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Research Article

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Effect of fraction 3 of *Portulaca oleracea* on plasma gonadotrophins and testosterone levels and their recoveries 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. The crude extracts of this plant caused significant decreases in testosterone levels 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 for fractionation. Out of the 5 fractions obtained, fraction 3 was then subjected to male rats' hormonal bioassays. Twenty male rats were divided into control and fraction 3(1, 2, 3 mg/kg) treated groups (5 per group) for hormonal assay study. The animals were orally treated on daily basis for 50 days and allowed a recovery (withdrawal) period of 50 days after which hormonal assays were carried out at the end of dosing and recovery periods. Plasma LH, FSH and testosterone levels were assayed using Enzyme – Linked Immunosorbent Assay (ELISA) technique. Treatment of rats for 50 days with fraction 3 (1 mg/kg, 2 mg/kg) caused significant ($p<0.05$) reductions in LH levels relative to the control. Fraction 3 (3 mg/kg) caused significant ($p<0.05$) increase in FSH level relative to the control. There was a significant ($p<0.05$) decrease in LH level of fraction 3 (3 mg/kg) recovery group relative to the control. Fraction 3 (1 mg/kg, 2 mg/kg, 3 mg/kg) caused significant ($p<0.05$) increase in testosterone levels relative to the control. It can therefore be concluded that fraction 3 of Portulaca oleracea probably induced significant changes on plasma gonadotrophins and testosterone levels through the hypothalamic – pituitary – testicular axis which could result in sterility.

Keywords: Fraction 3, Testosterone, Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Rats.

INTRODUCTION

Portulaca oleracea belongs to the family of Portulacaceae. It is a warm - climate annual herb and has cosmopolitan distribution. It is commonly called Purslane in English language and "Esanomode" or "Papasan" by the Yoruba tribe of South - West Nigeria [1].
It is used in Iranian folk medicine as a diuretic, vermifuge, antiscorbatic, antitussive, analgesic and gastroesophageal reflux [2].

Pharmacologically, *Portulaca oleracea* extracts have been reported to decrease morphine dependence in mice [3]. Its extracts have been reported to have analgesic and anti-inflammatory effects [4]. The aqueous and methanol extracts of this plant have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations [5]. Its extracts have been reported to cause reduction in locomotor activity and an increase in the onset time of

pentylenetetrazole (PTZ) – induced convulsion in rats [6]. Its crude extracts have also been reported to have beneficial effects on the hematological functions and blood chemistry of rats [7].

Since the crude extracts of this plant have been reported to cause significant decreases in testosterone levels in male rats [8], this study therefore aims at investigating the effect of chromatographic fraction 3 of *Portulaca oleracea* on plasma gonadotrophins and testosterone levels and their recoveries 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 3 was then subjected to bioassay, *vis-à-vis*, its effect on hormonal profiles in male rats were evaluated.

Acute Toxicity Test of Chromatographic Fraction
The acute toxicity test of chromatographic fraction 3 of *Portulaca oleracea* was evaluated in mice as described by [9]. 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 3
Group II rats received 2 mg/kg of fraction 3
Group III rats receive 3 mg/kg of fraction 3
Group IV rats received 0.5 ml of distilled water as the control group.

Collection of Blood Samples
Twenty four hours (day 51) after the last dosing of the four groups and also twenty four hours after the last day of the 50 days recovery period(day 101), blood samples were collected from all the animals through the medial cantus into EDTA bottles for the determination of plasma gonadotrophins and testosterone levels using ELISA technique with Fortress Kit.

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 $p<0.05$.

