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https://dx.doi.org/10.4314/nvj.v39i2.5 **ORIGINAL ARTICLE**

Studies on Crude Powder and Ethanolic Extract of *Citrullus lanatus* Seeds: Phytochemical Analysis, Effects on Haemogram and Some Reproductive Characteristics of Male Albino Rats

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SUMMARY
Effects of crude powder and ethanolic extract of *Citrullus lanatus* seeds on haemogram, spermiogram, testicular and epididymal histology of forty-eight male wistar rats were investigated following phytochemical analysis. The rats, divided into three test groups (A, B and C; n=16) were administered distilled water (Group A), ground powder (Groups B; 200mg/kg) and ethanol extract of *Citrullus lanatus* seed (Group C; 200mg/kg) for 28 days. Blood samples were collected from all rats after expiration of extract administration for haemogram. Subsequently, four rats from each group were sacrificed weekly post treatment for a period of four weeks for semen analysis and histology. Phytochemical analysis revealed alkaloids, flavonoids, saponins, anthraquinones, terpenoids, and cardiac glycosides. Haematological parameters were not significantly different across the groups. Mean sperm motility of the control group was significantly ($p<0.05$) higher compared with treatment groups (A and B) each week post treatment. Also, initial lower sperm motility recorded in week 1 and 2 post treatment in the treatment groups followed by increase at week 3 and 4 were observed. However, there were significant post treatment reductions ($p<0.05$) each week in sperm morphological abnormalities comparing the treatment groups with control group. Histology of the testes of Treatment groups showed numerous closely packed seminiferous tubules with normal architecture containing spermatogenic cells. The study revealed that ground powder and ethanol extract of *Citrullus lanatus* seeds administered at 200mg/kg have no deleterious effect on haemogram, the organs of male reproduction and sperm morphology. Hence, it may be considered safe for use in breeding animals.

Key words: *Citrullus lanatus*, crude powder, ethanolic, semen, haemogram.

INTRODUCTION

The species *Citrullus lanatus* (generally known as watermelon) belongs to the family *Cucurbitaceae* and consists of about 120 genera and about 825 species (Edwards *et al.*, 2003). The *Cucurbitaceae* family is also known as the gourd family and apart from *Citrullus lanatus* consists of important sources of food such as pumpkin (*Cucurbita*

pepo), melon (*Cucumis melo)* and cucumber (*Cucumis sativa)* [Adesanya *et al.,* 2011]. Watermelon is thought to have originated from Southern Africa because it is found growing in the wild throughout the area and has been cultivated in Africa for over 4,000 years (Dauda *et al.,* 2008).

Citrullus lanatus fruit is composed of about 92% water, hence the name "water" melon (Naz *et al.,* 2014). The fruit has a smooth exterior rind and a juicy, sweet, interior flesh (Kolawole *et al.,* 2014). The flesh colour may vary from yellow to red, although the red-fleshed varieties are the most popular (Gwana *et al.,* 2014). In Nigeria, *Citrullus lanatus* is commonly referred to as Kankana by the Hausas, Esobara by the Yorubas and Anyu by the Igbos.

Citrullus lanatus has been reported to be mildly diuretic and an excellent source of vitamins, proteins, oil, calories, minerals, essential fatty acids, carotenoids such as lycopene and beta-carotene, and amino acids such as arginine and lysine (Inuwa *et al.*, 2011; Erhirhie and Ekene, 2013). *C. lanatus* also contains a significant amount of citrulline, which is an important amino acid (Collins *et al.,* 2007). Watermelon seeds contain cucurbocitrin which aids in lowering blood pressure and improving kidney function (Bird, 2015). The rind is usually discarded or applied as feeds or fertilizers, but they are edible and can be used as a vegetable (Erukainure *et al.,* 2010).

