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Research Article

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Effect of fraction 2 of *Portulaca oleracea* on reproductive functions in male wistar rats

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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. The crude extracts of this plant have been reported to have deleterious effects on reproductive parameters in male rats. Air-dried specimen of Portulaca oleracea was cold-extracted in methanol for 72 hours. The resulting methanol extract was then subjected to open column chromatography on silica gel for fractionation. Out of the 5 fractions obtained, fraction 2 was then subjected to male rats' reproductive bioassay. Twenty male rats were divided into control (distilled water) and fraction 2 (1, 2, 3 mg/kg) treated groups (5 per group) for hormonal assay and andrological studies. The animals were orally treated on daily basis for 50 days. Plasma testosterone level was assayed using Enzyme-Linked Immunosorbent Assay (ELISA) and semen analysis was done microscopically. Treatment of rats for 50 days with fraction 2 (1 mg/kg, 3mg/kg) caused significant ($p<0.05$) reductions in testosterone levels relative to the control. Fraction 2 (1 mg/kg, 2 mg/kg, 3 mg/kg) caused significant ($p<0.05$) decrease in sperm motility, sperm viability and sperm counts of rats relative to their respective controls. It can therefore be concluded that fraction 2 of Portulaca oleracea probably has deleterious effects on reproductive parameters in male rats.

Keywords: Fraction 2, Testosterone, Sperm count, Sperm motility, Rats.

INTRODUCTION

Portulaca oleracea belongs to the family of Portulacaceae. It is a fleshy annual herb, much-branched and attaining 30 cm long. It is commonly called Purslane in English language, "Babbajibji" in Hausa language and "Esanomode or Papasan" by the Yoruba tribe of Nigeria[1].

It is used medicinally in Ghana for heart - palpitations [2]. The plant is used as a diuretic in Nigeria [3]. A tisane of the plant is drunk in Trinidad as a vermifuge [4]. At some areas near Benin City (Nigeria), the plant, along with other ingredients is taken as an aid to the development of the fetus [5].

It has been reported that the aqueous and methanol extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations [6]. The extracts of *Portulaca oleracea* have been reported

to have protective effects on hypoxic nerve tissue [7], anti-inflammatory effects [8] and wound-healing activity [9]. The skeletal muscle relaxant effect of this plant has also been reported [10].

Since the crude extracts of this have been reported to have deleterious effects on reproductive parameters in male rats [11], this study therefore aims at investigating the effect of chromatographic fraction 2 of *Portulaca oleracea* on reproductive parameters in male Wistar rats.

EXPERIMENTAL SECTION

Experimental Animals

Adult male albino rats weighing between 120 g and 150 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, AfeBabalola University were used. They were housed under standard laboratory conditions 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-gauze 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 (45 – 50 ^{0}C).

The methanol extract was then pre - absorbed with silica gel and placed in the oven at a reduced temperature (45 – 50 ^{0}C) overnight and then subjected to open column chromatography on silica gel (F_{254}, 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:

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)		

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

Thin Layer Chromatography (TLC)
The 21 fractions were spotted on pre - coated plates of silica gel GF_{254} (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 = distance compound has moved from origin
distance of solvent front from origin

Fraction 2 was then subjected to bioassay, *vis-à-vis*, its effect on reproductive profiles in male rats were evaluated.

Acute Toxicity Test of Chromatographic Fraction
The acute toxicity test of chromatographic fraction 2 of *Portulaca oleracea* was evaluated in 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, 5 mg/kg and 10 mg/kg 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 behavioral 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 of fraction 2
Group II rats received 2 mg/kg of fraction 2
Group III rats receive 3 mg/kg of fraction 2
Group IV rats received 0.5 ml of distilled water as the control group.

Twenty-four hours (day 51) after the last dosing of the four groups, blood samples were collected and the animals were then euthanized by overdose of diethyl ether for semen analysis.
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 ^{O}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) [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 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 calculated [14].

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 magnification [14]. 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 was expressed as a percentage of the total number of spermatozoa.

Sperm count: This was done by removing the caudal epididymis from the right testis and blotted with filter paper. The caudal epididymis was immersed in 5mlformol-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 5mlformol-saline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the Improved Neubauerhemocytometer under the microscope.

