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Fertility Assessment of Male Wistar Rats Orally Exposed to Chloroform Stem Extract of *Portulaca Oleracea* Linn. (Purslane)– An Experimental Study

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Abstract

Portulaca oleracea Linn. has a history of extensive use as a medicinal plant and is frequently used by traditional medicine practitioners in Nigeria. Different parts of *P. oleracea* L. have been studied for their effect on the reproductive physiology of animals but there is a paucity of information on the stem as it relates to male fertility. This study, therefore, investigated the sub-chronic effect of oral administration of *P.oleracea* stem extract on male fertility indices (semen analysis, testosterone concentration, and testicular histology) in Wistar rats. Twenty animals were randomly divided into 4 groups of 5 rats each. Group A(Control) received 0.5 ml of olive oil(vehicle) while Groups B, C & D received 125, 250 & 500 mg/kg of extract respectively for 21 days by oral gavage. In the end, the animals were anesthetized and blood samples were collected for testosterone assay, caudal epididymis for semen analysis, and testes for histology. *P.oleracea* stem extract had no significant ($p>0.05$) effect on serum testosterone concentration, testicular histoarchitecture, and sperm viability, morphology & motility relative to control. However, there was a significant ($p=0.014$) reduction in the sperm cell count of rats exposed to the highest dose (500mg/kg) in relation to the control. Oral administration of *P.oleracea* stem extract as used in this study may harm male fertility; thus further study is recommended to ascertain if prolonged exposure will validate this finding.

Keywords: Testis, Testosterone, Sperm Count, Portulaca, Medicinal plants

Introduction

Reproductive physiology research in animals originated from man's desire to study factors that optimize the growth and production of animals. With time, many plants used as food were discovered to have the capacity to boost the growth, physical performance, and reproductive potential of animals. The use of extracts from plants in the study of reproductive physiology is becoming popular globally as researchers are targeted at evaluating the effects of different plant parts on reproductive physiology. According to Raji *et al.* (1), the role of medicinal plants in the induction of infertility in experimental animals and their possible development into contraceptive agents has continued to receive significant attention globally. Animal studies have shown anti-steroidogenic, anti-spermatogenic, anti-fertility, abortifacient or pro-fertility activities with some indigenous plant parts (2). The effect of different plant parts in modulating the reproductive physiology of animals has also been extensively demonstrated. Several researchers have also reported on diverse beneficial effects of plant formulations which have consequently resulted in the increasing use of plants in both traditional and orthodox medicine for the management of health-related issues including reproductive challenges (3, 4). *Portulaca oleracea* is one of these medicinal plants and is frequently used by traditional medicine practitioners in Nigeria. *Portulaca oleracea* Linn. (commonly called purslane) a member of the family Portulacaceae, is a warm climate green herb, with obovate leaves, and small yellow flowers which open individually in the middle of the leaves for some hours on sunny days, especially in the mornings, and branched succulent stems which are decumbent near the base (5).

All parts of the plant, especially the leaves and stems are useful as remedies for many ailments and they are usually used in fresh or dried state (6). In the Eastern part of Nigeria, the aerial parts of the plants are crushed to extract the juice which is taken with or without raw egg to improve fertility both in males and females (6, 7).

No doubt, the effect of different parts of *Portulaca oleracea* on reproductive physiology has been studied by some authors. However, literature is scarce on the effect of *Portulaca oleracea* stem extract on fertility. It is on this premise that this study was designed to investigate the effect of chloroform stem extract of *P.oleracea* (CSEPO) on male fertility indices (semen analysis, testosterone concentration, and testicular histology) using a Wistar rat as an animal model.

Materials and Methods

Ethics approval and consent to participate

The study protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt with the Ref. No: UPH/CEREMAD/REC/04. The rats for the study were humanely handled following the Ethics and Regulations guiding the use of research animals as approved by the University.

