



Effect of Recovery Period on Plasma Testosterone Levels in Male Rats Treated with Crude Extracts of *Portulaca oleracea*

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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 distilled water and 70 % methanol respectively for 72 hours and concentrated using steam bath. The resulting aqueous and methanol extracts (AEPO and MEPO) were then subjected to male rats' hormonal bioassay. 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 50 days and allowed a recovery (withdrawal) period of 50 days after which hormonal assay was carried out. Plasma testosterone level was assayed using Enzyme-Linked Immunosorbent Assay (ELISA) technique. There were significant ($p < 0.05$) increases in testosterone levels after 50 days recovery period from 50 days treatment of rats with all the doses of AEPO and MEPO relative to the control. It can therefore be concluded that the inhibitory effects of the crude extracts on testosterone levels are probably reversible after the recovery period.

Keywords: *Portulaca oleracea*, Testosterone, Recovery period, ELISA, 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 "Papasani" by the Yoruba tribe of South-West Nigeria¹.

It is used in Iranian folk medicine as a diuretic, vermifuge, antiscorbutic, antitussive, analgesic and gastroesophageal reflux².

Pharmacologically, *Portulaca oleracea* extracts have been reported to decrease morphine dependence in mice³. Its extracts have been reported to have analgesic and anti-inflammatory effects⁴.

The aqueous and methanol extracts of this plant have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations⁵.

Its extracts have been reported to cause reduction in locomotor activity and an increase in the onset time of pentylenetetrazole (PTZ) – induced convulsion in rats⁶. Its crude extracts have also been reported to have beneficial effects on the hematological functions and blood chemistry of rats⁷.

Since the crude extracts of this plant have been reported to cause significant decreases in testosterone levels in male rats⁸, this study therefore aims at investigating the effect of recovery period on testosterone levels in male rats treated with aqueous and methanol extracts of this plant.

MATERIALS AND METHODS

Experimental Animals

Adult male albino rats weighing between 150 g and 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 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 (FRIN), Jericho, Ibadan, where it was identified and assigned a voucher specimen number FHI 108334.

Preparation of the Extracts

Large quantity (2.0 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 the dry specimens were pulverized using laboratory mortar and pestle, and then divided into two samples A and B.



Aqueous Extract of *Portulaca oleracea* (AEPO)

Weighted Portion (400.0 g) of sample A was 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. 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.

Methanol Extract of *Portulaca oleracea* (MEPO)

Weighted portion (350.0 g) of sample B was 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. 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 gram of AEPO and MEPO were dissolved in 100 ml of distilled water to give a concentration of 0.1 g/ml. The dosages of AEPO and MEPO administered in these studies were in accordance with those reported by⁹.

Animal Grouping and Extracts Administration

Thirty-five adult male albino rats weighing between 150-200 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 50 days and allowed a recovery (withdrawal) period of 50 days:

Group I received 25mg/kg of AEPO

Group II received 50mg/kg of AEPO

Group III received 75mg/kg of AEPO

Group IV received 25mg/kg of MEPO

Group V received 50mg/kg of MEPO

Group VI received 75mg/kg of MEPO

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

Collection of Blood Samples

Twenty four hours (day 101) after the last day of the recovery period, blood samples were collected from all the animals through the medial cantus into EDTA bottles for the determination of plasma testosterone level using ELISA technique with Fortress kit.

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 Duncan's Multiple

Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

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

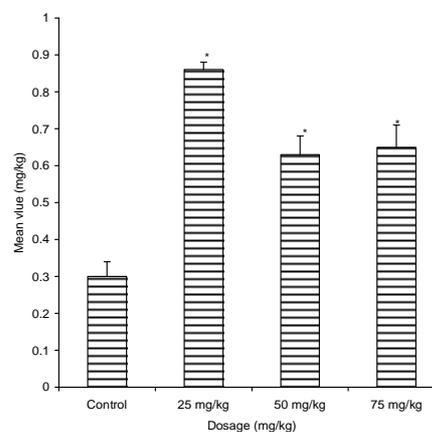


Figure 1: Plasma testosterone levels after 50 days recovery period from 50 days treatment of rats with AEPO (n=5, * $p < 0.05$).

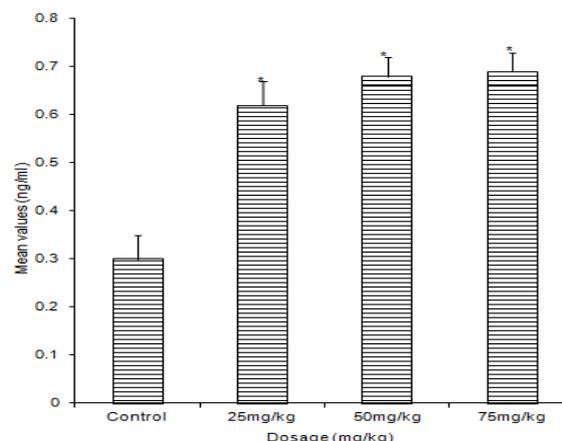


Figure 2: Plasma testosterone levels after 50 days recovery period from 50 days treatment of rats with MEPO (n=5, * $p < 0.05$).

DISCUSSION

These significant increases in testosterone levels after the recovery period could probably be due to self-induced activation of androgen receptors by testosterone, since testosterone has been known to activate the androgen receptor by itself. Similar result was reported by¹⁰ in *Ricinus communis* extract treated rats. The increases in testosterone levels could also be due to the influence of the recovery period to intervene (stimulate) in the Leydig cells steroidogenic process, since these cells are the main source of testosterone in rats¹¹. It could also be related to the hypersensitization of the hormone receptors during the recovery period. It could also be as a result of

metabolism and excretion of the active constituents of this plant after the fifty days recovery period.

This recovery observation could also be due to the reversible binding of these crude extracts to their receptors in the seminiferous tubules.

CONCLUSION

This study has shown that the testosterone inhibitory effects of these crude extracts (AEPO and MEPO)⁸ are probably reversible after 50 days recovery period, *vis-a-vis*, these crude extracts are potential reversible male contraceptives.

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