

Research Article

**Effect of Fraction 3 of *Portulaca Oleracea* on Reproductive Functions in Male Albino Rats**OYEDEJI K.O.¹, ADEGOKE A.O.², OLADOSU I. A.³¹Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.³Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan, Nigeria.

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ABSTRACT

The effect of oral administration of chromatographic fraction 3 of *Portulaca oleracea* at doses of 1 mg/kg BW, 2 mg/kg BW and 3 mg/kg BW on reproductive parameters in male albino rats were investigated. The fraction was administered on daily basis for 50 days with distilled water (0.5 mL) serving as the control. Plasma testosterone levels were assayed and semen analyses were carried out. Treatment of rats with all the treatment doses of the fraction (1 mg/kg BW, 2 mg/kg BW, 3 mg/kg BW) caused significant ($p < 0.05$) increase in testosterone levels relative to the control. Treatment of rats with all the treatment doses of the fraction caused significant ($p < 0.05$) reductions in % progressive sperm motility, sperm counts, as well as significant increase in the percentage of abnormal sperm cells relative to their respective controls. It can therefore be suggested that chromatographic fraction 3 of *Portulaca oleracea* has deleterious effect on the reproductive functions in male albino rats.

Keywords: Fraction, Testosterone, Sperm count, Sperm motility, Albino 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 foetus⁵.

It has been reported that aqueous and methanolic extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations⁶.

It has also been reported that aqueous and methanolic extracts of *Portulaca oleracea* have some toxic and beneficial potentials on the blood chemistry of albino rats⁷.

The extracts of *Portulaca oleracea* have been reported to have protective effects on hypoxic nerve tissue⁸, anti-inflammatory effects⁹ and wound-healing activity^{10, 11}. It also reported the skeletal muscle relaxant effect of the plant.

This study aims at investigating the effect of chromatographic fraction 3 of *Portulaca oleracea* in male reproductive functions in albino rats.

MATERIALS AND METHODS**Experimental Animals**

Adult male albino rats weighing between 160 g and 180 g bred in the Animal House of Physiology Department, LAUTECH, Ogbomoso were used. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water; they were acclimatized to laboratory conditions for two weeks 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, Jericho, Ibadan, and was authenticated in the above named institute where a voucher specimen (No FHI 108334) was deposited.

Extraction and Fractionation of *Portulaca oleracea*

About 3.2 kg of air-dried specimen of *Portulaca oleracea* was cold-extracted in methanol for 72 hours. The mixture was filtered using a wire-guaze and a sieve with tiny pores (0.25 mm) and concentrated at room temperature by exposing the extract for six days. The resulting solution was then placed in the oven at a reduced temperature (50°C).

The methanolic extract was then preabsorbed with silical gel and placed in the oven at a reduced temperature (50°C) overnight and then subjected to open column chromatography on silical gel (F₂₅₄, 50-200 mesh, E. Merck) for fractionation. The solvents (mobile phases)



were hexane (non-polar), ethylacetate (partially polar) and methanol (polar). The gradients of the mobile phases involved hexane with an increasing percentage of ethylacetate (hexane/ethylacetate mixture) and then ethylacetate with an increasing percentage of methanol (ethylacetate/methanol mixture) as shown below:

Twenty-one fractions were obtained after the column chromatographic procedure.

Hexane	Ethylacetate	Methanol
100% (50 mL) :	0% (0 mL)	
90% (45 mL) :	10% (5 mL)	
80% (40 mL) :	20% (10 mL)	
70% (35 mL) :	30% (15 mL)	
60% (30 mL) :	40% (20 mL)	
50% (25 mL) :	50% (25 mL)	
40% (20 mL) :	60% (30 mL)	
30% (15 mL) :	70% (35 mL)	
20% (10 mL) :	80% (40 mL)	
10% (5 mL) :	90% (45 mL)	
0% (0 mL) :	100% (50 mL)	0% (0 mL)
	90% (45 mL)	10% (5 mL)
	80% (40 mL)	20% (10 mL)
	70% (35 mL)	30% (15 mL)
	60% (30 mL)	40% (20 mL)
	50% (25 mL)	50% (25 mL)
	40% (20 mL)	60% (30 mL)
	30% (15 mL)	70% (35 mL)
	20% (10 mL)	80% (40 mL)
	10% (5 mL)	90% (45 mL)
	0% (0 mL)	100% (50 mL)

Thin Layer Chromatography (TLC)

The 21 fractions were spotted on precoated plates of silica gel GF₂₅₄ (20 x 20, 0.5 mm thick; E. Merck) using capillary tubes. The spotted TLC plates were developed in a tank that contained a mixture of ethylacetate/methanol (9:1) as the mobile phases.

