Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 2, 2023, pp. 1313-1324.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

BULL. PHARM. SCI. Assiut Univ.

EFFECT OF COLOCASIA ESCULENTA (L.) SCHOTT (ARACEAE) ETHANOL LEAF EXTRACT ON SPERM CONCENTRATION IN MALE WISTAR RATS

Temitayo O. Ajayi*[1], Gideon O Alade[2], Kemelayefa O. James[3] and Kola' K. Ajibesin[2]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria*
[2]*Department of Pharmacognosy & Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria*
[3]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria*

Colocasia esculenta (L.) Schott (Araceae) is a staple food which supply needed nutrients in Africa and is therefore, employed in ethnomedicine. This study aimed to evaluate the effect of the leaf ethanol extract as well as its partitioned fractions (ethyl acetate, butanol and aqueous) on some male reproductive parameters such as sperm count, motility and morphology as well as the histology of testes in male Wistar rats after 15 days of administration of the extracts/fractions. The ethanol extract showed significant increases of 66 and 213% in sperm concentration at 500 and 1000 mg/kg respectively. This activity was largely retained in the ethylacetate with 200, 213 and 300% in sperm concentration at 50, 100 and 200 mg/kg, respectively and aqueous fractions with 274 and 657% increase at 50 and 100 mg/kg, respectively. Motility and morphology were not significantly altered. The histology of the tests revealed that spermatozoa cells were the positively affected cells.

Keywords: *Histology, motility, morphology, fractions*

INTRODUCTION

Colocasia esculenta (L) Schott of the family Araceae is an herbaceous annual plant which is extensively cultivated in Southeast Asia and commonly known as Arbi, Arvi and Eddoe. The corms and leaves of *C. esculenta* are ethnomedicinally employed for liver diseases[1]. The juice from the leaf is also useful in scorpion sting, snake bite and food poisoning of plant origin. This plant is also important in Ayurveda identified ailments such as constipation, alopecia, stomatitis, haemorrhoids and general body weakness[1].The leaf juice has rubefacient, stimulant and styptic property and is useful in internal haemorrhages, adenitis, otalgia, asthma, arthritis, diarrhoea, internal hemorrhage, neurological and skin disorders [2]. The corm juice has demulcent, laxative and anodyne and contraceptive property in female[3], it is also employed to treat stomach swelling, general body ache, baldness and fever[1-2]. The plant (all the parts including the leaf) is a staple food throughout Africa due to its notable dietary benefits; the leaves are vital sources of protein, dietary fibre, ascorbic acid and some nutritionally important minerals. Among the chemical constituents of this plant are cyanidin- 3- rhamnoside, cyanidin-3-glucoside anthocyaninsperlargonidin 3-glucoside. and pelargonidin-3-glucoside. Others include Apigenin, luteolin, anthocyanins[4] 14α-methyl-5α-cholesta-9, 24-diene-3b, 7α-diol, 14α-methyl-24-methylene-5α-cholesta-9, 24-diene-3α, 7 α-diol, b-sitosterol, stigmasterol, nonacosane, tetracos-20-en-1, 18-diol; 25-methyl triacont-10-one; octacos-10-en-1, 12-diol; pentatriacont-1, 7-dien-12-ol and 25-methyl-tritriacont-2-en-1, 9, 11-triol[5]. Biological activities such as antimicrobial[6], antifungal[7], diabetic[8], anti-inflammatory[9], antihelminthic[10], antioxidant[11]

Received in 6/3/2023 & Accepted in 16/6/2023

*Corresponding author: Temitayo O. Ajayi, E-mail: to.ajayi@mail1.ui.edu.ng or tayomiajayi@gmail.com

and hypolipidaemic[12], antihelminthic[13] have been reported for the extracts of the plants. Many plants such as which had medicinal effects on female reproductive parameters have also been reported to show pro or anti effect in the male[14-17]. The leaf of *Bambusa vulgaris* L. (Poaceae)[18], *Sacoglottis gabonensis* (Bail.) Urb. (Humiriaceae) stem bark[19], *Senna alata* (L.) Roxb. Leguminosae leaf [20] and *Peperomia pellucida* (L.) Kunth, (Piperaceae) leaf[20] have been reported to reduce sperm concentrations while plants like *Acanthus montanus* (Nees) T. Anderson (Acanthaceae), *Commelina diffusa* Burm.f. (Commelinaceae) and *Alchornea cordata* Benth. (Euphorbiaceae) showed sperm count boosting effect [21]. While estrogen like activity has been reported in the female[22], the effect on the male has not been carried out and this has necessitated this study.

METHODS

Collection and Identification of plant material

Colocasia esculenta was identified and authenticated by Dr. A.T. Oladele of the Department of Forestry and Wildlife Management, University of Port Harcourt, Nigeria. Voucher specimen number NDUP235 was deposited at the herbarium of the Department of Pharmacognosy and herbal medicine, Niger Delta University, Bayelsa, Nigeria. The leaves of *C. esculenta* were collected from Amassoma, Bayelsa State; latitude 4°58'13" N and longitude 6°6'32" E in April, 2021..