Study site

This study was carried out in the Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

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Plant Material and Authentication

Fresh stems of Portulaca oleracea were collected from Alakahia axis of Port Harcourt, Nigeria. The plant was identified at the University of Port Harcourt Herbarium, Department of Plant Science and Biotechnology, with Herbarium number - UPH/V/1,302.

Preparation of the stem Extract

The collected plant stems were shade-dried at room temperature to constant weight over a period of twelve weeks. The dried stems were weighed and ground to fine powder. The stem extract was obtained by cold maceration using chloroform with the following procedure: the powdered stems were soaked in chloroform (500g stem powder in 1.5L solvent) for 72 hours with fresh replacement of solvent every 24 hours. The pooled extract was filtered with the Watman's No. 1 filter paper. The filtrate was concentrated with a rotary evaporator (Model No: RE-52A) at 45°C in vacuo and later transferred to an evaporating dish and dried over a water bath (Digital thermostatic water bath, Jinotech instruments). The resulting extract was stored in a desiccator. All reagents used were of analytical grades.

Acute Toxicity Testing

The acute toxicity testing of the extract was evaluated according to the method of Lorke (8)in order to determine the LD50 and also the doses to be used in the study . Briefly, a total of eighteen wistar rats were randomly divided into six groups of three rats each. The first three groups were used for the first phase of the study while the last three groups were used for the 2nd phase. In the first phase, the three groups, each made up of three rats, were orally treated with CSEPO at the doses of 10, 100 and 1000 mg/kg and observed for 24hours. In the absence of mortality or other signs of toxicity, the last three groups, made up of three rats each, were orally treated with CSEPO at the doses of 1600, 2900 and 5000 mg/kg for the second phase and also observed for 24 hours for mortality and other signs of toxicity.

Determination of sample size

The determination of sample size was done using the 'resource equation' approach, which is based on the acceptable range of the degrees of freedom in an analysis of variance (ANOVA), thus giving a total sample size of twenty (20) animals of five per group. This approach is suitable for exploratory studies of this nature where the standard deviation and effect size are impossible to assume (9, 10).

Animals, source and handling

Twenty (20) sexually mature male wistar rats with an average weight of 190g were procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria for the study. They were acclimatized for two (2) weeks before commencing the study. The rats were housed in wooden cages (five rats per cage) under standard conditions of 28oC ambient temperature and approximately 12- hr natural light-dark cycle. They were fed ad libitum with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study.

Inclusion and Exclusion Criteria

Only apparently healthy sexually mature male wistar rats with descended testes, and within the weight of 190±10g were used for the study. Whilst cryptorchid rats were excluded from the study.

Experimental Procedure

The experimental procedures are in compliance with ARRIVE guidelines as reported by Kilkenny *et al*. (11).

Following acclimatization, the animals were assigned to four (4) groups of five (5) animals each for treatment using randomisation:

Group A (Control): 1ml/kg body weight of olive oil (vehicle).

Group B: 125 mg/kg body weight of CSEPO

Group C: 250 mg/kg body weight of CSEPO

Group D: 500 mg/kg body weight of CSEPO

The $1/40^{th}$, $1/20^{th}$ and $1/10^{th}$ of the maximum dose (5000mg/kg) used in the acute toxicity testing = 125, 250 and 500 mg/kg doses were adopted for this study.

Administration of extract and vehicle were by oral gavage daily for 21 consecutive days. Animals' weights were taken weekly and the doses adjusted accordingly.

At the end of the experiment, the animals were anaesthetized in a desiccator jar containing cotton wool ball soaked with chloroform (analytical grade). Blood samples were collected into sterile plain bottles while the epididymides were harvested and the caudal parts were used to determine the epididymal sperm count and characteristics**.** The testes were carefully resected and promptly fixed in Bouin's fluid for histology.

Testosterone assay

Sera were harvested from the collected blood samples and subjected to hormonal assay for assessment of Testosterone levels using Accu-bind ELISA kits (Testosterone Test System Product Code: 3725-300) from Monobind Inc. Lake Forest, CA 92630, USA.