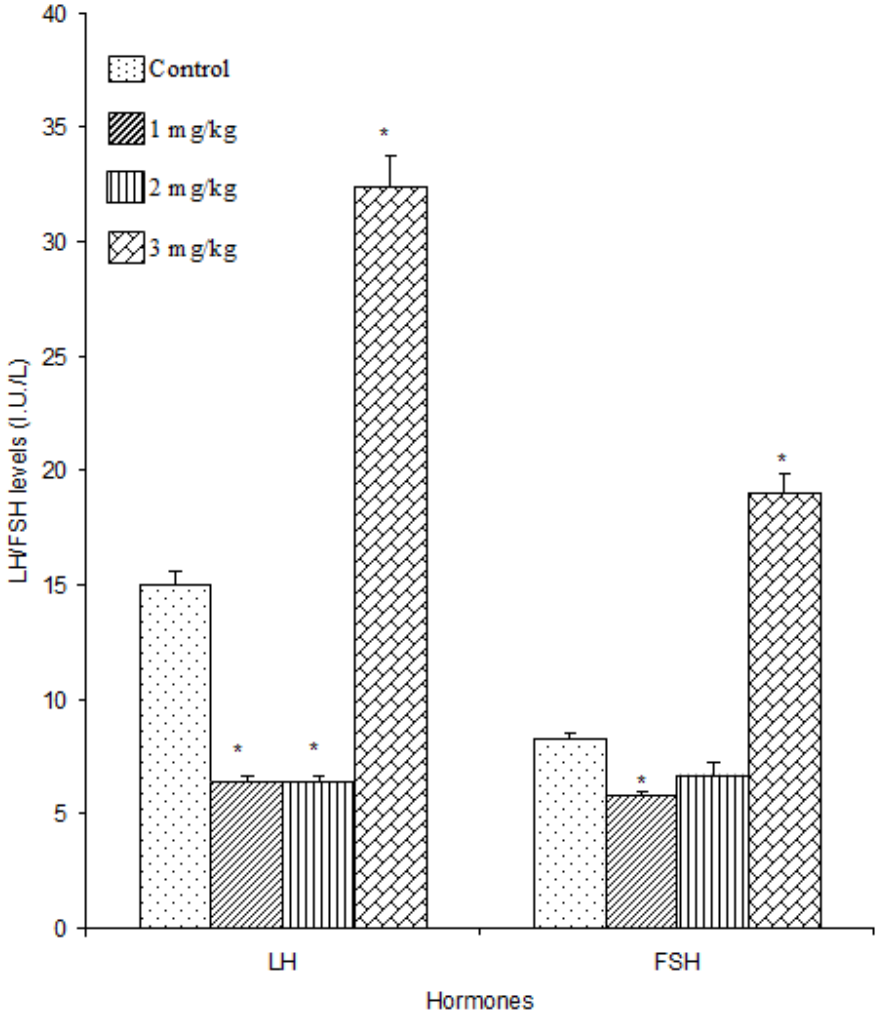
RESULTS AND DISCUSSION

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

Treatment of rats for 50 days with fraction 3 (1 mg/kg, 2 mg/kg) caused significant ($p<0.05$) decreases in plasma LH levels relative to the control, while fraction 3 (3 mg/kg) caused significant ($p<0.05$) increase in plasma LH level relative to the control. Treatment of rats for 50 days with 1 mg/kg and 3 mg/kg of fraction 3 caused significant ($p<0.05$) decrease and increase respectively in plasma FSH levels relative to the control, while fraction 3 (2 mg/kg) caused no significant ($p>0.05$) change in FSH level relative to the control (Figure 1).

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

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



There were no significant (p>0.05) changes in LH levels of fraction 3 (1 mg/kg, 2 mg/kg) recovery groups relative to the control, but there was a significant (p<0.05) decrease in LH level of fraction 3 (3 mg/kg) recovery group relative to the control. There were significant (p<0.05) decreases in FSH levels of fraction 3 (1 mg/kg, 3 mg/kg) recovery groups relative to the control, but there was no significant (p>0.05) change in FSH level of fraction 3 (2 mg/kg) recovery group relative to the control (Figure 3).

There were no significant (p>0.05) changes in testosterone levels of fraction 3 (1 mg/kg, 3 mg/kg) recovery groups relative to the control, but there was a significant (p<0.05) increase in testosterone level of fraction 3 (2 mg/kg) recovery group relative to the control (Figure 4).