Drying and extraction and partitioning of *C. esculenta* leaf

The leaves were cut into small bits and dried in an oven at a temperature of 40°C. They were the pulverized and stored in air-tight container for until further studies. Exactly 600 g of powdered plant material was macerated with 50 % ethanol for 72 h with occasional agitation in the first six hours. It was thereafter decanted, filtered and concentrated *in vacuo* at 30°C. The marc was re extracted with fresh 50 % ethanol, repeating the previous process. The two extracts were mixed to obtain a yield of 112 g (18.6 %w/w). Distilled water was used to prepare the different concentrations of the extracts to be administered. The ethanol leaf extract of *C. esculenta* (100 g) was suspended uniformly in distilled water and was partitioned into ethyl acetate (500 mL x 3), butanol (500 mL x 3) to obtain 16 g ethylacetate, 16 g of butanol and 68 g aqueous fractions. These were separately concentrated *in vacuo* and kept in the desiccator for biological assay.

MATERIALS AND METHODS

Animals

Male Wistar rats were purchased from the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State. They were kept in a chamber at 25±3°C and 55±5 % humidity for 12 h light/ dark illumination schedule. They were fed with standard diet feed for rodent. All animals were allowed free access to food and water. All the experiments were performed between 9.00 am and 12.00 noon daily as adapted from Baumans (2004)[23] study record. Ethical approval to carry out this study was sought and obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria ethical committee.

Determination of LD_{50}

The Lorke's method (1983)[24] was used for the determination of the oral median lethal dose (LD_{50}). All animals were fasted overnight prior to the experiment but had free access to water until an hour before the experiment. Feeding resumed two hours after commencement of the experiment. The experiment was divided into two phases. In the first phase, nine rats were used with doses of 10, 100 and 1000 mg/kg of extract administered orally. They were observed for signs of acute toxicity and death for 24 h. In the second phase, the procedure was repeated using three rats allotted into three groups of a rat each. Doses (1600, 2900 and 5000 mg/kg) were administered. They were likewise observed for signs of acute toxicity and mortality for 24 h.

Reproductive effect *C. esculenta* leaf ethanol extract

A total of 24 male Wistar rats were employed for the study. They were divided at random into 4 groups of 6 animals each. Animals in groups A - C were administered orally with 250, 500 and 1000 mg/kg body

weight of *C. esculenta* leaf extract while group D serving as negative control distilled water which was the vehicle used for the dissolution of extracts. The administration of the extract was carried out daily for 15 days. At the end of the treatment, animals were fasted overnight and thereafter anaesthetized using chloroform, they were sacrificed; and testes and the epididymis were separately excised and used for sperm count and histopathological evaluation.

Epididymal sperm count

From each epididymis, the caudal part was removed and placed in a beaker containing 1ml physiological saline solution. The section was rapidly macerated with 5 mL saline solution for about five to ten minutes for spermatozoa to be released into the saline solution. Determination of sperm count, motility and morphology was carried out using standard method[19]. A few drops of semen were dropped onto the microscope slide and viewed under the microscope using x10 objective to evaluate sperm morphology and motility. Total sperm count was done with a 1 in 20 dilution with seminar fluid by employing improved Neubauer haemocytometer[19]. Data were referred as $x10^6$ sperm per epididymis. The testes were preserved in 10.0% forma-saline for histological evaluation.

Routine histological preparation

The histology of the testes was done by the method described by Alade *et al.,* 2022[25]. The organ was cut in slaps of about 0.5 cm thick transversely and fixed in 10.0% buffered formalin for a day after which it was transferred to 70.0% alcohol for dehydration. The tissues were passed through 90.0% and absolute alcohol and xylene for different durations before they were transferred into two changes of molten paraffin wax for 1hr each in an oven at 65°C for infiltration. They were thereafter immersed and serial tissue sections trimmed with the aid of a rotary microtome at 6 microns after which they were fixed into albumerized slide. They were then given time to get dried using a hot plate for 2 minutes. The slides were dewaxed using xylene after which they were passed through absolute alcohol (2 changes), 70 and 50.0% alcohol and finally water for 5 minutes. The photomicrographs were taken using x 100 objectives.

Data analysis

Data were presented in mean, standard error of mean and graphically using graph pad prism 8.3 comparison of data were done with post hoc test (Tukey).