Watermelon fruit is a great source of antioxidants and is commonly consumed worldwide but the seeds are often discarded perhaps due to a lack of knowledge about the potential nutritional and medicinal benefits though they have been reported (Etim *et al*., 2013). The fruit is fast becoming a source of feed in livestock especially as a source of water during the dry season (Shayo *et al.,* 1996), while the seeds have been reported to be incorporated in the feed of broiler chicks and fingerlings (Shazali *et al.,* 2013; Tiamiyu *et al.,* 2014) to enhance growth performance. This study aimed at investigating the haemogram profile and semen characteristics of male wistar rat following prolonged oral administration of *Citrullus lanatus.*

MATERIAL AND METHODS

Experimental animals

Forty-eight adult male Wistar rats were used for this study. The rats were housed at the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Oyo State. The rats were kept in standard pen and fed with commercially prepared ration. Water was given *ad libitum*.

Crude powder processing and ethanolic extraction

Citrullus lanatus fruit and seeds were obtained from a local market and identified at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State with voucher number *Citrullus lanatus* Thunb. Mansf. 110362.

The seeds were carefully removed from the fruit and were thoroughly washed with water to remove fruit portions. The seeds were spread on trays and were air-dried at room temperature after which they were ground into powder using a grinding machine (Adesanya *et al.,* 2011). The powder was stored at room temperature in air tight plastic containers prior to use. The ethanolic extract was prepared by soaking 500 grams of the dry seed powder in 2.5 litres of ethanol at room temperature for 48 hours. The extract was filtered after 48 hours through a Whatmann filter paper No.1 (185mm). The filtrate was thereafter concentrated using a rotary evaporator at a temperature of 40^0C (Kolawole *et al.,* 2014). The concentrate was further concentrated using a vacuum oven set at a temperature of 40^0C and a pressure of 600mmHg so as to further remove any trace of the solvent (ethanol) left in the concentrate. The weight and volume of the extract was then determined.

Qualitative phytochemical screening
Both the crude powder and the ethanolic extract of C*itrullus lanatus* seeds were subjected to qualitative phytochemical evaluations for the presence of alkaloids, flavonoids, saponins, triterpenoids, cardiac glycosides, anthraquinones and tannins following the procedure as described by Ayoola *et al.* (2008).

Extract administration
The experimental animals were divided into three groups (A, B and C) containing 16 rats each. Group A rats served as control and were administered 0.1 mls of distilled water orally; the rats in group B were administered orally with the finely milled *Citrullus lanatus* seed powder using distilled water as solvent at a dosage of 200mg/kg (Kolawole *et al.*, 2014). This mixture was prepared freshly on a daily basis before administering to the rats. The rats in group C were administered with 200mg/kg of the ethanolic extract of *Citrullus lanatus* seeds orally by using a rat oral cannula (Kolawole *et al.*, 2014). Extracts were administered for 28 days. Blood samples for haematology were collected at the end of extract administration through the ocular vein into ethylene ditetra-acetic acid (EDTA) - containing sample bottles.

Gonad excision and semen analysis
Weekly sacrifice of the rats began one-week post cessation of extract administration and lasted for four weeks with four rats each sacrificed per group for semen analysis. Rats from each group were weighed and euthanized weekly by cervical dislocation after stunning. The peritoneal cavity was opened through a lower transverse abdominal incision, thereafter, the testes and epididymides in the control and experimental groups were immediately removed and weighed using a digital scale. Semen was collected from the right cauda epididymis from the control and treatment groups and evaluated for sperm motility, liveability and sperm morphological abnormalities (Oyeyemi *et al.*, 1996).

Histology
The excised testes and epididymides samples collected were fixed in Bouin's fluid and embedded in paraffin blocks. Sections of 10 μm thick were stained with Haematoxylin and Eosin (Akinloye *et al.*, 2002). The slides of the testes and epididymides were thereafter examined under the light microscope for any pathologic lesion.