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 AND DISCUSSION

No mortality and changes in behavior were observed in all the treated and control groups of rats.

Treatment of rats for 50 days with fraction 2 (1 mg/kg, 3 mg/kg) caused significant ($p<0.05$) decreases in testosterone levels relative to the control (Figure 1).

Treatment of rats for 50 days with all the doses of fraction 2 (1 mg/kg, 2 mg/kg, 3 mg/kg) caused significant ($p<0.05$) reductions in sperm motility, sperm viability and sperm counts relative to their respective controls. Fraction 2 (1 mg/kg, 2 mg/kg, 3 mg/kg) also caused significant ($p<0.05$) increases in the percentage of abnormal sperm cells relative to the control(Figures 2 and 3).

Figure 1: Effect of 50 days treatment of rats with fraction 2 of *Portulaca oleracea* on plasma testosterone levels (n=5, *p<0.05)

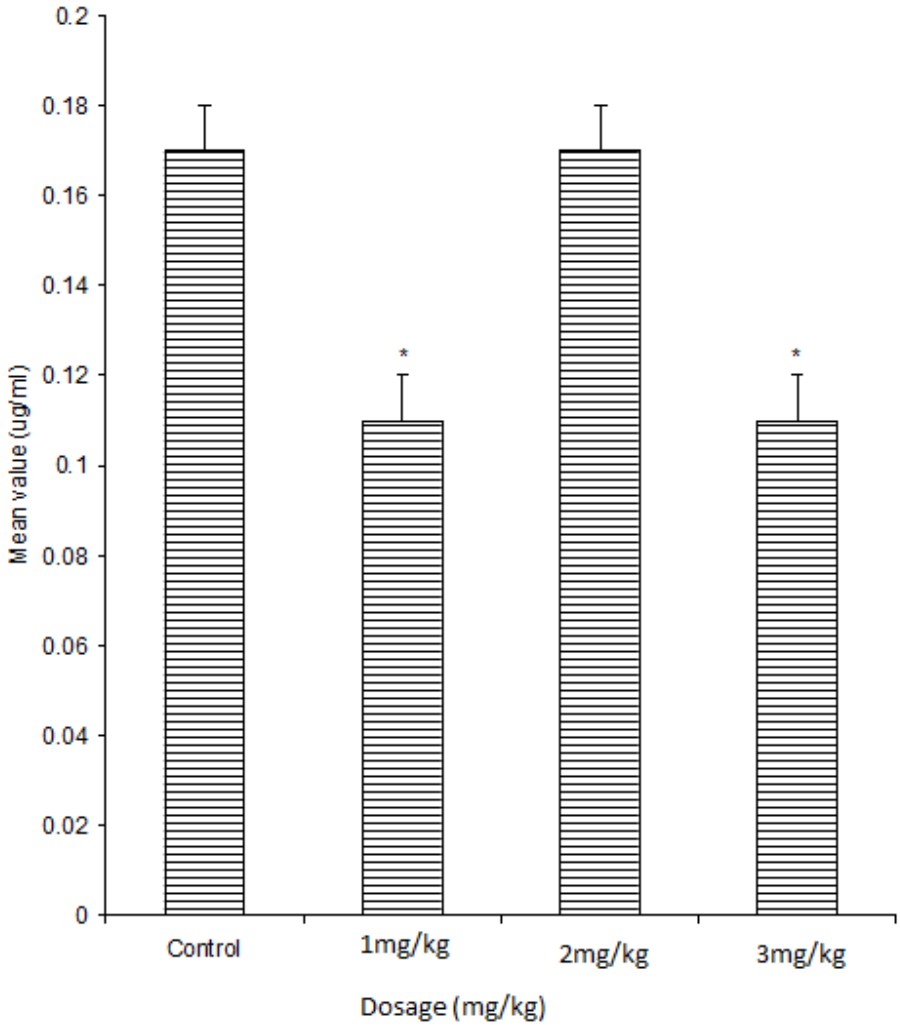
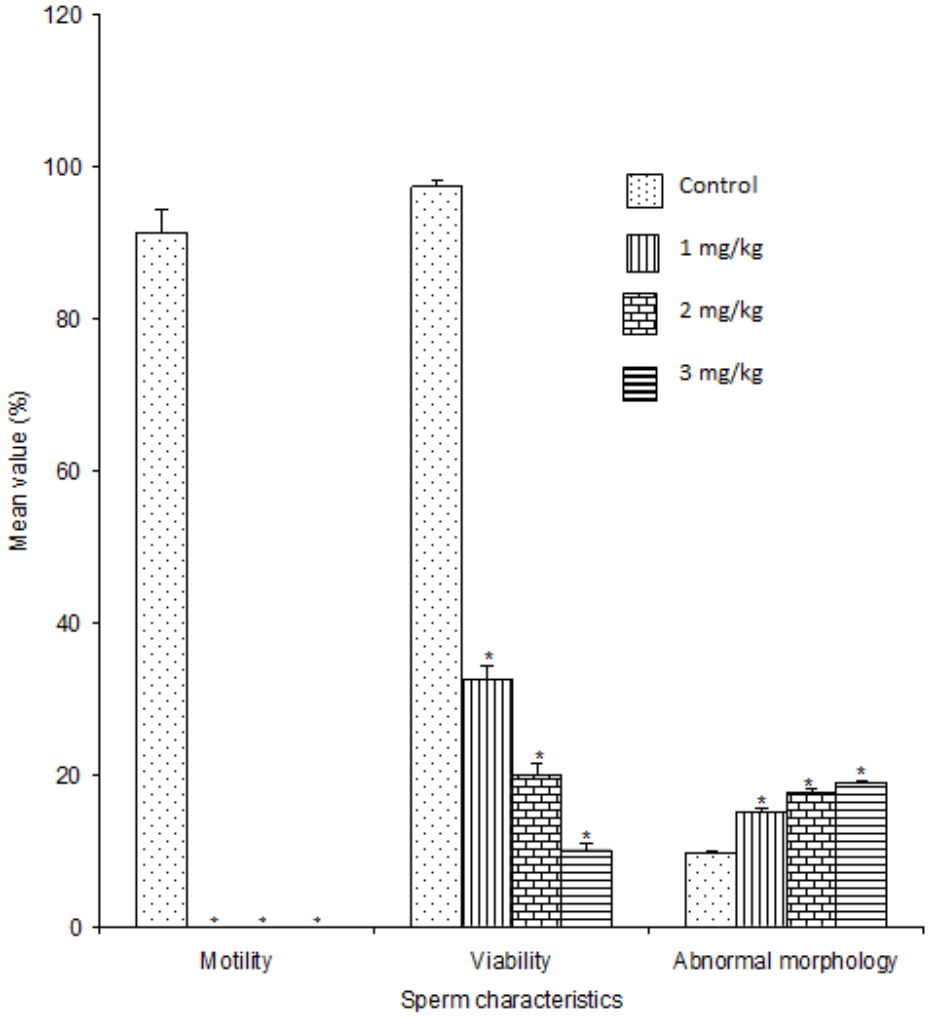