RESULTS AND DISCUSSION

Generally, there was no sedation, immobility, salivation, seizure, death among other toxicity signs recorded at 5 g/kg in the acute toxicity study. It therefore showed that the lethal dose is greater than 5 g/kg **(Table 1)**. The ethanol leaf extract showed a dose dependent increase in sperm count which was only significant at 500 mg/kg ($P< 0.02$) and 1000 mg/kg ($P< 0.001$) with 66 and 213% increase, respectively **(Fig. 1)**. Sperm motility of the ethanol extract also followed the same pattern, which is dose dependent **(Fig. 2)**. The sperm morphology was similar to those of the control **(Fig. 3)**. The partitioned fractions showed that the butanol fraction is devoid of activity while activity was retained in the ethyl acetate with significant increases of 200, 213 and 300% ($P< 0.002$) at 50, 100 and 200 mg/kg dose. The aqueous fraction had significant sperm count significant increases ($P< 0.001$) of 274 and 657% at 50 and 100 mg/kg dose, respectively while the 200 mg/kg dose was devoid of activity **(Fig. 4)** and the histology of the testes showed a distorted structural architecture. This suggests that ethylacetate presents a better choice compared to the aqueous fraction. The motility and morphology were not affected in these active fractions **(Fig.s 5 & 6)**. The histology of the testes also corroborated these findings **(Tables 2 & 3 and plates 1-4)**. The main target cells are the spermatozoa as shown in the histology. It therefore showed that the ethanol extract of *C. esculenta* only has sperm boosting activity especially by increasing the spermatozoa and does not affect the motility and morphology. Comprehensive studies have been carried out on flavonoids in the management of reproductive system dysfunction in male especially in the area of testicular architecture and low sperm quality[26]. Luteolin and apigenin which are flavonoids that have been isolated from this plant may therefore be responsible for the sperm boosting activity.

Table 1: General appearance of rats administered orally with *Colocasia esculenta* ethanol leaf extract in the acute toxicity study.

mg/kg	**10**	**100**	**1000**	**1600**	**2900**	**5000**
Sedation	None	None	None	None	One	One
Immobility	None	None	None	None	One	One
Faeces	Dry	Dry	Dry	Wet	Dry	Wet
Skin	Intact	Intact	Intact	Intact	Intact	Intact
Hair form	Intact	Intact	Intact	Intact	Intact	Intact
Death	None	None	None	None	None	None

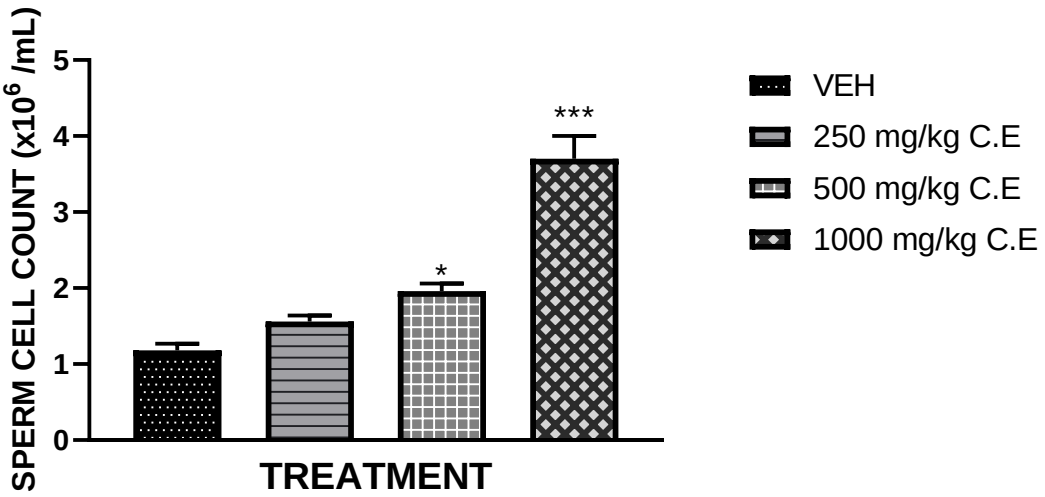

Fig. 1: Effect of *Colocasia esculenta* leaf ethanol extract on sperm concentration of male Wistar rats. Data showed statistically significant increase in the sperm cell at higher dose *P<0.025, ***P<0.001.

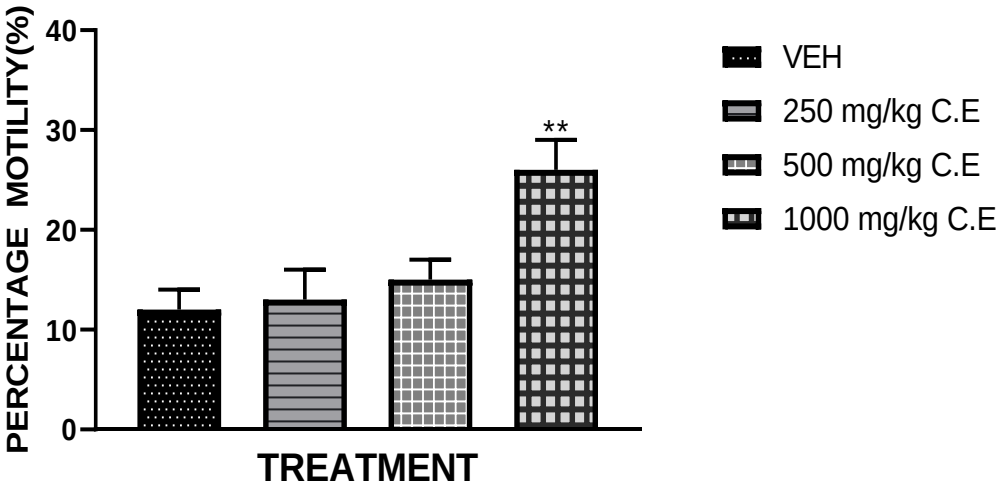

Fig. 2: Effect of *Colocasia esculenta* leaf ethanol extract on sperm motility of male Wistar rats. Data showed statistically significant increase in motility of the sperm cells at higher dose **P<0.005.

