

Effects of *Portulaca oleracea* Stem Extract on Male Reproduction in Albino Rats (*Rattus norvegicus*)

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Abstract

Portulaca oleracea (Purselane) is a green leafy vegetable that is said to have some health promoting nutrients, however data on the safety and toxicology profile are scarce hence this study. The aim of this study was to evaluate the reproductive effect and toxicological profile of methanol stem extracts of *Portulaca oleracea* using albino rats as model. Fresh stem of *Portulaca oleracea* were collected and dried at room temperature to constant weight over 6 weeks, the dry stem weighed and grounded to fine powder. Methanol solvent (1.5L) was used and successive solvent extraction by cold maceration was done for 72hours. It was filtered with a filter paper and filtrate obtained, concentrated with rotary evaporator, transferred to evaporating dish and dried over a water bath. 82 albino rats were used: 12 for the acute toxicity study and 64 for the experimental study proper. Animals were acclimatized for 2 weeks before the study. In the experiment proper, animals were divided into four groups of 16 animals each. **Group A:** (control) received 1.5ml of clean water, **Group B:** 125mg/kg body weight of extract, **Group C:** 250mg/kg of extract, **Group D:** 500mg/kg body weight of extract. Administration was via oral gavage for 60days. On days 15, 30, 45 and 60, 4 rats from each group were anaesthetized and dissected and blood samples and testes were collected for testosterone assay and histopathology respectively. The result of the study showed that methanol stem extract of P.O showed no acute toxicological effect, that the extract has the propensity to increase testosterone level especially when administered over a long period of time, the extract caused an increase in the sperm cell count and no obvious toxic effect on sperm morphology thus may be used as fertility booster.

INTRODUCTION

It's not unusual to come upon a plant so remarkable that you have to pinch yourself to believe it's real. One such plant is the purslane, or *Portulaca oleracea*, Purslane, pigweed, duckweed, and small hogweed are all common English names for this plant. This plant has green leaves and may survive in a variety of climates. Some people may see it as a weed, although it has been used for food and medicine in many civilizations for ages. Because of its rich nutritional content and claimed medical benefits, many people are curious in this plants health benefits include cancer prevention, prevention of headaches including migraine, speeds up wound healing, useful in gastrointestinal and cardiovascular diseases, immunity and vision booster, improves circulation among others. (Nayaka et al 2014, Foster & Duke, 2000; Chiej, 1988; Rashed et al., 2003).

It is grown through the world due to its health promoting nutrients, vitamins and minerals. It is also said to be a rich source of omega-3-Fatty acids which has protective effect from cardiovascular diseases. It contains antioxidants such as vitamins A,C,E, glutathione, melatonin (Simopoulos et al; 1995).

AIM

To evaluate the reproductive effect and toxicological profile of methanol stem extracts of *Portulaca oleracea* in albino rats.

Objectives

- 1) To evaluate the effects of extract of *Portulaca oleracea* on male reproductive hormone (Testosterone)
- 2) To evaluate toxicological effect of *Portulaca oleracea* on the tests.
- 3) To investigate the effect of *Portulaca oleracea* on the seminal fluid: sperm cell count, sperm morphology and viability

SIGNIFICANCE OF THE STUDY

- 1) The study will provide insight on the safety profile of *Portulaca oleracea* stem extract.
- 2) It will provide information on the effect of *Portulaca oleracea* stem extract on male reproduction
- 3) It will provide information on whether or not *portulaca oleracea* stem extract has any toxicological effect on the testes
- 4) The results will serve as guide to either justify or refute the use of *Portulaca oleracea* in enhancing fertility in humans (males).

Effects of plant extracts on animal reproduction

Several plants formulation are used for reproduction related purposes. Many of them are used as fertility enhancer, contraception, infertility or abortifacient agents in traditional medical practice of many developing. Countries (Akaneme, 2008).

METHODOLOGY

After collection of the plant, the stems were shade-dried at room temperature to constant weight over a period of six weeks. The dried stem of *Portulaca oleracea* were weighed and ground to fine powder. Successive Solvent extraction by cold maceration was done for 72 hours. Methanol solvent was used.

A 500g portion of the pulverized stem of *Portulaca oleracea* was macerated by soaking in 1.5L of methanol for 72hrs with fresh replacement of solvent 24hourly. The combined filtrate was obtained by filtration using whatman's No1 filter paper and then concentrated with rotary evaporator at 45°C *in vacuo* and later transferred to an evaporating dish and dried over a water bath. At 45°C, the dried methanol stem extract is obtained.

18 animals were used for the Acute oral Toxicity study and 64 for the experimental study proper making a total of 82 animals.

For the experimental test proper, following acclimatization, the animals were randomly assigned to four groups of sixteen (16) animals each for treatment as follows:

Group A (Control): received 1.5 ml of clean water.

Group B: Received 125mg/kg body weight of extract

Group C: Received 250mg/kg body weight of extract

Group D: Received 500mg/kg body weight of extract

Administration of extract or water was by oral gavage daily for 60 days. Animals' weights were taken weekly on days of sacrifice and the dose adjusted accordingly. On days 15, 30, 45 and 60, four (4) rats from each group were anaesthetized and dissected. Blood samples were collected with plain bottle while the testes were harvested and placed in plane bottles containing formalin. The collected blood samples were used for estimation of testosterone level in the blood and the testes were used for sperm analysis (sperm count, sperm viability & sperm morphology) and histology.

RESULTS AND DISCUSSION

Acute toxicity test showed no mortality, morbidity or other apparent signs of toxicity at the doses used which were 1600mg/kg 2900mg/kg and 5000mg/kg.