Figure 2: Spermogram showing the effect of 50 days treatment of rats with fraction 2 of *Portulaca oleracea* on sperm characteristics (n=5, *p<0.05)



It was observed that the highest dose of fraction 2 caused no mortality or behavioral changes in all the treated animals which indicates that the fraction has a wide safety margin.

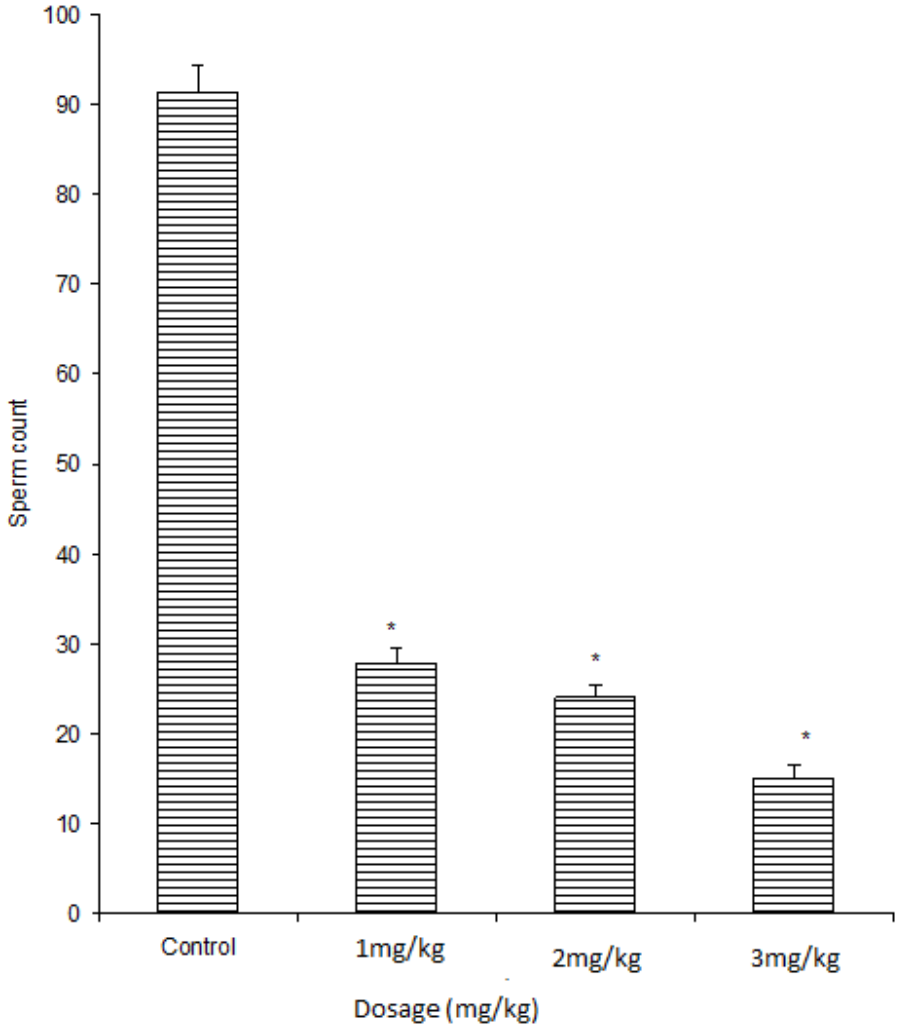
The fraction caused significant reductions in testosterone levels. These decreases in the testosterone levels could probably indicate that the fraction inhibits the mechanism intervening in the process of hormone synthesis in the Leydig cells. Similar report was given by [15] in rats treated with *Aegle mermelos* extract.

The fraction caused significant decreases in sperm motility. This suggests that the fraction was able to permeate the blood - testis barrier with a resultant alteration in the micro environment 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 barrier [16] and thus creating a different microenvironment in the inner part of the wall of the seminiferous tubules from the outer part [17]. Similar report was given by [18] in rats treated with *sarcotem maacidum* extract.

There were significant reductions in sperm viability as well as significant increases in the percentage of morphologically abnormal sperm cells induced after treatment of rats with the fraction. This could probably be due to the ability of the fraction to either interfere with the spermatogenic processes 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 [19, 20]. Similar result was reported by [21] in isolated tetracyclic steroid treated rats.

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



Sperm count is considered to be an important parameter with which to access the effect of chemicals on spermatogenesis [22]. Spermatogenesis is influenced by the hypothalamic adenohypophysial – Leydig cell system relating gonadotropin releasing hormone, luteinizing hormone and androgen. This implies that the decrease in sperm count caused by fraction 2 in the treated rats might be as a result of decrease in plasma level of testosterone, because this hormone has been reported to be important in the initiation and maintenance of spermatogenesis [23]. Similar report was given by [24] in *Terminalia chebula* extract treated rats.

In conclusion, this study has shown that chromatographic fraction 2 of *Portulaca oleracea* probably has spermatotoxic or antispermatogenic effects in male rats. However, the effect of fraction 2 of this plant on human reproductive function is unknown. Nevertheless, considering these findings in animal model, it is recommended that men with infertility or reproductive problems should abstain from eating *Portulaca oleracea* during the treatment period.

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