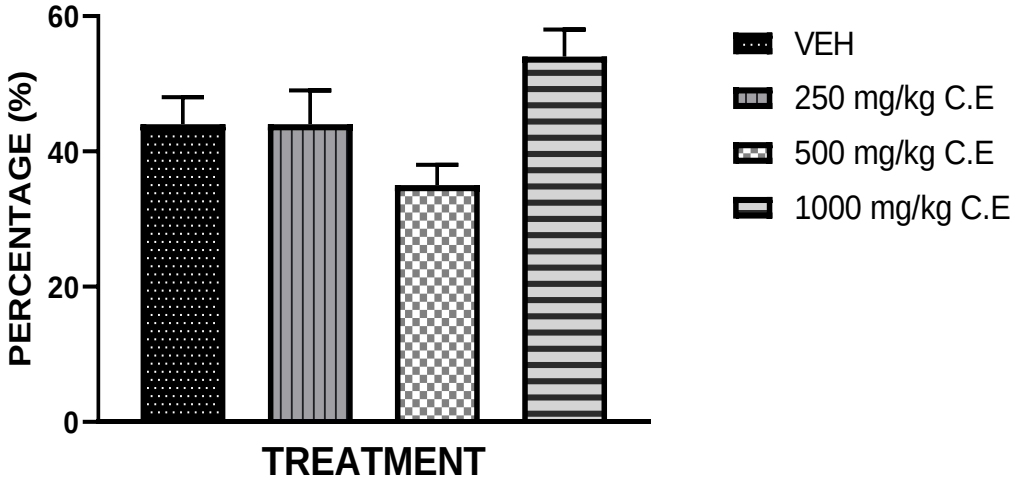

Fig. 3: Effect of *Colocasia esculenta* leaf ethanol extract on sperm morphology of male Wistar rats. Showed no statistically significant different in the morphology dose. P>0.05.

Key: Percentage normal sperm.

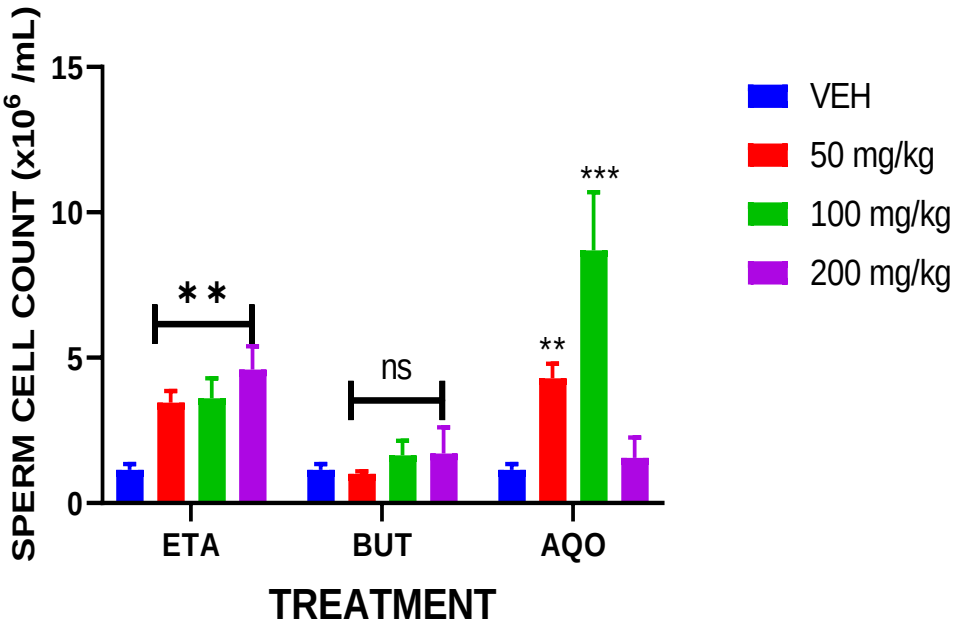

Fig. 4: Effect of *Colocasia esculenta* leaf partition fractions on sperm concentration of male Wistar rats. Data showed statistically significant increase in the sperm cell in the ethyl acetate and the aqueous fractions. **P<0.002, ***P<0.001,ns P>0.05 compared with the control group.

Key: ETA- ethylacetate fraction, BUT- butanol fraction, AQO- Aqueous fraction.