Figure 2: Effect of 50 days recovery period from 50 days treatment of rats with fraction 3 of *Portulaca oleracea* on plasma LH and FSH levels (n=5, *p<0.05)

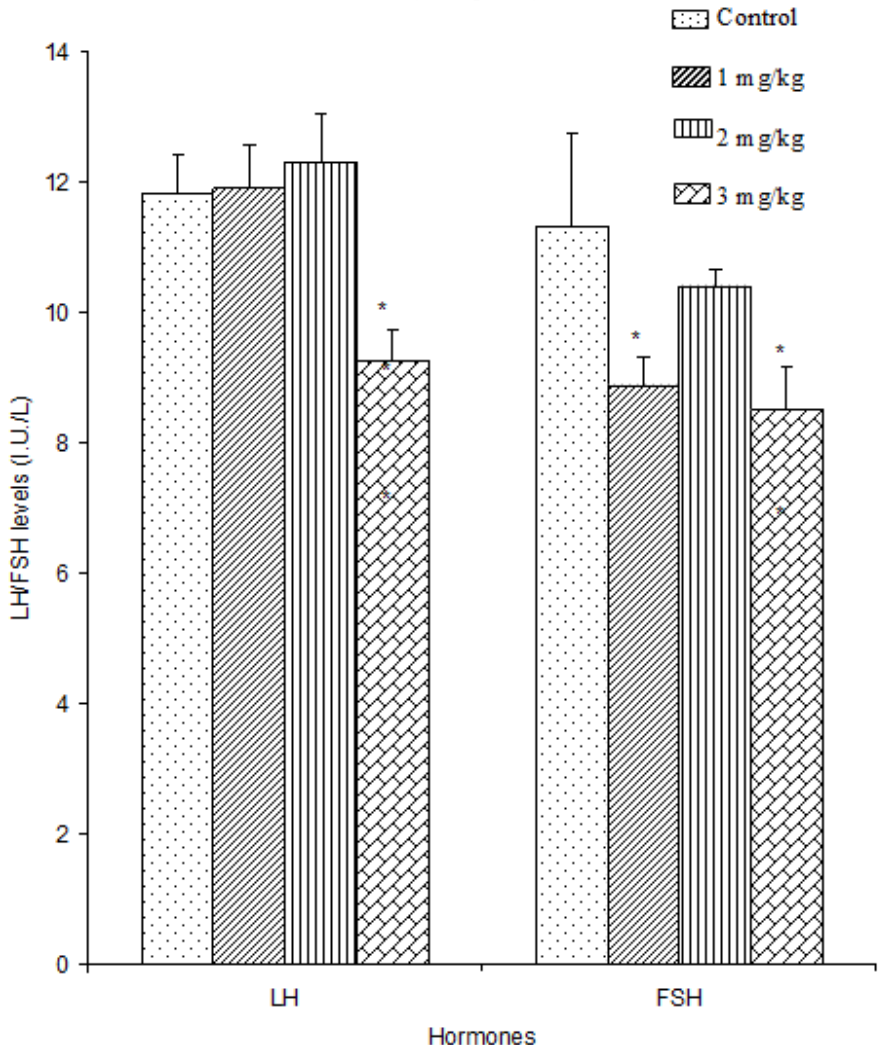
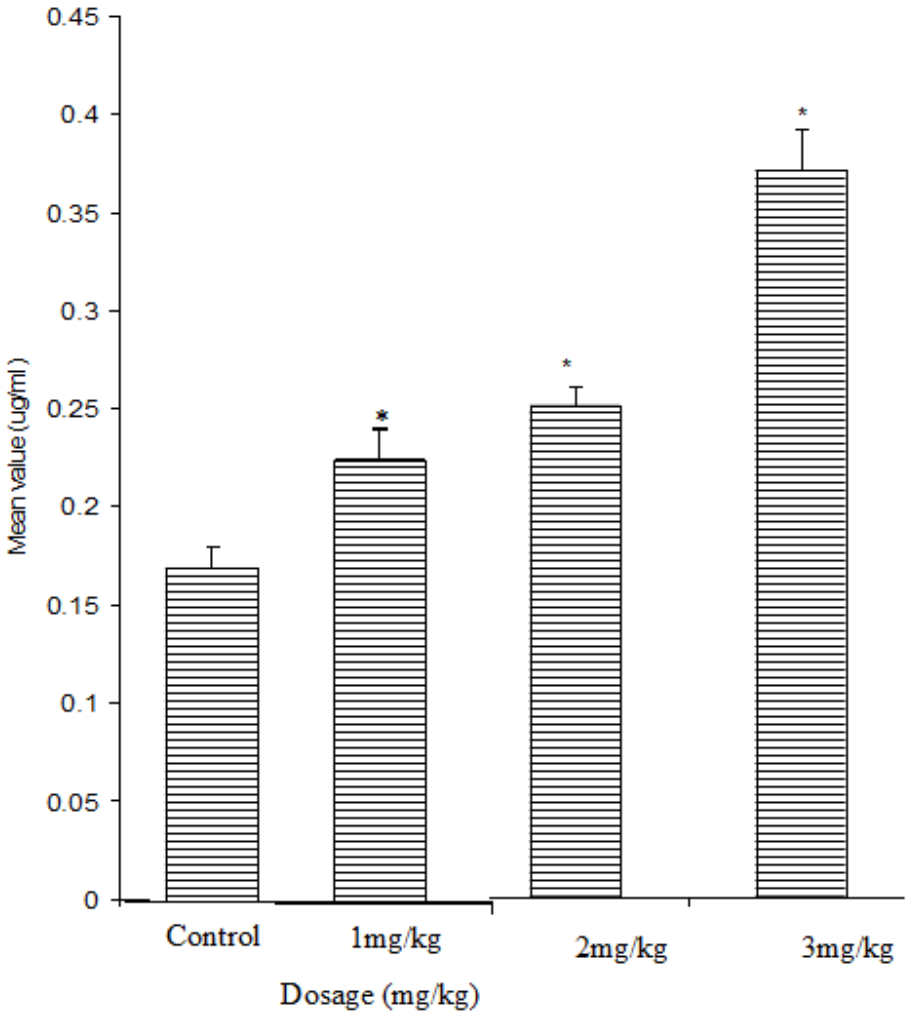


