

Research Article



Recovery of Reproductive Functions in Male Rats Treated with Crude Extracts of *Portulaca oleracea*

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Accepted on: 10-04-2016; Finalized on: 30-04-2016.

ABSTRACT

Portulaca oleracea is a fleshy annual herb which is distributed throughout the temperate and tropical areas of the world. Its crude extracts have been reported to have deleterious effects on reproductive parameters in male rats. This study investigates the effect of 50 days recovery period on reproductive parameters in male rats treated with Aqueous and Methanol Extracts of *Portulaca oleracea* (AEPO, MEPO). Thirty-five male rats (150 – 200 g) were divided into control (distilled water), AEPO (25, 50, 75 mg/kg) and MEPO (25, 50, 75 mg/kg) treated groups (5 per group). The animals were orally treated on daily basis for fifty days and allowed a recovery (withdrawal) period of 50 days after which hormonal assay, andrological and histopathological studies were carried out. There were no significant ($p > 0.05$) changes in sperm count at all the treatment doses relative to the control after 50 days recovery period from 50 days pre-treatment with AEPO and MEPO. It can therefore be concluded that the deleterious effects induced by *Portulaca oleracea* extracts on the reproductive parameters in male rats were probably reversible after a recovery period of 50 days.

Keywords: *Portulaca oleracea*, Recovery, Testosterone, Sperm count, Rats.

INTRODUCTION

Portulaca oleracea belongs to the family of Portulacaceae. It is commonly called Purslane in English language, “Babbajibi” in Hausa language and “Esan omode” or “Papasan” in Yoruba language. It is a fleshy annual herb, much-branched and attaining 30 cm long¹.

It is used medicinally in Ghana for heart-palpitations². The plant is used as a diuretic in Nigeria³. A tisane of the plant is drunk in Trinidad as a vermifuge⁴. At some areas near Benin City (Nigeria), the plant, along with other ingredients is taken as an aid to the development of the fetus⁵.

It has been reported that aqueous and methanol extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations⁶. It has also been reported that aqueous and methanol extracts of *Portulaca oleracea* have some toxic and beneficial effects on the hematological functions and blood chemistry of rats⁷.

Since this plant extracts have been reported to have deleterious effects on the reproductive parameters in male rats⁸, this study therefore aims to investigate the effect of recovery period on the reproductive parameters in male rats treated with aqueous and methanol extracts of *Portulaca oleracea*.

MATERIALS AND METHODS

Experimental Animals

Adult male rats weighing between 150 g–200 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola University were used. They were housed under standard laboratory conditions and had free access to feed and water. They were acclimatized for two weeks to laboratory conditions before the commencement of the experiments.

All experiments were carried out in compliance with the recommendations of Helsinki's declaration on guiding principles on care and use of animals.

Plant Material

Fresh specimens of *Portulaca oleracea* were collected from the Botanical Garden of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, where it was identified and assigned a voucher specimen number FHI 108334.

Preparation of the Extracts

Large quantity (2 kg) of fresh specimens of *Portulaca oleracea* were washed free of soil and debris, and the roots were separated from the leaves and stems. The leaves and stems were air-dried for six weeks and then pulverized using laboratory mortar and pestle and was later divided into two samples A and B.



(i) Aqueous Extract of *Portulaca oleracea* (AEPO)

Weighted portion (400.0 g) of sample A were macerated and extracted with distilled water (1:2 wt/vol) for 72 hours at room temperature (26–28 °C).

The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The distilled water was later evaporated using - steam bath to give a percentage yield of 11.8 % of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

(ii) Methanol Extract of *Portulaca oleracea* (MEPO)

Weighted portion (350.0 g) of sample B were macerated and extracted with 70 % methanol (1:2 wt/vol) for 72 hours at room temperature (26 – 28 °C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm).

The 70 % methanol was later evaporated using steam-bath to give a percentage yield of 10.2 % of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

Ten grams of AEPO and MEPO were dissolved in 100 ml of distilled water to give a concentration of 0.1 g/ml.

Animal Grouping and Extracts Administration

Thirty-five male rats weighing between 150 g - 200 g were randomly divided into seven groups, with each group consisting of five animals.