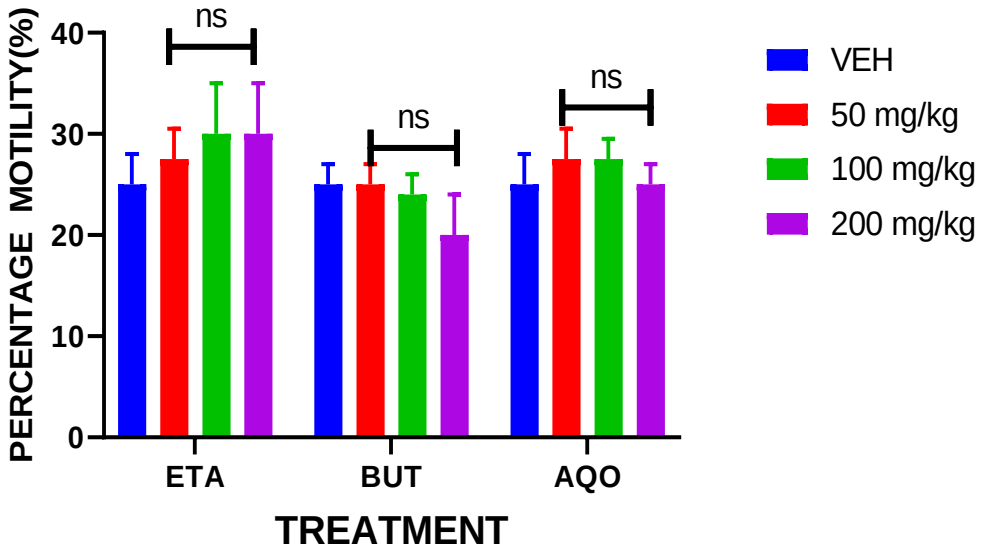

Fig. 5: Effect of *Colocasia esculenta* leaf partition fractions on sperm motility of male Wistar rats. Data showed no statistically significant (P>0.05).

Key: ETA- ethylacetate fraction, BUT- butanol fraction, AQO- Aqueous fraction.

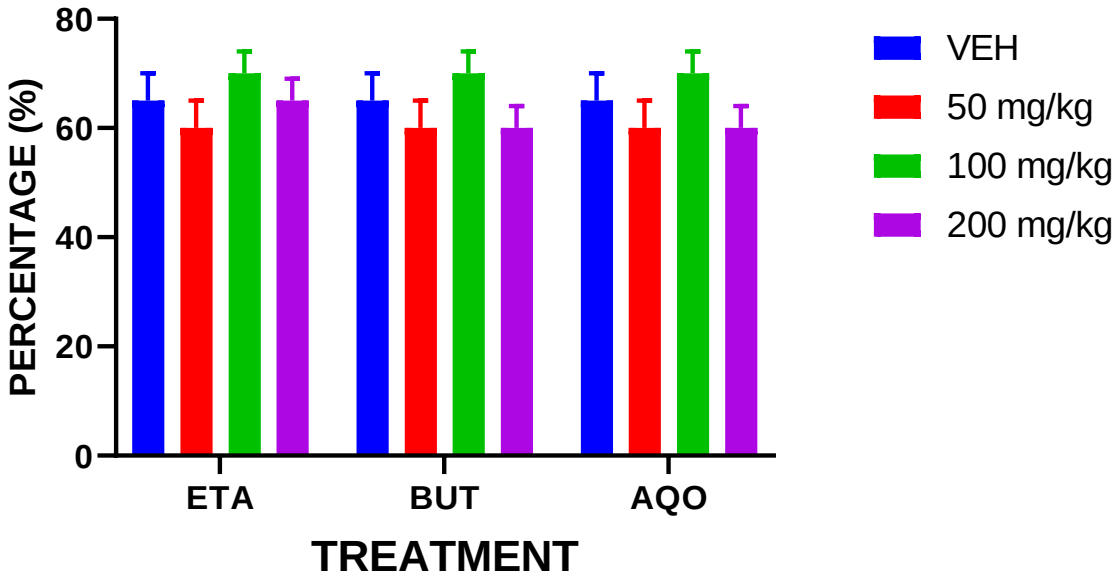

Fig. 3: Effect of *Colocasia esculenta* leaf partition fractions on sperm morphology of male Wistar rats. Data showed no statistically significant (P>0.05).

Key: ETA- ethylacetate fraction, BUT- butanol fraction, AQO- Aqueous fraction.

Table 2: Description of the histology of the testes of male Wistar rats administered with *Colocasia esculenta* leaf ethanol extract.

Dose	**250**	**500**	**1000**
	Histological normal testes similar to control	Histological normal testes similar to control, interstitial cells contain leydig cells with seminiferous tubules containing spermatogonia, spermatocyte and spermatozoa	Histological normal testes similar to control but with increased concentration of spermatozoa
	Histological normal testes similar to control with increased concentration of spermatozoa	Histological normal testes similar to control	Histological normal testes similar to control, interstitial cells contain leydig cells with seminiferous tubules containing spermatogonia, spermatocyte and spermatozoa but with increased number in spermatozoa concentration
	Histological normal testes similar to control with increased concentration of spermatozoa compared to control, 50 and 100 mg/kg	Histological normal testes similar to control with increased concentration of spermatozoa compared to control, 50 and 100 mg/kg	Histological distorted testes similar to control, interstitial cells contain leydig cells with seminiferous tubules containing distorted spermatogonia, spermatocyte and spermatozoa,

Table 3: Description of the histology of the testes of male Wistar rats administered with *Colocasia esculenta* leaf partitioned fractions .

