative necrosis of the renal tubules
Liver	No visible lesion	Portal congestion	Severe central venous congestion	Severe portal congestion with periportal cellular infiltration by mono-nucleus cell	Severe portal congestion with periportal cellular infiltration by mono-nucleus cell	Severe portal congestion with periportal cellular infiltration by mono-nucleus cell
Spleen	No visible lesion	No visible lesion	No visible lesion	No visible lesion	No visible lesion	No visible lesion
Testes	No visible lesion	No visible lesion	No visible lesion	No visible lesion	No visible lesion. Germinal epithelium height reduced	No visible lesion marked sloughing of the germinal epithelium

TOLE = *Telfairia occidentalis* Leaves Extract.

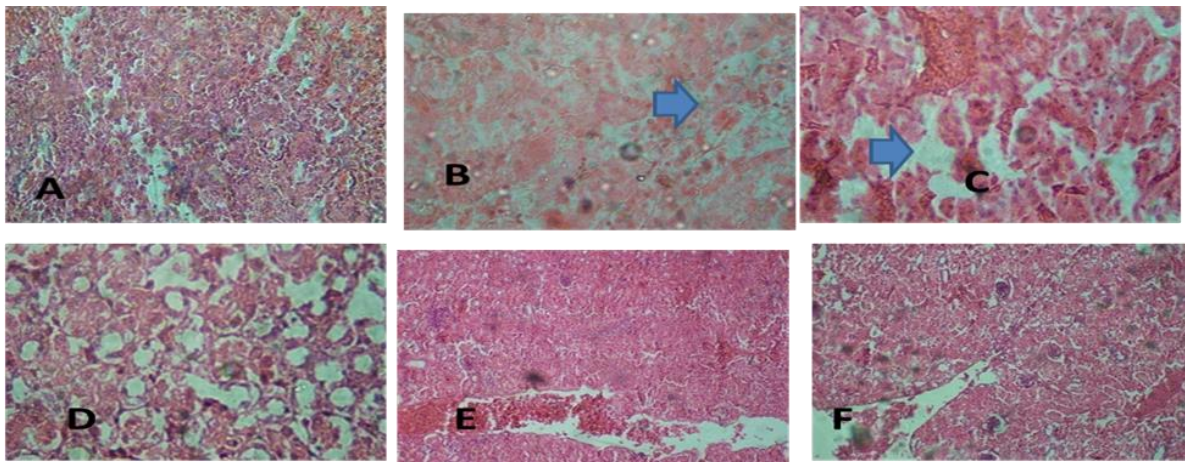


Figure 1. Micrographs of the kidneys of Nera black cocks served fluted pumpkin leaf extract at 4 weeks interval for 24 weeks A= 0mL/ TOLE/L (no visible lesions seen). B= 30mL/ TOLE/L (wide spread coagulative necrosis of the renal tubules (arrow); C= 60mL// TOLE/L (severe generalized interstitial congestion), D = 90mL/ TOLE/L (mild to moderate loss and sloughing of renal tubular epithelium), E= 120mL/ TOLE/L (severe generalized interstitial congestion), F= 150mL/ TOLE/L (widespread necrosis of the renal tubules. (mg× 400)