Analysis of epididymal spermatozoa characteristics

Sperm morphology

One drop of the caudal epididymal sperm was applied to a clean glass slide, followed by two drops of normal saline to make a suspension, which was mixed with one drop of 0.5% eosin solution. After a few seconds, the slide was examined under light microscope at ×40 magnification. Morphological appearance of normal and abnormal spermatozoa (abnormality in the head, mid-piece, or tail) was determined and their percentages were estimated (12).

Sperm motility

One drop of the caudal epididymal sperm was applied to a clean glass slide and two drops of normal saline were added to emulsify it before it was covered with a cover-slip. The slide was examined under a light microscope at ×40 magnification. The sperm motility was assessed by counting both active progressive movements (motile) and non-progressive movements (immotile) of sperms. The motile and non-motile cells were counted in 10 random fields and the number expressed as a percentage of the total number of sperms (12).

Sperm count

The caudal epididymis was lacerated, and carefully pressed on a clean grease free glass slide in order to express the sperms. Two drops of normal saline were added to emulsify it to form a suspension. The suspension was diluted with formal saline in the ratio of 1:20. The new improved Neubauer counting chamber (Hemocytometer) was charged with a drop of the

diluted sperm. The counting chamber was mounted on the slide stage of a light microscope and viewed under the magnification of ×40. The count was expressed as million/ml of suspension (11).

Sperm viability

A smear of sperm cells from the caudal epididymis was applied to a clean glass slide, with two drops of normal saline added to it to form a suspension. The suspension was mixed with one drop of 0.5% eosin solution. After a few seconds, the slide was examined under light microscope at ×40 magnification. Eosin-stained non-viable sperm was differentiated from unstained viable sperm (12).

Histomorphological study of the tissues

The fixed testes were processed using the method of Lillie (13). The tissues were processed with time by dehydration in ascending grades of alcohol, followed by clearing in xylene. After infiltration and embedding in paraffin, they were sectioned at 4–5 µm, followed by deparaffinization in xylene, dehydration in descending grades of alcohol, and stained with Haematoxylin and Eosin blue dyes. The processed tissues were mounted on glass slides, covered with cover-slip, and then examined under a standard light microscope. The photomicrographs were captured using Olympus® CX31 digital camera.

Statistical Analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM and data were assessed by one-way ANOVA followed by the Tukey post-test. The significance level was set at $p<0.05$.

Results

The acute toxicity test did not cause mortality, morbidity or other apparent signs of toxicity in the animals at the doses used. This is a proof that the extract was not noxious at the maximum dose of 5000mg/kg hence $1/40^{th}$, $1/20^{th}$ and $1/10^{th}$ of this maximum dose (5000mg/kg) = 125, 250 and 500 mg/kg doses were used in the study.

The exposure of male wistar rats to chloroform stem extract of *Portulaca oleracea* by oral gavage for 21days at the doses of 125, 250 and 500 mg/kg produced no significant ($p>0.05$) effect on serum testosterone concentration as well as the sperm cell viability, motility and morphology in comparison with the control as shown in Figures 1-3.

Although, CSEPO had no significant ($p>0.05$) effect in the sperm cell count at the doses of 125 and 250 mg/kg, it caused a significant ($p<0.05$) decrease in the sperm cell count at the highest dose of 500mg/kg relative to the control (Figure 4).

The photomicrographs in Figures 5-7 showed the absence of obvious lesions in the histology of the testes of rats treated with 125, 250 and 500 mg/kg of CSEPO.

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at 95% confidence interval ($p > 0.05$). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 125 mg/kg CSEPO-treated rats, 250mg/kg CSEPO and 500 mg/kg CSEPO-treated rats, respectively.

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at 95% confidence interval ($p > 0.05$). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 125 mg/kg CSEPO-treated rats, 250mg/kg CSEPO and 500 mg/kg CSEPO-treated rats, respectively.