fraction	**Ethylacetate**	**Butanol**	**Aqueous**
50mg/kg	Histological normal testes similar to control	Histological normal testes similar to control, intetstitial cells contain leydig cells with seminiferous tubules containing spermatogonia, spermatocyte and spermatozoa	Histological normal testes similar to control but with increased concentration of spermatozoa
100mg/kg	Histological normal testes similar to control with increased concentration of spermatozoa	Histological normal testes similar to control	Histological normal testes similar to control, interstitial cells contain leydig cells with seminiferous tubules containing spermatogonia, spermatocyte and spermatozoa but with increased number in spermatozoa concentration
200mg/kg	Histological normal testes similar to control with increased concentration of spermatozoa compared to control, 50 and 100 mg/kg	Histological normal testes similar to control with increased concentration of spermatozoa compared to control, 50 and 100 mg/kg	Histological distorted testes similar to control, interstitial cells contain leydig cells with seminiferous tubules containing distorted spermatogonia, spermatocyte and spermatozoa,

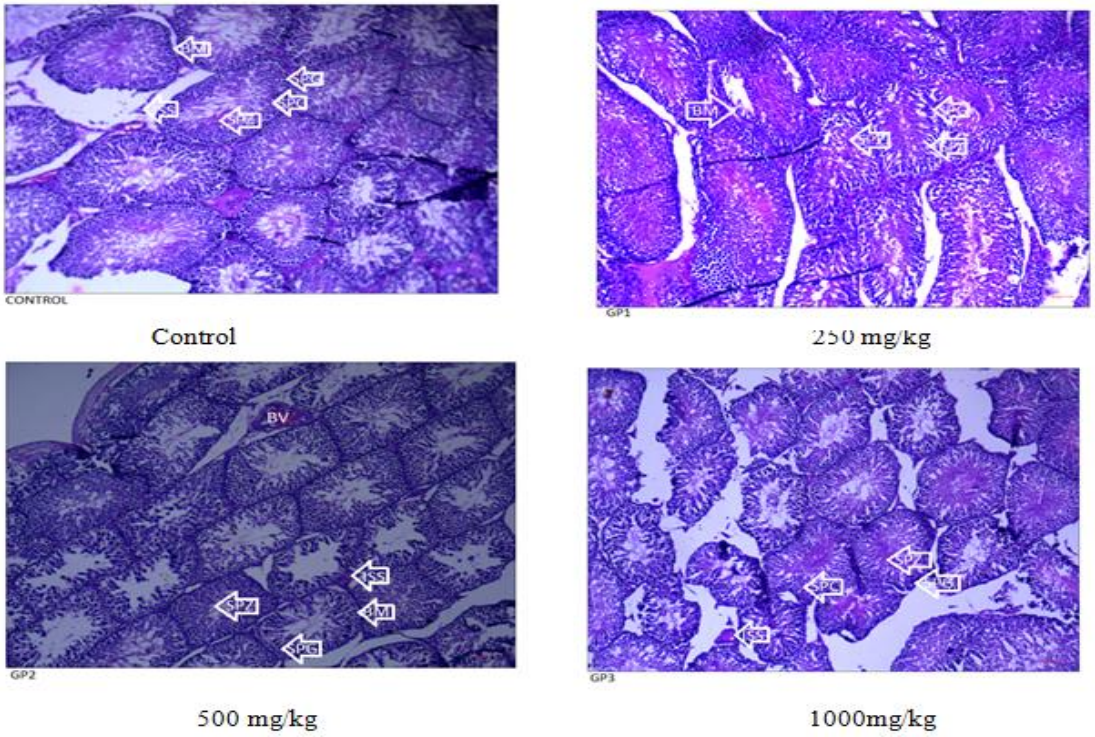

Plate 1: Histology of the testes of male Wistar rats administered with *Colocasia esculenta* leaf ethanol extract.

BM- Basement membrane, SPG-spermatogonia, SPC- spermatocytes, SPZ- spermatozoa, SC- sertoli cells, ISS- interstitial spaces.