Statistical analysis
Data generated from this study was presented as mean ±SE. The differences between the means in the treated and in the control groups were compared by two-way analysis of variance (ANOVA) while that of haematology was compared using one-way analysis of variance using Statistical Package for Social Sciences (SPSS) analytical package. A value of $p<0.05$ was considered significant.

RESULTS
Qualitative phytochemical analyses revealed the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides and anthraquinones in both the crude powder and ethanolic extract of *Citrullus lanatus* seeds. No saponins were present in the ethanolic extract of the seeds and no tannins were present in both the crude powder and ethanolic extract of *Citrullus lanatus* seeds as shown in Table 1. There were no significant differences ($p>0.05$) in the values obtained for the various blood parameters across the three groups (Table 2).
Comparing the mean sperm motility values per group within the four weeks post cessation of treatment, it was observed that the mean sperm motility in group A was not significantly different ($p>0.05$) from week 1 (88.3±4.41) to week 4 (92.5±1.44). In group B, there was a progressive increase in the mean sperm motility values from week 1 (72.5±2.50) to week 4 (77.5±2.50) post

cessation of treatment, with weeks 2 (72.5±2.50) and week 1 (72.5±2.50) significantly lesser ($p<0.05$) compared to weeks 3 and 4 (77.5±2.50) within the group. In group C, there was a progressive increase in the mean sperm motility values from week 1 (67.5±2.50) to week 4 (82.5±2.50) post cessation of treatment. Within the group, weeks 2 (67.5±2.50) and 1 (67.5±2.50) were significantly less ($p<0.05$) compared to weeks 3 (77.5±2.50) and 4 (82.5±2.50) (Table 3).

In comparing the mean sperm motility values for the treatment groups within each week post cessation of treatment, mean sperm motility values for groups B (72.5±2.50) and group C (67.5±2.50) were significantly lower ($p<0.05$) compared to group A in week 1. In week 2, mean sperm motility values for groups B (72.5±2.50) and C (67.5±2.50) were significantly lower ($p<0.05$) than group A (93.75±1.25) while there was no significant difference ($p>0.05$) between groups B and C, though group B was greater in value than C. In week 3, mean sperm motility values for group B (77.5±2.50) and C (77.5±2.50) were significantly lower than group A (91.7±1.67) while there wasn't any significant difference between groups B and C. In week 4, the mean sperm motility values for Groups B (77.5±2.50) and C (82.5±2.50) were significantly lower ($p<0.05$) than group A (92.5±1.44) while there was no significant difference

TABLE 1: Qualitative phytochemical analysis result of ethanolic extract and crude powder of *Citrullus lanatus* seeds

ALKALOID TEST	Dragenduff's reagent	Mayer's reagent	Wagner's reagent
C. lanatus seed powder	+	+	+
C. lanatus ethanolic extract	+	+	+
FLAVONOID TEST	Ammonia/ H_2SO_4 test	Aluminium solution test	Ethyl acetate test
C. lanatus seed powder	+	+	+
C. lanatus ethanolic extract	+	+	+
SAPONINS TEST	Frothing test		
C. lanatus seed powder	+		
C. lanatus ethanolic extract	-		
TANNINS	Ferric chloride test		
C. lanatus seed powder	-		
C. lanatus ethanolic extract	-		
ANTHRAQUINONES TEST	Chloroform/ Ammonia test		
C. lanatus seed powder	+		
C. lanatus ethanolic extract	++		
TERPENOIDS	Chloroform/ H_2SO_4 test		
C. lanatus seed powder	+		
C. lanatus ethanolic extract	+		
CARDIAC GLYCOSIDES TEST	Keller-killiani's test		
C. lanatus seed powder	+		
C. lanatus ethanolic extract	+		

+ =present
- =absent

TABLE 2: Effects of treatment with crude powder and ethanolic extract of *Citrullus lanatus* seeds on haemogram after 28 days of administration in Wistar rats