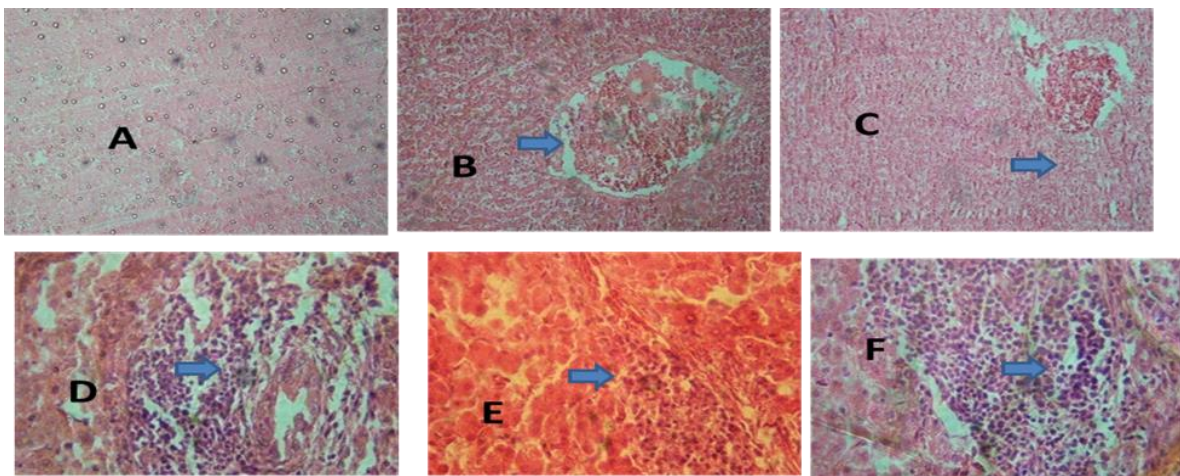


Figure 2. Micrographs of the liver of Nera black cocks served fluted pumpkin leaf extract at 4 week interval for 24 weeks. TOLE = *Telfairia occidentalis* Leaves Extract. A: 0mL/L (No visible lesions seen), B: 30mL TOLE/L (Portal congestion (arrows), C: 60mL TOLE/L (Severe central venous congestion), D: 90mL TOLE/L (Severe portal congestion with periportal cellular infiltration by mono-nuclear cells), E: 120mL TOLE/L (Severe portal congestion with periportal cellular infiltration by mono-nuclear cells). F: 150mL TOLE/L (Severe portal congestion with periportal cellular infiltration by mono-nuclear cells) (Mg× 400).

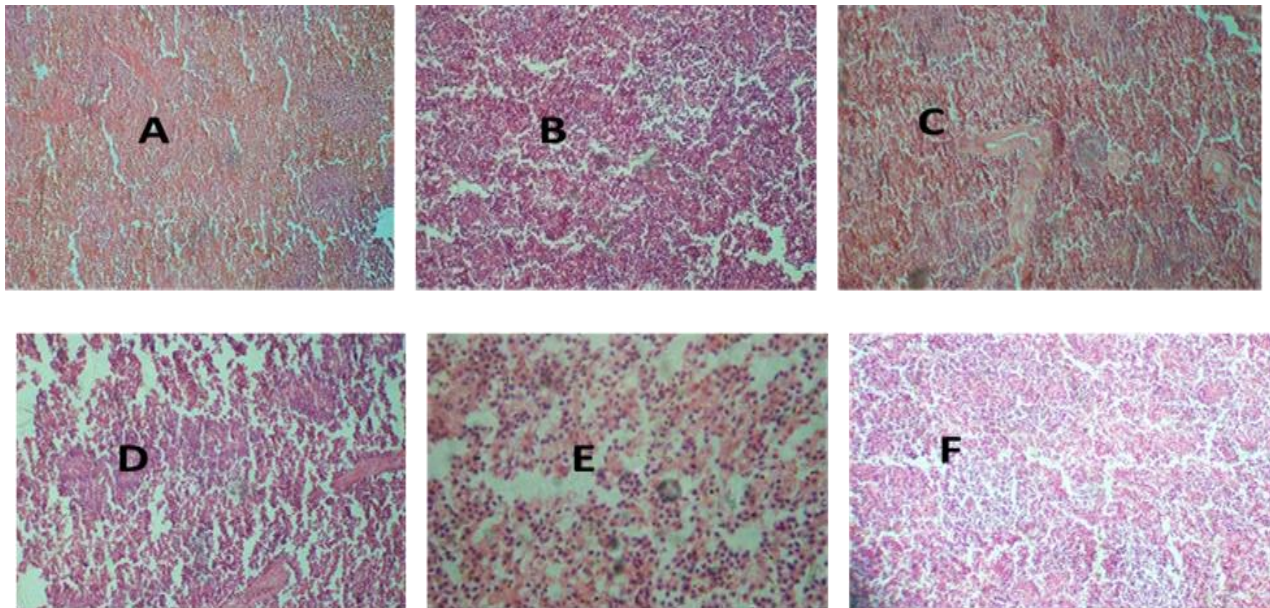


Figure 3. Micrographs of the spleen of Nera black cocks served fluted pumpkin leaf extract at 4 week interval for 24 weeks A to F: No visible lesions seen, (Mg×400). TOLE = *Telfairia occidentalis* Leaves Extract. A= (0ml TOLE/L), B= (30mL TOLE/L), C= (60mL TOLE/L), D= (90mL TOLE/L), E= (120mL TOLE/L), F= (150 TOLE/L)