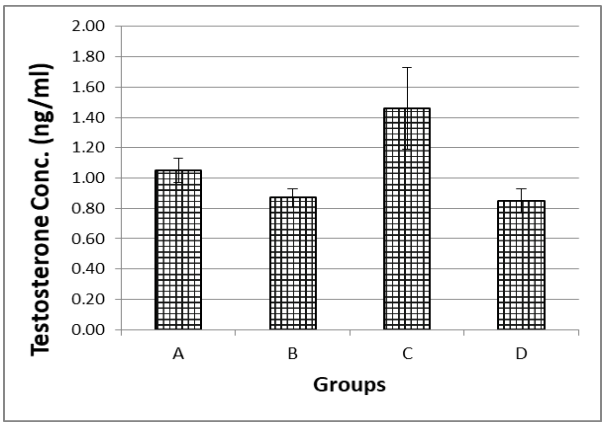

Figure 1. Effect of chloroform stem extract of P. oleracea on testosterone concentration in rats.

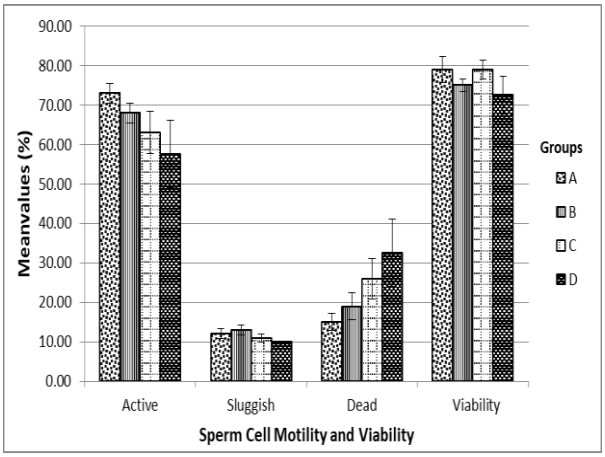

Figure 2. Effect of chloroform stem extract of P. oleracea on sperm cell motility and viability in rats.

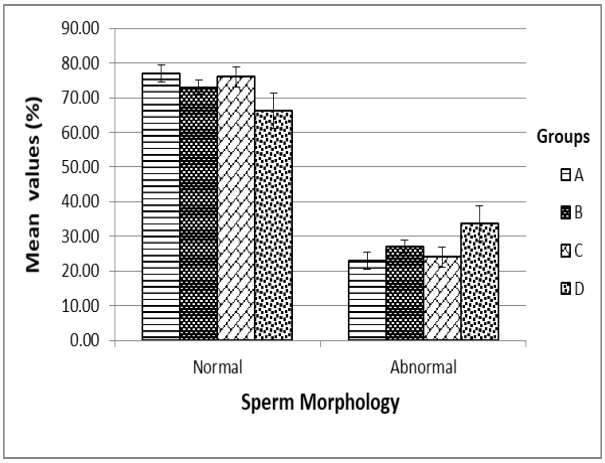

Figure 3. Effect of chloroform stem extract of P. oleracea on sperm cell morphology in rats.

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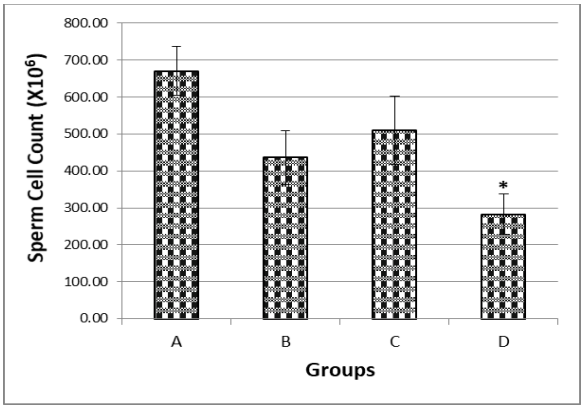

Figure 4. Effect of chloroform stem extract of P. oleracea on sperm cell count in rats.