	GROUP A	GROUP B	GROUP C	*Normal reference intervals
PCV (%)	43.13±0.80	44.13±0.68	42.88±0.75	36-54
Hb (g/dl)	14.54±0.28	14.76±0.23	14.58±0.26	11-19.2
RBC (X 10^6/µL)	7.23±0.13	7.41±0.13	7.20±0.16	
MCV (fl)	59.68±0.39	59.57±0.49	59.68±0.52	48-70
MCH (pg)	20.12±0.14	19.94±0.25	20.29±0.17	-
MCHC (%)	33.74±0.18	33.47±0.21	34.01±0.15	40
WBC (X10^3 cells/µL)	9.36±0.76	8.58±0.56	9.16±0.53	6-18
LYMPHOCYTES (%)	67±1.14	63.3±2.53	67.00±2.16	65-85
NEUTROPHILS (%)	28.63±1.20	32.25±2.71	29.38±2.43	10-30
MONOCYTES (%)	2.06±0.21	2.06±0.21	2.19±0.26	0-5
EOSINOPHILS (%)	2.38±0.20	2.38±0.33	2.13±0.29	0-6

*Research Animal Resources, University of Minnesota, 2013

TABLE 3: Table showing comparison of mean sperm motility across the three treatment groups of treatment

	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GROUP A	88.3±4.41^{ai}	93.75±1.25^{bl}	91.7±1.67^{cn}	92.5±1.44^{dm}
GROUP B	72.5±2.50^{amo}	72.5±2.50^{bef}	77.5±2.50^{cem}	77.5±2.50^{dfo}
GROUP C	67.5±2.50^{ijk}	67.5±2.50^{lgh}	77.5±2.50^{ngj}	82.5±2.50^{mhk}

Means with same superscripts across rows and within columns are significantly different ($p<0.05$)

($p>0.05$) between groups B and C, though group C was greater in value than B (Figure 1).

Comparing the mean sperm liveability values, it was observed that there was no significant difference ($p>0.05$) in the mean sperm liveability per treatment group within the four weeks post cessation of treatment and no significant difference ($p>0.05$) also in the mean sperm liveability values for the treatment groups within each week post cessation of treatment (Figure II).

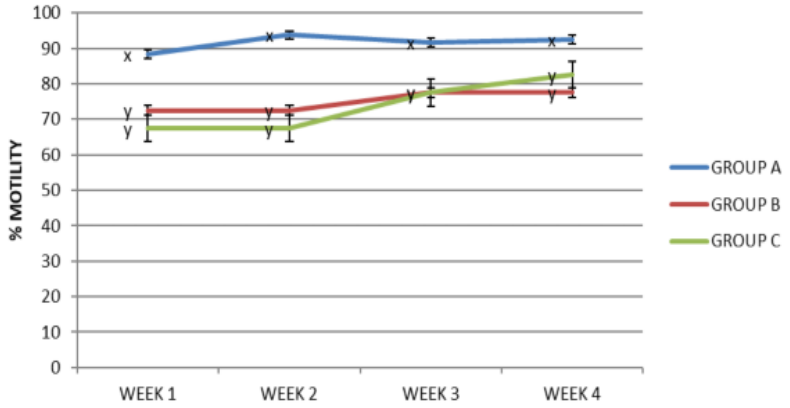

FIGURE 1: Line graph showing comparison of mean percentage sperm motility across the three treatment groups for the four weeks post cessation of treatment

Means with different letters are significantly different ($p<0.05$)

Comparing the mean sperm morphological abnormalities values per group within the four weeks post cessation of treatment, it was observed in Group A that there was a non-significant ($p>0.05$) progressive decline in the mean sperm morphological abnormalities from weeks 1 (15.43 ± 1.68) to week 3 (10.37 ± 0.90) with a significant

TABLE 4: Table showing comparison of mean percentage sperm morphological abnormalities across the three groups of treatment