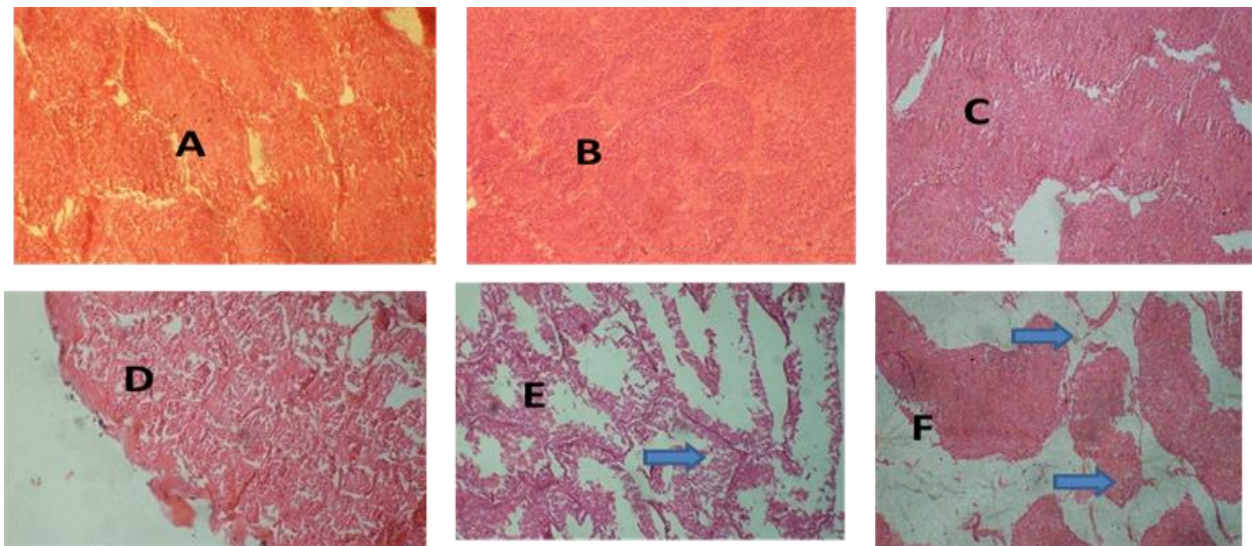


Figure 4. Micrographs of the testes of Nera black cocks served fluted pumpkin leaf extract at 4 week interval for 24 weeks A to D: No visible lesions seen, E and F: Marked sloughing of the Germinal Epithelium (arrows) (Mg × 400). TOLE = *Telfairia occidentalis* Leaves Extract. A= (0ml TOLE/L), B= (30mL TOLE/L), C= (60mL TOLE/L), D= (90mL TOLE/L), E= (120mL TOLE/L), F= (150 TOLE/L)

DISCUSSION

This study showed that the aqueous extract of the plant produced significant ($P<0.05$) increase in the levels of the PCV, Hb, WBC, MCV, MCH and monocytes when compared with the control. The best PVC (38.39%) and Hb (12.79 g/dL) were obtained for birds served 60 ml/l TOLE while the least parameters (35.20% and 11.73g/dL respectively) were obtained for birds on the control. This showed that animals suffering from low blood volume

may benefit from the administration of this plant extract. This observation of increased level of erythron supports the earlier research findings that this plant has haematinic potency (Adedapo et al., 2002; Nworgu et al., 2007; Ifeanyi et al., 2014). The increase in the level of erythron produced by this extract may be due to the fact that the leaves of this plant are rich in many minerals (Burkill, 1994; Aletor et al., 1995; Archibong, 2002) high in crude protein, essential and non-essential amino acid in the leaf (Alabi et al., 2017; Fasuyi, 2007). This result agrees with

the study of Alada (2000) and lends validity to the use of the leaves in the treatment of anaemia (Gbile, 1986). Birds on treatment D recorded the highest value of $13.92 \times 10^6/\text{mm}$ for WBC though it was not significantly different ($P>0.05$) from the values obtained for birds on treatments A, C and F but significantly different ($P<0.05$) from those on B and E. The WBC helps to defend the body against foreign organism or extraneous materials so the higher the WBC the better the ability of animal to fight diseases since the WBC values fall within the normal range as reported by Mitruka and Rawnsley (1981), it then suggests that the health status of the birds was not challenged by the administration of this extract.

Birds on treatment C recorded the highest MCV (142.48μ) value which was similar to the values obtained for birds on treatment B but different ($P<0.05$) from the values obtained for birds on treatments A, D, E and F. The highest MCH value of $48.50\mu\text{g}$ was also obtain for birds on treatment C though it was similar to the values obtained for birds on treatments A and D. The result obtained for the corpuscular constants in this study did not follow any particular trend and they were within the normal physiological range as reported by Mitruka and Rawnsley (1981) for normal cocks. The values obtained for the monocytes and eosinophils were significantly different but did not follow any particular pattern.