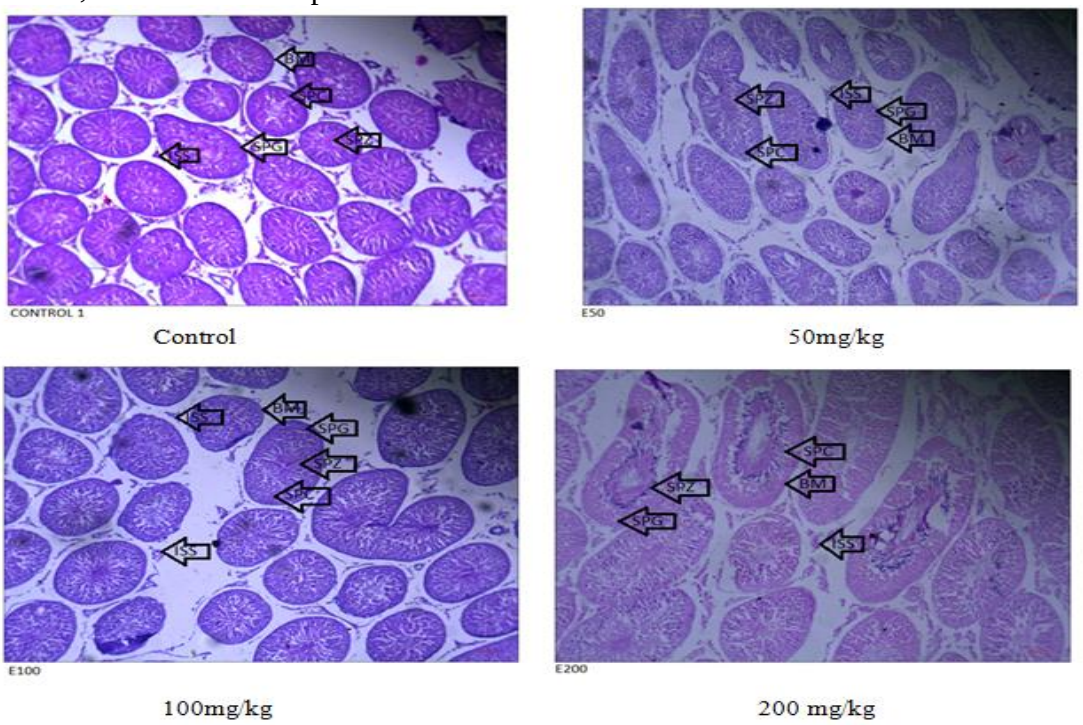

Plate 2: Histology of the testes of male Wistar rats administered with *Colocasia esculenta* leaf ethyl acetate fraction.

BM- Basement membrane, SPG-spermatogonia, SPC- spermatocytes, SPZ- spermatozoa, SC- sertoli cells, ISS- interstitial spaces.

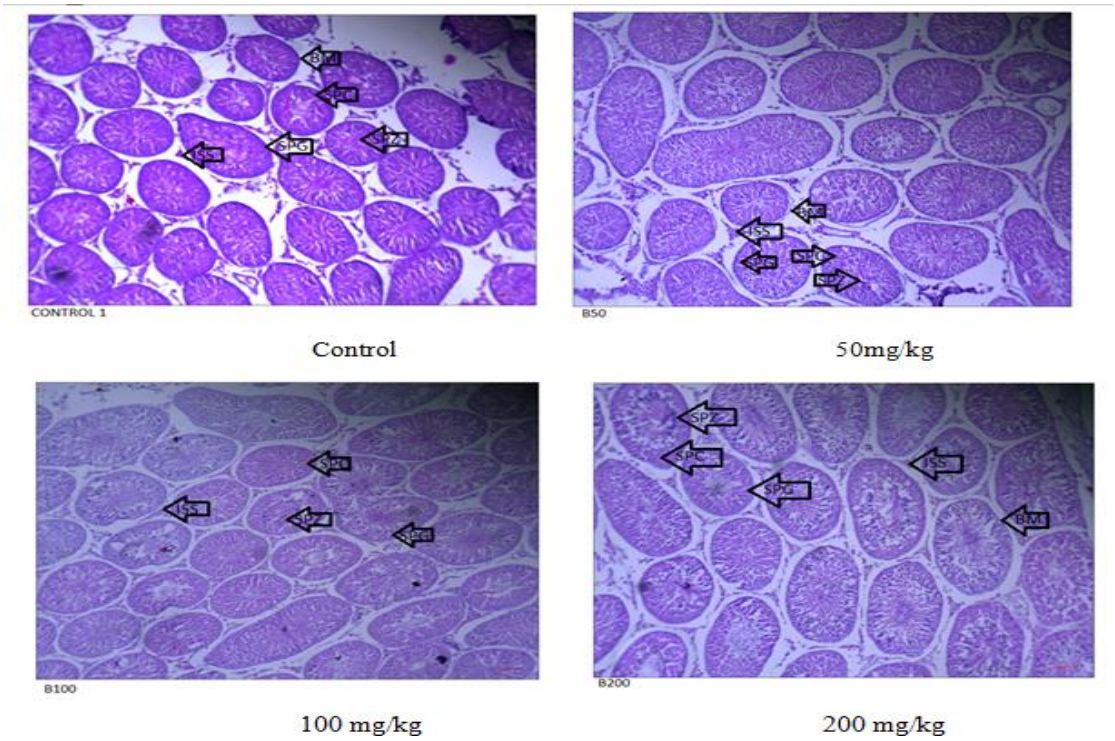

Plate 3: Histology of the testes of male Wistar rats administered with *Colocasia esculenta* leaf butanol fraction.