	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GROUP A	15.43 ± 1.68^{a}	13.38 ± 2.57^{bq}	10.37 ± 0.90^{afgi}	13.80 ± 2.48^{chj}
GROUP B	6.33 ± 1.04^{ad}	4.21 ± 0.70^{be}	8.66 ± 0.79^{cfg}	6.35 ± 1.04^{igh}
GROUP C	4.12 ± 0.77^{adklmn}	7.09 ± 0.66^{qem}	8.37 ± 0.55^{il}	7.82 ± 0.93^{jkm}

Means with same superscripts across rows and within columns are significantly different ($p<0.05$)

decrease ($p<0.05$) in week 3. In group B, there was no significant difference ($p>0.05$) in mean sperm morphological abnormalities values from weeks 1 (6.33 ± 1.04) to week 4 (6.35 ± 1.04) though there was a slight increase in week 3 (8.66 ± 0.79) which became significantly lower ($p<0.05$) in week 4 (6.35 ± 1.04). In group C, there was a significant difference ($p<0.05$) in the mean sperm morphological abnormalities values from week 1 (4.12±0.77) to week 4 (7.82±0.93) (Table 4).

Comparing the mean morphological abnormalities values for the treatment groups within each week post cessation of treatment, groups B (6.33±1.04) and C (4.12±0.77) were significantly lower ($p<0.05$) compared to group A (15.43±1.68) in week 1; there was also a significant decrease ($p<0.05$) in the mean morphological abnormalities between groups B and C. In week 2, Groups B (4.21±0.70) and C (7.09±0.66) were significantly lower ($p<0.05$) than group A (13.38±2.57) while there was a significant difference ($p<0.05$) comparing groups B and C. In week 3, the mean morphological abnormalities values for group B (8.66±0.79) and C (8.37±0.55) were significantly lower ($p<0.05$) than group A (10.37±0.90) while there wasn't any significant difference ($p>0.05$) between groups B and C. In week 4, the mean morphological abnormalities values for Group B (6.35±1.04) and C (7.82±0.93) were significantly lower ($p<0.05$) than group A (13.80±2.48) while there was no

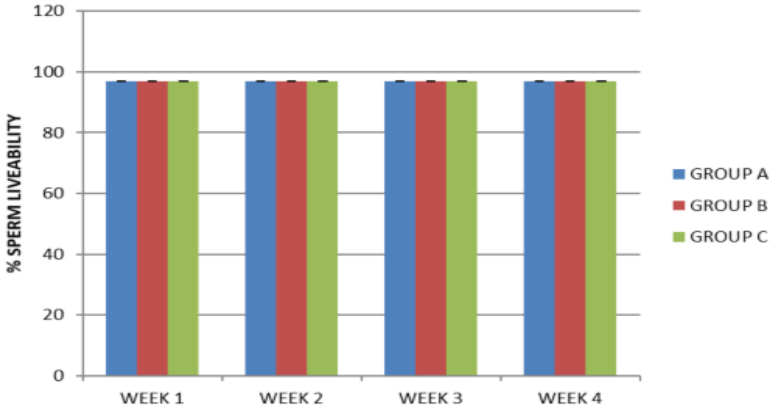

FIGURE 2: Bar chart showing comparison of mean percentage sperm livability across the three groups for the four weeks post cessation of treatment

No significant difference between and across groups

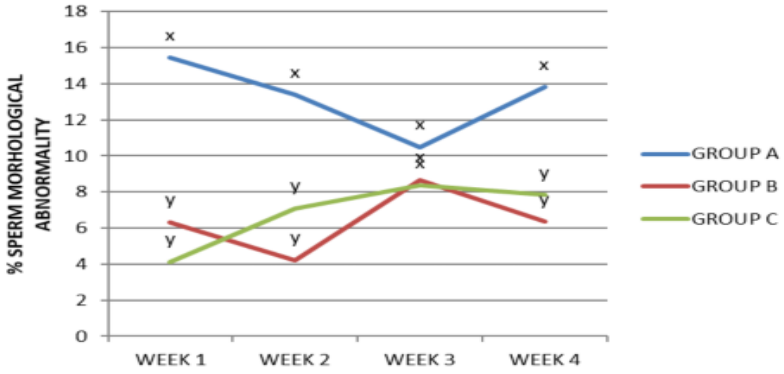

FIGURE 3: Line graph showing comparison of mean percentage sperm morphological abnormalities across the three groups for the four weeks post cessation of treatment