The seven groups of rats were subjected to the following oral treatments once a day for fifty days and were allowed a recovery period of fifty days and the dosages of AEPO and MEPO administered in these studies were in accordance with those reported by⁹:

Group I received 25 mg/kg of AEPO

Group II received 50 mg/kg of AEPO

Group III received 75 mg/kg of AEPO

Group IV received 25 mg/kg of MEPO

Group V received 50 mg/kg of MEPO

Group VI received 75 mg/kg of MEPO

Group VII received 0.5 ml of distilled water as the control group.

Collection of Blood Samples

Twenty four hours (day 51) after the last day of the recovery period, blood samples were collected from all the animals through the medial cantus for the determination of plasma testosterone level.

All the animals were later sacrificed by overdose of diethyl ether and the testes were removed along with the epididymides for semen analysis.

Hormonal Assay

Plasma samples were assayed for testosterone using the enzyme-linked immunosorbent assay technique using the Fortress kit.

Semen Collection

The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen.

Semen Analysis***Progressive sperm motility***

This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27 °C) and two drops of warm 2.9 % sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using x 400 magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labeled as motile, sluggish, or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100)¹⁰.

Sperm viability (Life/dead ratio)

This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope using x 400 magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated¹¹.

Sperm morphology

This was done by adding two drops of warm Walls and Ewas stain (Eosin/Nigrosin stain can also be used) to the semen on a pre-warmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using x 400 magnification¹¹. Five fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the five fields; the number of abnormal spermatozoa were expressed as a percentage of the total number of spermatozoa.

Sperm count

This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5 ml formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5ml formol-saline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the Improved Neubauer hemocytometer under the microscope.

Testicular Histology

After removing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70 % alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and were dehydrated by passing through different grades of alcohol: 70 % alcohol for 2 hours, 95 % alcohol for 2 hours, 100 % alcohol for 2 hours, 100 % alcohol for 2 hours and finally 100 % alcohol for 2 hours. The tissues were then cleared to remove the alcohol, the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten Paraffin wax for 2 hours in an oven at 57 °C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at 5 microns (5µm). The satisfactory ribbons were picked up from a water bath (50–55 °C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1 % acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x 40, x100 and x 400 magnifications.

Statistical Analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparisons between the control and the treated groups were done using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

There were significant ($p < 0.05$) increase in testosterone levels at all doses of AEPO and MEPO relative to the control after 50 days recovery period from 50 days pretreatments with AEPO and MEPO (Figures 1 and 2).

There were no significant ($p > 0.05$) changes in sperm motility at all the treatment doses (except at 50 mg/kg AEPO and 75 mg/kg MEPO) relative to the control after 50 days recovery period from 50 days pre-treatments of rats with AEPO and MEPO. There were no significant ($p > 0.05$) changes in sperm viability at all the treatment doses relative to the control after withdrawal of treatments for 50 days after 50 days pre-treatments of rats with AEPO and MEPO. There were significant ($p < 0.05$) increments in the percentage of abnormal sperm cells at all the

treatment doses relative to the control after withdrawal of treatments for 50 days after 50 days pre-treatment of rats with AEPO and MEPO. There were no significant ($p > 0.05$) changes in sperm count at all the treatment doses relative to the control after 50 days recovery period from 50 days pre-treatments of rats with AEPO and MEPO (Figures 3, 4, 5, 6).

The histopathological study revealed that testes of control rats and rats that were allowed 50 days recovery period from 50 days pre-treatments with different doses of AEPO and MEPO presented with normal germinal epithelia with no visible lesions observed (Plates 1 and 2).

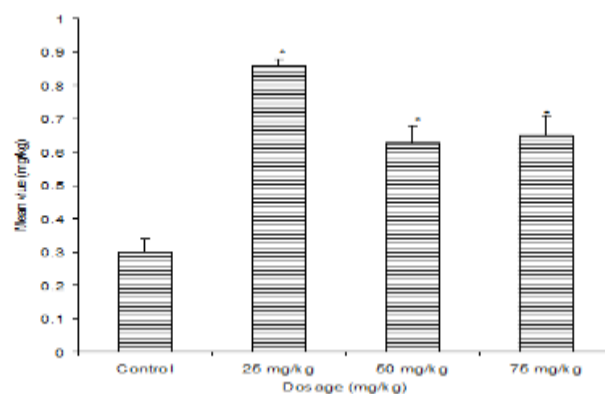


Figure 1: Testosterone level after 50 days recovery period from 50 days pre-treatment of rats with AEPO (n=5, * $p < 0.05$).