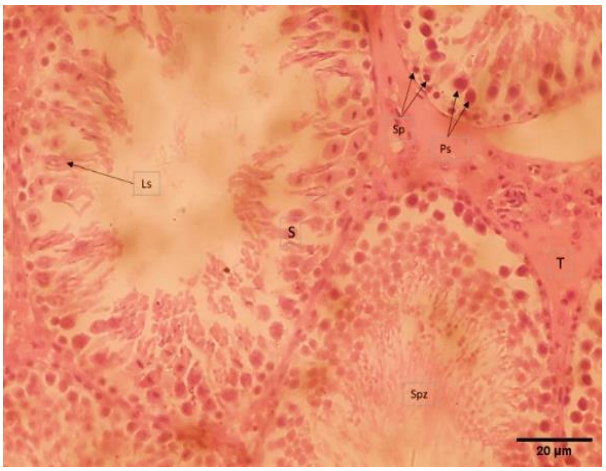

Figure 5. Histological appearance of testis of rat from group B given 125mg/kg of chloroform stem extract of P.oleracea, showing no obvious lesions. Note active seminiferous tubules (S) bearing spermatogonium (Sp), primary spermatocytes (Ps), late spermatids (Ls), Spermatozoa (Spz) and interstitium (T); Hematoxylin & Eosin (H&E) × 400.

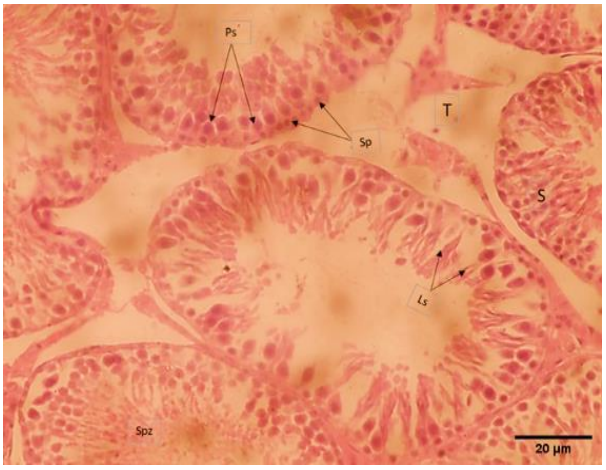

Figure 6. Histological appearance of testis of rat from group B given 250mg/kg of chloroform stem extract of *P.oleracea*, showing no obvious lesions. Note active seminiferous tubules (S) bearing spermatogonium (Sp), primary spermatocytes (Ps), late spermatids (Ls), Spermatozoa (Spz) and interstitium (T); Hematoxylin & Eosin (H&E) × 400

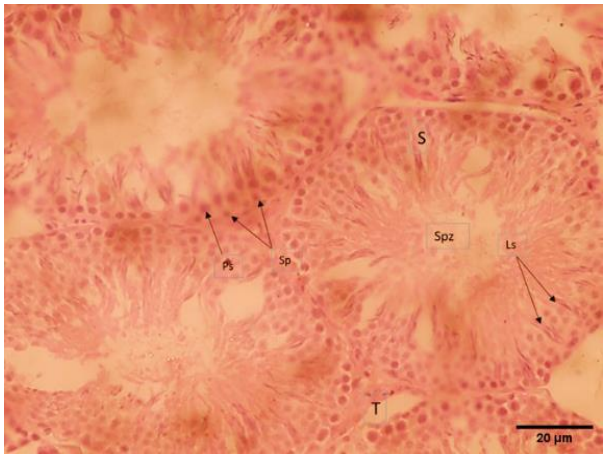

Figure 7. Histological appearance of testis of rat from group B given 500mg/kg of chloroform stem extract of P.oleracea, showing no obvious lesions. Note active seminiferous tubules (S) bearing spermatogonium (Sp), primary spermatocytes (Ps), late spermatids (Ls), Spermatozoa (Spz) and interstitium (T); Hematoxylin & Eosin (H&E) × 400.