Means with different letters are significantly different ($p<0.05$). Means with same superscripts are not significantly different

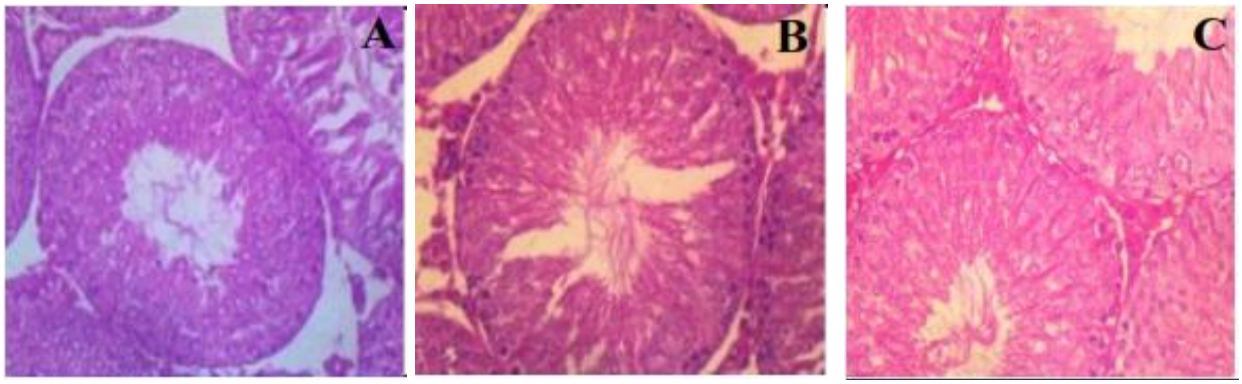

PLATE I: Photomicrograph showing the histology of the testes across the 3 groups typical for the four weeks post cessation of treatment using Haematoxylin and Eosinstain (H&E) stain (×400). Testicular structures are normal after treatment with 200mg/kg crude powder and ethanolic extract of *Citrullus lanatus* seeds

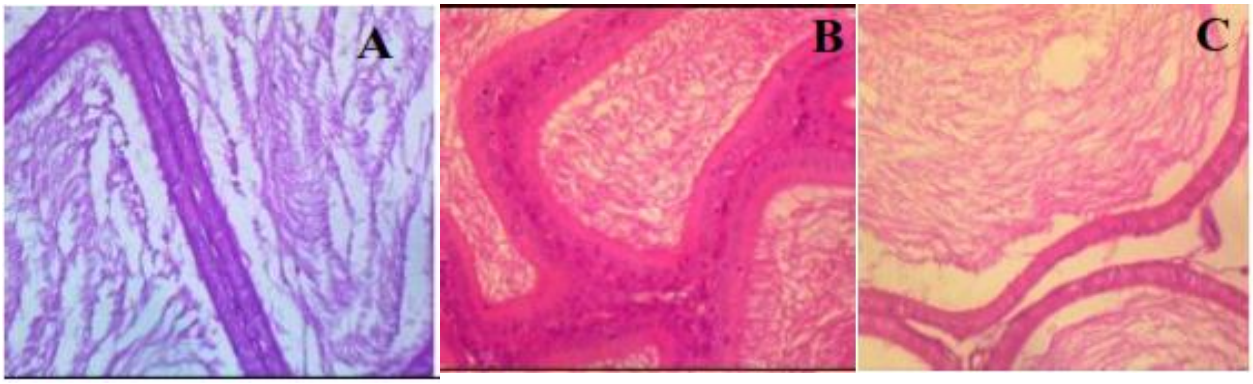

PLATE II: Photomicrograph showing the histology of the epididymides across the 3 groups typical for the four weeks post cessation of treatment using Haematoxylin and Eosinstain (H&E) stain (×400). Epididymal structures are normal after treatment with 200mg/kg crude powder and ethanolic extract of *Citrullus lanatus* seeds

significant difference ($p>0.05$) between groups B and C (Figure 3).