The TLC plates were then examined under the ultraviolet (UV) light at a wavelength of 365 nm and the well-defined spots of the components were then revealed by the UV light. Fractions with similar relative fronts or retention or retardation factors (R_f value) were then pooled or bulked together, this then reduced the number of fractions to five (fractions 1, 2, 3, 4, 5).

$$R_f = \frac{\text{distance compound has moved from origin}}{\text{distance of solvent front from origin}}$$

Fraction 3 was then subjected to bioassay, *vis-à-vis*, its effect on reproductive parameters in male albino rats were evaluated.

Acute Toxicity Test of Chromatographic Fraction

The acute toxicity test of chromatographic fraction 3 of *Portulaca oleracea* was evaluated in albino mice as described by ¹². Fifteen adult male mice weighing between 20-22g were divided into five mice per group. Three doses of the fraction: 1 mg/kg BW, 5 mg/kg BW and 10 mg/kg BW were given orally to the animals. The control group mice (n=5) received 0.5 ml of distilled water. The animals were observed for seven days for behavioural changes and mortality.

Experimental Design

Twenty animals were randomly divided into four groups with each group consisting of five rats. The four groups were subjected to the following oral daily treatments for 50 days:

Group I rats received 1 mg/kg BW of fraction

Group II rats received 2 mg/kg BW of fraction

Group III rats receive 3 mg/kg BW of fraction

Group IV rats received 0.5 mL of distilled water as the control group.

Collection of Blood Samples

Blood samples were collected through the medial cantus into EDTA bottles for hormonal assay.

Hormonal Assay

Plasma samples were assayed for testosterone using the enzyme-linked immunosorbent assay (ELISA) technique using the Randox 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 X400 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 labelled 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)¹³.

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 x400 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 calculated¹⁴.

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 prewarmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using x400 magnification (Laing, 1979). 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 5ml 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 haemocytometer under the microscope.

Statistical Analysis

The mean and standard error of mean (S.E.M) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with least significant difference (LSD). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Acute Toxicity

No mortality and changes in behaviour were observed in all the treated and control groups. Hence lower doses of the fraction were used for this study.

Effect of Fraction 3 on Hormonal Levels

Treatment of rats for 50 days with all the treatment doses of fraction 3 (1 mg/kg BW, 2 mg/kg BW, 3 mg/kg BW) caused significant ($p < 0.05$) increase in testosterone levels when compared to the control as shown in Figure 1.

Effect of Fraction on Sperm Characteristics

Treatment of rats for 50 days with the treatment doses of the fraction (1 mg/kg BW, 2 mg/kg BW, 3 mg/kg BW) resulted in significant ($p < 0.05$) decrease in sperm motility, sperm viability, sperm counts as well as significant ($p < 0.05$) increase in the percentage of abnormal sperm cells relative to their respective controls as shown in Figures 2 and 3.

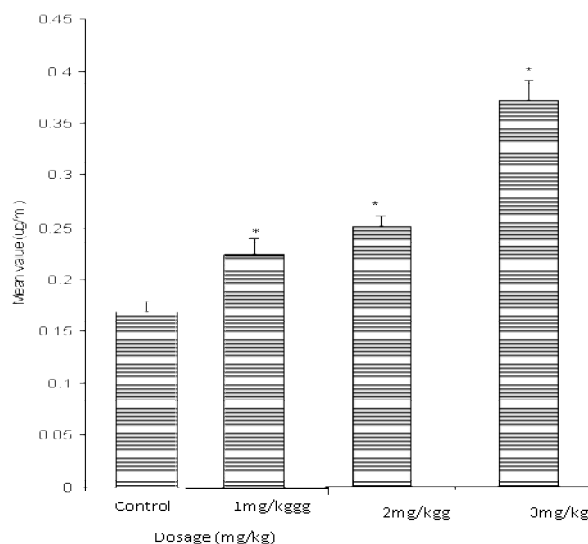
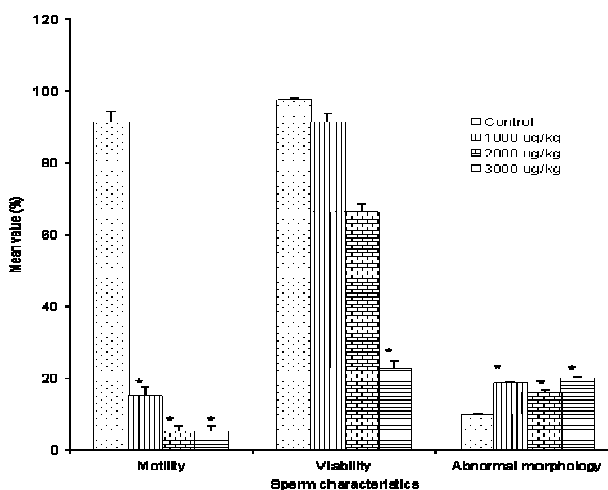
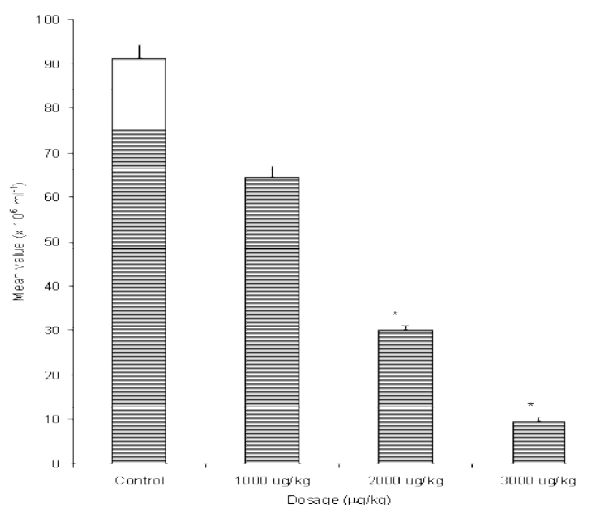