Figure 3: Effect of 50 days treatment of rats with fraction 3 of *Portulaca oleracea* on plasma testosterone levels (n=5, *p<0.05)

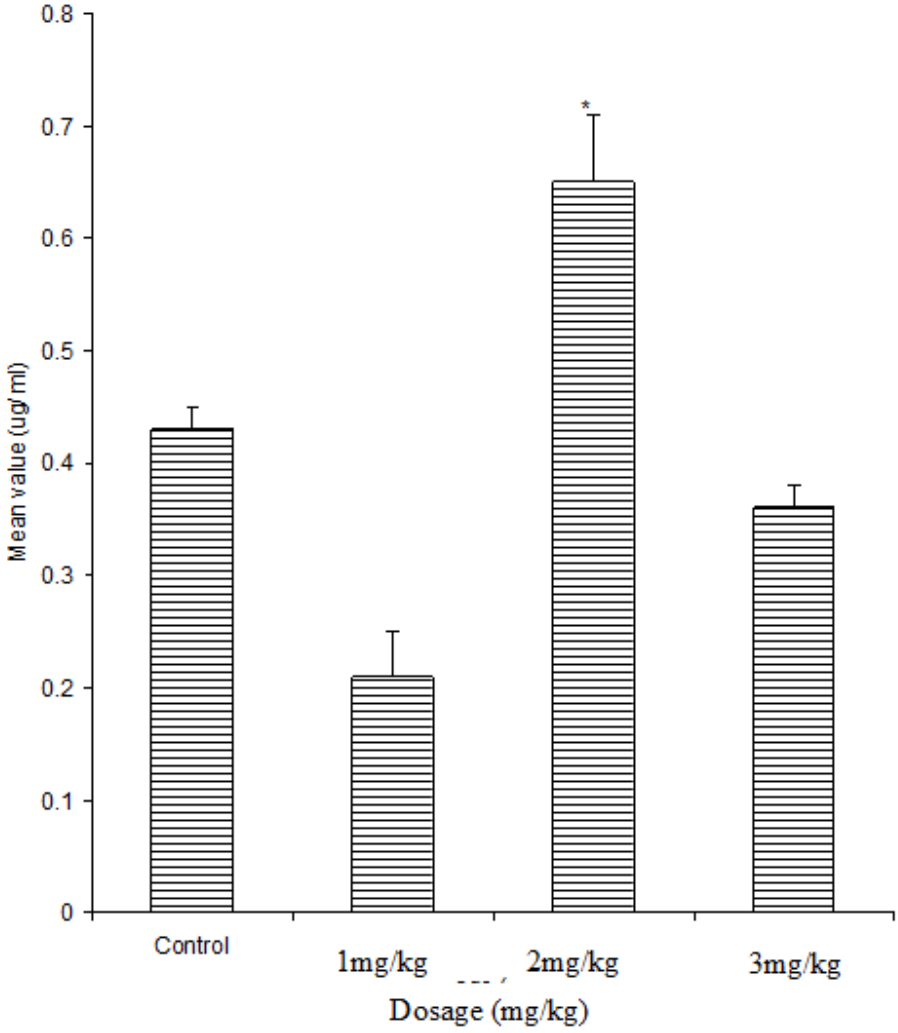


It was observed that the highest dose of fraction 3 caused no mortality or behavioral change in all the treated animals which probably indicates that the fraction has a wide safety margin.

At high dose, the fraction induced a significant increase in LH level which could be due to the indirect action of this fraction on the anterior pituitary gland thereby causing the stimulation of LH release by the adenohypophysis. Contrary result was reported by [10] in Caricapryl-99 extract treated rats. However, there was a significant decrease in LH level after the recovery period of treatment with the fraction which could be due to the stimulation or activation of the hypothalamic – pituitary – testicular axis negative feedback mechanism.

The fraction induced significant increase in FSH level which could be due to the indirect action of this fraction on the anterior pituitary, thereby causing stimulation of FSH release by the adenohypophysis. Similar result was reported by [11] in *Rutachalepensis* extract treated rats. However, there were significant reductions in FSH levels after withdrawal (recovery) of treatment with the fraction which could be due to the negative feedback effect on the anterior pituitary by the hormone called inhibin which in secreted by the Sertoli cells. This hormone (inhibin) has a strong direct effect on the anterior pituitary gland to inhibit the secretion of FSH and possibly a slight effect on the hypothalamus to inhibit the secretion of GnRH [12].

Figure 4: Effect of 50 days recovery period from 50 days treatment of rats with fraction 3 of *Portulaca oleracea* on plasma testosterone levels (n=5, *p<0.05)



At lower doses, the fraction caused significant increases in testosterone levels which was not expected due to the fact that this fraction induced significant decreases in LH levels at equivalent lower doses, as such it was expected that the fraction would cause some significant decreases in testosterone level, since it is generally believed that Leydig cells normally secrete testosterone by the stimulatory effect of LH. The plausible explanation for this observation could be as a result of direct damage to the testes by this fraction, which impaired gonadal response to the gonadotrophin (LH), since it has been reported that any direct damage to the testis is likely to impair gonadal response to FSH and LH [13]. However, there was still a significant increase in testosterone level after withdrawal of treatment (recovery) with the fraction which probably indicates that the damage done to the testes by this fraction is irreversible due to non-total renal clearance of this fraction leading to its accumulation in the ECF with a resultant manifestation of its deleterious activity.

CONCLUSION

It can therefore be concluded that chromatographic fraction 3 of *Portulaca oleracea* probably induced significant changes on plasma gonadotrophins and testosterone levels through the hypothalamic – pituitary – testicular axis which could result in sterility.

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