Histology of testes and epididymides in the control group revealed regularly and variably sized seminiferous tubules with a few having reduced germinal epithelium height and the epididymis containing moderate numbers of spermatozoa with the epithelium of a few showing mild cloudy degeneration of a few epithelial cells. The histology of the testes of the treatment groups (Plate I), showed numerous closely packed seminiferous tubules with normal architecture containing spermatogenic cells during the four weeks of monitoring. Moderate to marked numbers of sperms were present in the epididymal ducts of the treatment groups. Also, there was neither disruption nor visible lesion in the epididymal epithelium during the four weeks post treatment in the two treatment groups (Plate II).

DISCUSSION

The results of the phytochemical analysis obtained from this study appear to be in agreement with Gwana *et al.* (2014) and Rahman *et al.* (2013) who previously analysed the dry powder and the ethanolic extract of *Citrullus lanatus* seeds, respectively.

The haemogram from this study differs from that obtained by Wannang *et al.* (2007) who reported that *Cucumis metuliferus* which is also in the family *Cucurbitaceae*, produced dose-dependent alterations in the haematological indices evaluated in rats dosed with the powdered fruits at 500 and

1000mg/kg for 28 days. Though *Cucumis metuliferus* and *Citrullus lanatus* belong to the same *Cucurbitaceae* family and the extracts were both administered for the same number of days, the differences in the haematological indices may be attributed to the difference in the doses administered. Since there were no significant increases in any of the blood indices in the current study, *Citrullus lanatus* seeds extracts may have no deleterious effects on haematological parameters of albino rats if administered at 200mg/kg.

Results of the mean percentage sperm livability and morphological abnormalities evaluations by this study are consistent with reports from Kolawole *et al.* (2014). The reduced morphological defects reported for the treatment groups imply that *Citrullus lanatus* seeds have protective property over spermatozoa. Also, sperm liveability results suggest that the seeds had no deleterious effect on sperm cells. The initial reduction in mean percentage sperm motility in the treated groups during the first 2 weeks post cessation of extract administration and subsequent progressive increases from the third week may be an indication of reduced fertility upon prolonged ingestion of the plant's seed. The saponin content of the seed may be responsible for this. Saponin has been reported to reduce sperm motility significantly (Gupta *et al.*, 2005). However, during this two weeks of percent motility reductions, the values obtained from this study were still within World Health Organization (WHO) reference value for human semen forward motility which is ≥60% (Cooper *et al.*, 2010) and the ones recommended by Rouge, (2003) for the bulls (greater than 30% progressively motile sperm) stallions (greater than 60%) and dogs (greater than 70%). The results of sperm motility from this study disagree with that of Kolawole *et al.* (2014) who utilized methanolic extract of *Citrullus lanatus* rind; the solvent of extraction and the plant part being possible causes of the difference

Histology findings are in agreement with Kolawole *et al.* (2014) in which the methanolic extract of the rind of *Citrullus lanatus* administered at 200mg/kg to male albino rats enhanced normal testicular architecture with presence of several normal spermatocytes.

In conclusion, the findings from this study revealed that the powdered and ethanolic extract of *Citrullus lanatus* seeds administered at 200mg/kg have no negative effects on haematology, the architecture of the testes and epididymis and the percentage sperm liveabilty. It also significantly reduced sperm morphological abnormalities. However lengthy duration of exposure could have deleterious effect on sperm motility at the same dosage. Thus one could recommend the seed for inclusion in livestock ration served for a period of just 4 weeks.

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