Figure 1: Effect of 50 days treatment with fraction 3 of *Portulaca oleracea* on plasma levels of testosterone (n=5, * $p < 0.05$)



Spermogram showing the effects of fraction 3 of *Portulaca oleracea* on sperm characteristics on day 50 (n=5, * $p < 0.05$).

Figure 2: Spermogram showing the effect of fraction 3 of *Portulaca oleracea* on sperm characteristics after treatment of rats for 50 days (n=5, * $p < 0.05$)



Spermogram showing the effects of Fraction 3 of *Portulaca oleracea* on sperm characteristics on day 50 (n=5, * $p < 0.05$).

Figure 3: Spermogram showing the effect of fraction 3 of *Portulaca oleracea* on sperm counts after treatment of rats for 50 days (n=5, * $p < 0.05$)

DISCUSSION

It was observed that the highest dose of fraction 3 caused no mortality or behavioural changes in all the treated animals which indicates that fraction 3 has wide safety margins.

The fraction induced significant increase in testosterone levels which was not expected. The plausible explanation for this observation could be as a result of direct damage to the testes by fraction 3, since it has been reported that any direct damage to the testis is likely to impair gonadal response to FSH and LH¹⁵. Contrary report was given by¹⁶ in rats treated with aspirin. This increase in testosterone levels could indicate that fraction 3 did not inhibit the mechanism intervening in the process of hormone synthesis in the Leydig cells.

The andrological results show that treatment of rats with fraction 3 caused significant decrease in sperm motility. Similar report was given by¹⁷ in rats treated with *Sarcotemma acidum* extract. This suggests that the fraction was able to permeate the blood-testis barrier with a resultant alteration in the microenvironment of the seminiferous tubules, since it has been reported that the decrease in sperm motility caused by chemical agents was due to their ability to permeate the blood-testis barrier¹⁸ and thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in the outer part¹⁹.

There was a statistically significant decrease in sperm viability as well as a significant increase in the percentage of morphologically abnormal sperm cells induced after treatment of rats with the fraction. This could be due to the ability of the fraction to either interfere with the spermatogenic processes in the seminiferous tubules and epididymal functions which may result in alteration of spermatogenesis^{20,21}.

Sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis²². Spermatogenesis is influenced by the hypothalamic-adenohypophyseal – Leydig cell system relating gonadotrophin releasing hormone, leutinizing hormone and androgen. This implies that the decrease in sperm count caused by fraction 3 in treated rats could not be as a result of plasma level of testosterone, because this hormone has been reported to be important in the initiation and maintenance of spermatogenesis²³. Contrary report was given by²⁴ in *Terminalia chebula* extract treated rats.

In conclusion, this study has shown that chromatographic fraction 3 of *Portulaca oleracea* could have some toxic potentialities on the male reproductive functions of albino rats. However, its effect on human reproductive functions are unknown; nevertheless, considering these findings in animal models, it is recommended that men with infertility or reproductive problems should abstain from eating *Portulaca oleracea* during the treatment period.

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