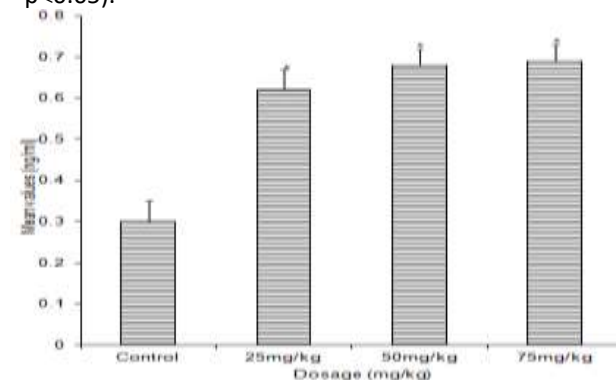


Figure 2: Testosterone level after 50 days recovery period from 50 days pre-treatment of rats with MEPO (n=5, * $p < 0.05$).

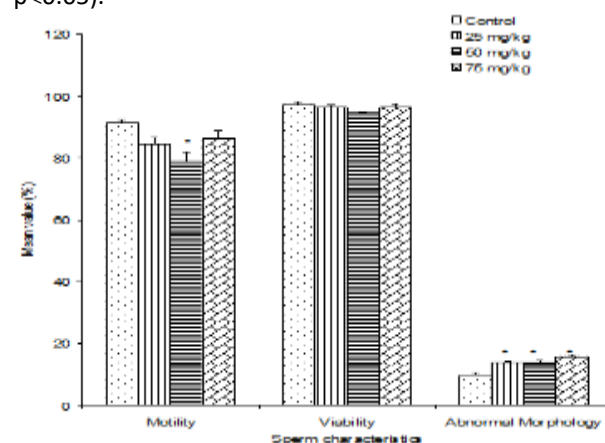


Figure 3: Sperm characteristics after 50 days recovery period from 50 days pre-treatment of rats with AEPO (n=5, * $p < 0.05$).

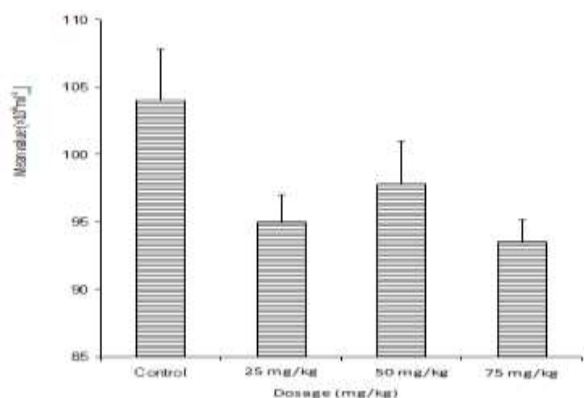


Figure 4: Sperm count after 50 days recovery period from 50 days pre-treatment of rats with AEPO (n=5).

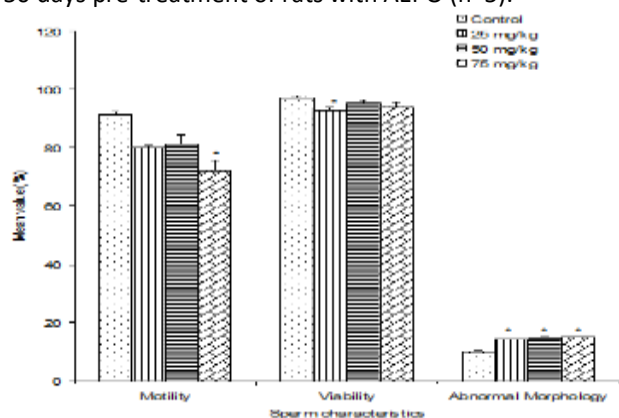


Figure 5: Sperm characteristics after 50 days recovery period from 50 days pretreatment of rats with MEPO (n=5, *p<0.05)