Serum biochemical investigations have been explored extensively to distinguish normal state from stress and diseased conditions in animals. Except for the albumin and AST, all other serum parameters investigated in this study were significantly ($P<0.05$) influenced by the administration of the TOLE.

The total protein, globulin and ALT increased with increasing levels of TOLE while triglyceride decreased with increasing levels of TOLE. The total proteins were higher for birds on the TOLE treatments than for birds on the control. This increase could be attributed to the additional protein supplied by the TOLE since the birds were on the same diets. This observation was similar to the report of Adedapo et al. (2008) and Alabi et al. (2017) who reported that aqueous extract of fluted pumpkin leaves caused a significant increase in the levels of the total protein and globulin of finisher broilers and rats respectively that were on fluted pumpkin leaves extract treatment. Although the albumin values in this present study were not significant ($P>0.05$), Nworgu et al. (2007) reported an increase in the albumin levels of birds on treatment F (150 mL/L) having the values of 10.6 IU/L. The slight increase in the serum enzyme activities suggest that there was little breakdown of muscle tissues on the

birds on the TOLE treatments. The values of the triglycerides decreased with increasing levels of TOLE, this points to the ability of the extract to lower the lipids content as earlier reported by Adaramoye et al. (2007) and Nworgu et al. (2012).

Telfairia occidentalis leaves extract showed various cellular effects on histological characteristics of the tissues examined. The liver and kidney were the most affected while in the spleen and testes no lesion was observed. The hepatic lesion was characteristic by mild to severe portal congestion with periportal cellular infiltration by mononuclear cell and severe central venous congestion while the renal lesion were characterized by widespread coagulative necrosis of the renal tubule with generalized intestinal congestion and sloughing of renal tubular epithelium. This could be traced to the presence of alkaloids (Burkill, 1994) which have been observed with *Telfairia occidentalis*. This corroborates with the findings of Akubue et al. (1980) who reported venous congestion and in some cases burst vessels in the liver, spleen, lungs and heart of rats given aqueous extract of *Telfairia occidentalis* while Iweala and Obioda (2009) also reported irregularities in the liver of rats fed *Telfairia occidentalis* supplemented diet. Acute toxicity including hepatic toxicity have been reported (Akindele et al., 2018; Imosemi, 2018) in mice given *Telfairia occidentalis* up to 5000 mg/kg orally, with signs of decreased locomotion, calmness, writhing and increased breathing at higher doses of LD50 of intraperitoneal administration at 3000-5000 mg/kg. Although, there were no lesion observed in the testes in all the treatments, cocks on treatments E and F (120 and 150 mL TOLE/L) showed reduced germinal epithelium height and marked sloughing of the germinal epithelium respectively. The implication of this is disordered spermatogenesis and infertile/immature sperm cells. However, this effect is dose dependent since it was not observed at levels between 30-90 mL TOLE of water implying that, at higher doses of TOLE there was cell damage which could be detrimental to the fertility of cocks. This was similar to the findings of Adedapo et al. (2008) who reported testicular degeneration with severe disorganization of seminiferous tubules which were devoid of spermatid cells in rats served fluted pumpkin leaves extract. However, Iweala and Obioda (2009) reported the presence of large spermatogonia in the testes of rats fed *Telfairia occidentalis* supplemented diet while Nwangwa et al. (2007) reported a regenerative effect on the histology of rat's testes served the extract of fluted pumpkin leaves extract. Imosemi (2018); Sakpa et al. (2015) and Saalu et al. (2010) also testified to the

testiculo-protective attributes of TOLE at lower doses up to 200mg/kg in adult male Wister rats that showed increased sperm count, sperm viability and motility, enhanced spermatogenesis with elevated levels of testosterone and luteinizing and follicle stimulating hormones; but found aqueous TOLE to be testiculo-toxic at high dose of 800mg/kg body weight of male Wister rat.

CONCLUSION

Long term supplementation of TOLE for cockerel production should not exceed 60mL of TOLE per liter of water as the administration in excess of this can bring about tissue breakdown and reduced fertility. Animals suffering from blood loss can benefit from the administration of fluted pumpkin leaves extract as the extract increased erythron production.

DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

Author's contributions

All the authors have made substantive contribution to the study from its design to implementation, collection of data, statistical analysis, writing reports and manuscript preparation.

Consent to publish

All the authors gave their informed consent prior to their inclusion in the study.

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