Discussion

From this study which was carried out solely on the stem of *Portulaca oleracea*, the result showed that *Portulaca oleracea* stem extract administered to male wistar rats for 21 days had no significant effect on serum testosterone concentration and sperm cell characteristics – the viability, morphology and motility, at all the doses used in the study. Only the sperm cell count was decreased at the highest dose of 500mg/kg. The histoarchitecture of the testes was not distorted by the extract. This significant decrease in sperm cell count suggests that *P.oleracea* stem extract has the potential to reduce the male fertility. Sperm count and characteristics are known as major markers of male fertility since they are indicators of testicular spermatogenesis and epididymal sperm maturation (7).

In earlier study on the leaves of *Portulaca oleracea*, it was found that oral ingestion of chloroform leaf extract markedly increased the sperm count of male wistar rats on days 14 and 28, only at the dose of 250mg/kg with no significant effect on serum testosterone level and testicular histomorphology in all the treated rats for the duration of 60 days (7).

From these findings, the stem extract of *P.oleracea* decreased the sperm count at a higher dose of 500mg/kg while the leaf extract increased the sperm count at a lower dose of 250mg/kg. This disparity may be associated with the phytochemicals in both plant parts which may differ in terms of the quantity and presence. Phytochemicals are naturally occurring bioactive constituents present in plants and plant parts (14,15) hence the therapeutic effect of the plant part is dependent on the individual phytochemicals which produce definite actions in a biological system. In line with this finding, researchers have established that in *Portulaca oleracea* plant, the phytochemical concentrations vary according to the plant parts (16, 17).

Our result of a non-significant effect on the serum testosterone concentration and the testicular histoarchitecture as well as a significant decline in sperm count is consistent with the recent findings by Okafor., et al. (18) who treated male wistar rats with methanol extract of aerial parts (stem and leaves) of *P. oleracea* for 14 days at the doses of 400 and 800mg/kg.

On the other hand, the marked reduction in sperm cell count reported in this study which suggests that *P. oleracea* stem extract may have a deleterious effect on male fertility agrees with the study by Nayaka, *et al* (19) which reported that chloroform extract of aerial parts of *Portulaca oleracea* has anti-fertility effect on ovulation in female albino rats by reducing the number of ova in ovary following a 14-day treatment at the doses of 250 and 500 mg/kg via intra-gastric tube.

A number of activities of stem extract of *P. oleracea* are yet to be discovered with regards to male fertility. Possibly, a chronic study considering the testicular hormonal level, testicular and epididymal morphometry, seminiferous tubule luminal diameter and seminiferous epithelial height, in addition to the parameters evaluated in this study may suffice in that regards. The major limitation to this study is the fact that the baseline values for the sperm cell count for the rats was not taken since the caudal epididymis (site of sperm cell collection) cannot be assessed in a live rat except via surgery, this would have given a better insight on the influence of the extract on spermatogenesis. Secondly, the male fertility assessed in this study was centered on serum testosterone level, the caudal epididymal sperm count as well as sperm cell characteristics, and testicular histomorphology; these could also be limiting.

Generally, this study which evaluated the fertility of male rats administered with *Portulaca oleracea* stem extract, will serve as a useful literature on the effect of *P. oleracea* stem on male fertility indices since the fertility assessments of different parts (leaves, leaves + stem and aerial parts) of *P. oleracea* have been reported by several authors in earlier studies (19-22).

Conclusion

Oral ingestion of Portulaca oleracea stem extract as used in this study may be injurious to male fertility. In this era of unguided and constant resort to medicinal plants which has led to abuse of crude herbal formulations, it is advised that Portulaca oleracea stem should be taken with caution by human subjects sequel to its antifertility potential as demonstrated in this study. Further study, for a longer duration of exposure, is recommended to validate this finding.

List of abbreviations

CSEPO – chloroform stem extract of Portulaca oleracea, SEM – standard error of mean, ANOVA – analysis of variance, ELISA – enzyme linked immunosorbent assay.

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Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare that there is no conflict of interest.

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No funding was received for this study.

Authors' contributions

VCO designed the experiment, did the work and wrote the manuscript. GOA managed the analyses and supervised the work. All authors read and approved the final manuscript.

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