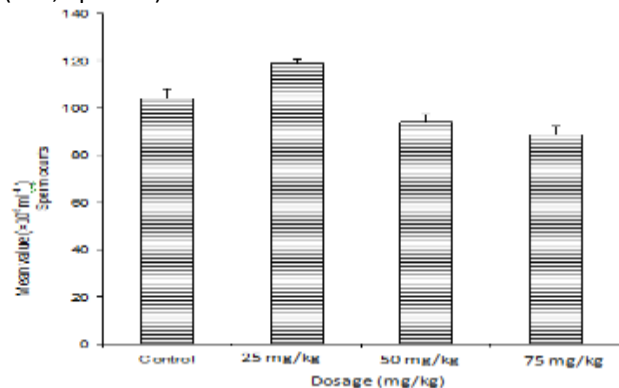


Figure 6: Sperm count after 50 days recovery period from 50 days pre-treatment of rats with MEPO (n=5)

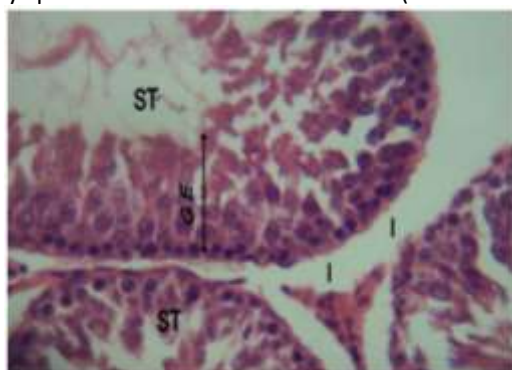


Plate 1: Histological evaluation of the testis after 50 days recovery period from 50 days pre-treatment with 0.5 ml of distilled water (control) (x 40)

Photomicrograph showing normal viable germinal epithelia (GE) with no visible lesions observed at the interstitia (I) and the seminiferous tubules (ST).



Plate 2: Histological evaluation of the testis after 50 days recovery period from 50 days pretreatment with 75 mg/kg of AEPO (x 400).

Photomicrograph showing normal germinal epithelia (GE) with no visible lesion observed.

DISCUSSION

There was a significant increase in testosterone level after the recovery period. This increase in testosterone level could probably be due to self-induced activation of androgen receptor by testosterone since testosterone has been known to have the capability to activate the androgen receptor by itself. Similar result was reported by¹² in *Ricinus communis* extract treated rats.

There were no significant changes in sperm motility after the recovery period. This suggest that the extracts were able to permeate the blood - testis barrier but unable to alter the micro environment of the seminiferous tubules, since it has been reported that the decrease the in sperm motility caused by chemical agents was due to their ability to permeate the blood-testis barrier¹³ and thus creating a different microenvironment in the inner part of the wall of the seminiferous tubules from the outer part¹⁴. Contrary report was given by¹⁵ in rats treated with *sarcotemma acidum* extract.

There were significant increases in the percentage of morphologically abnormal sperm cells after the recovery period. This could be due to the non-total renal clearance of the extracts after the fifty days recovery period with a resultant interference of the extracts with the spermatogenic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis^{16,17}. Similar result was reported by¹⁸ in isolated tetracyclic steroid treated rats.

Sperm count is considered to be an important parameter with which to access the effect of chemicals on spermatogenesis¹⁹. The insignificant changes in sperm counts and sperm viability in rats as well as the normal

germinal epithelia with no visible lesion observed at the histopathological level after the recovery period could be as a result of metabolism and excretion of the active constituents of this plant after the fifty days recovery period. Contrary report was given by²⁰ in *Terminalia chebula* extract treated rats.

CONCLUSION

In conclusion, this study has shown that the deleterious effects induced by *Portulaca oleracea* extracts on the reproductive parameters in male rats were reversible after a recovery period of 50 days.

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Source of Support: Nil, Conflict of Interest: None.