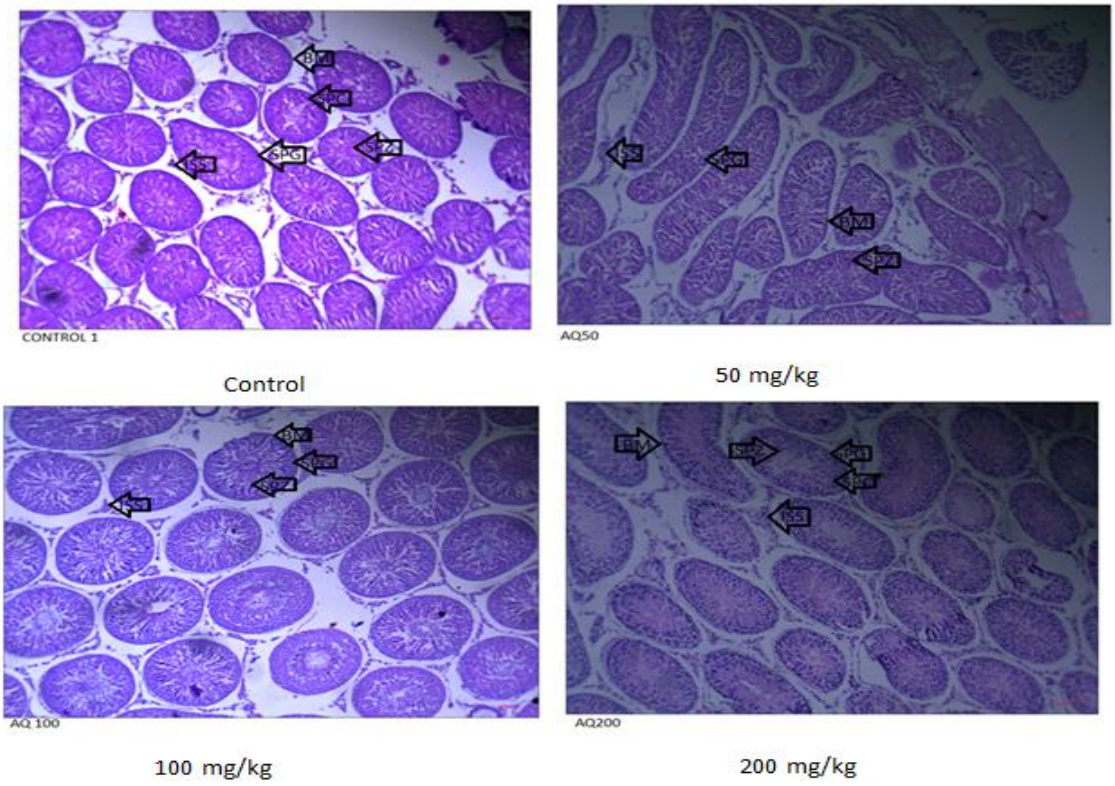

Plate 4: Histology of the testes of male Wistar rats administered with *Colocasia esculenta* leaf aqueous fraction.

Conclusion

This study showed the sperm boosting potential of the leaf of *C. esculenta* which resides mainly in the ethyl acetate fraction. A further study is hereby recommended in order to isolate the sperm cell boosting constituent(s) through bioactivity guided fractionation. On the interim, the use of *C. esculenta* leaf can be encouraged as food for men with infertility.

Acknowledgement

The authors are grateful to Dr. T.O. Alade of the Department of Medical Laboratory Science, Faculty of Basic Science, Niger Delta University for assisting in the semen evaluation.

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Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 2, 2023, pp.1313-1324.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

تأثير مستخلص أوراق الإيثانول لنبات كولوكاسيا اسكيولنتا (ال.) شوت (العائلة النخلية) على تركيز الحيوانات المنوية في ذكور فئران ويستار

تيميتايو أو أجايي[1*] – جدعون أو ألادي[2] – كيميلايفا أو جيمس[3] – كولا ك. أجيبيسين[2]

[1]قسم العقاقير ، كلية الصيدلة ، جامعة إبادان ، نيجيريا

[2]قسم العقاقير وطب الأعشاب ، كلية الصيدلة ، جامعة دلتا النيجر ، جزيرة ويلبرفورس ، نيجيريا

[3]قسم علم الادوية والسموم ، كلية الصيدلة ، جامعة دلتا النيجر، جزيرة ويلبرفورس ، نيجيريا

يعتبر نبات كولوكاسيا اسكيولنتا (ال.) شوت (العائلة النخلية) هو غذاء أساسي يوفر العناصر الغذائية اللازمة في أفريقيا، وبالتالي يستخدم في الطب العرقي. هدفت هذه الدراسة إلى تقييم تأثير مستخلص أوراق الإيثانول و الخلاصات (خلات الإيثيل والبيوتانول والمائي) على بعض المؤشرات التناسلية الذكورية مثل عدد الحيوانات المنوية وحركتها وشكلها وكذلك دراسة أنسجة الخصية في ذكور فئران ويستار بعد ١٥ يومًا من تناول المستخلصات. وقد أظهر مستخلص الإيثانول زيادة معنوية بلغت ٦٦ و ٢١٣% في تركيز الحيوانات المنوية عند تركيز ٥٠٠ و ١٠٠٠ ملجم/كجم على التوالي. تم الاحتفاظ بهذا النشاط إلى حد كبير في خلاصة خلات الإيثيل بنسبة ٢٠٠ و٢١٣ و٣٠٠% في تركيز الحيوانات المنوية عند تركيز ٥٠ و١٠٠ و٢٠٠ ملجم/كجم على التوالي وفي الخلاصة المائية بزيادة ٢٧٤ و٦٥٧% عند ٥٠ و١٠٠ ملجم/كجم على التوالي. اما الحركة والشكل فلم يتم تغييرهما بشكل كبير. وأظهرت دراسة أنسجة الخصية أن الخلايا المنوية هي الخلايا المتأثرة إيجابيا.