Male Reproductive Parameters

From this study aqueous methanol stem extracts of P. O. caused an increase in testosterone level of all the test groups except the ones treated with 250mg/kg of the extract on day 30 which showed a slight decrease in level relative to the control.

Significant increase occurred in 125mg/kg, 250mg/kg & 500mg/kg of day 45; and of day 60.

This indicates that the extract has the propensity to increase serum testosterone level especially when used for a long duration and at increased doses.

The reduction observed in test group treated with 250mg/kg on day 30 may be due to a systemic reaction on the Hypothalamus – pituitary – gonadal pathway following a mimicking of the hormone by the extracts, Inhibiting the enzyme/pathway for the hormone production. This reaction was probably overwhelmed with continued administration of the extract resulting in increase in testosterone level in the remaining days of the study.

The sperm cell counts of all the test groups on all the days of the study were higher than that of the control with that of the 250 and 500mg/kg groups being significant ($p < 0.05$) This indicates that the extracts had no adverse effect on the sperm cell count and characteristics but rather boosted the counts and can therefore have a pro-fertility effects especially when used for a longer duration and at higher doses. The significant increased was noticed on days 45 and 60 of the study indicating

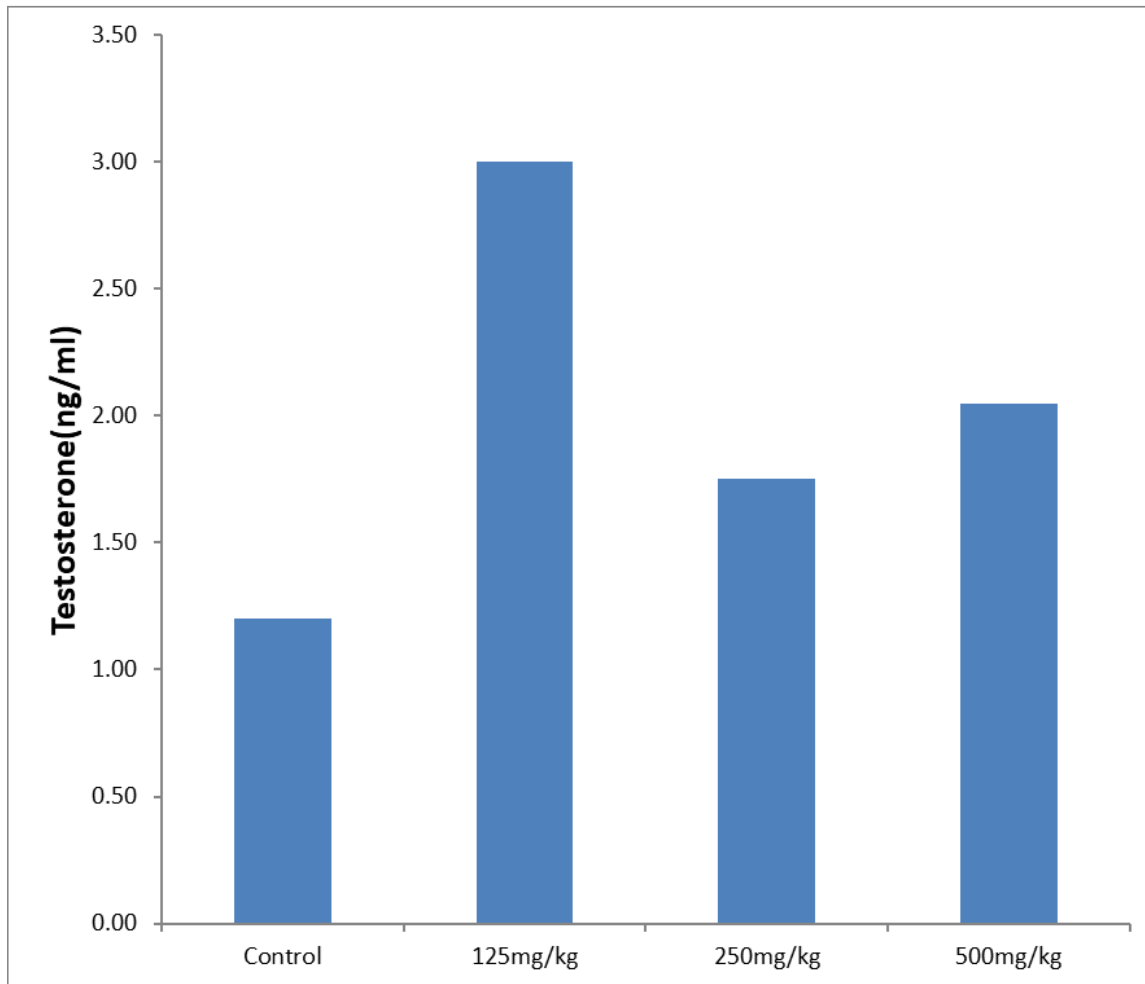
that increasing the duration of administration has a positive effect which is an increase effect on the sperm counts. This therefore means that the extracts can be used in men with infertility secondary to low sperm counts as it has the ability to boost sperm counts. The significant increase was also noticed in groups treated with 250mg/kg and 500mg/kg meaning that the beneficial effect on fertility will be seen on higher doses.

It was also observed in the study that except on day 30 where there was a decline in testosterone level, there was a significant increase in testosterone in the remaining days with significant increase in sperm cell counts on these days as well. It therefore means that testosterone can cause a stimulatory effect on sperm cell production as supported by Kagbo and Obinna, (2017).

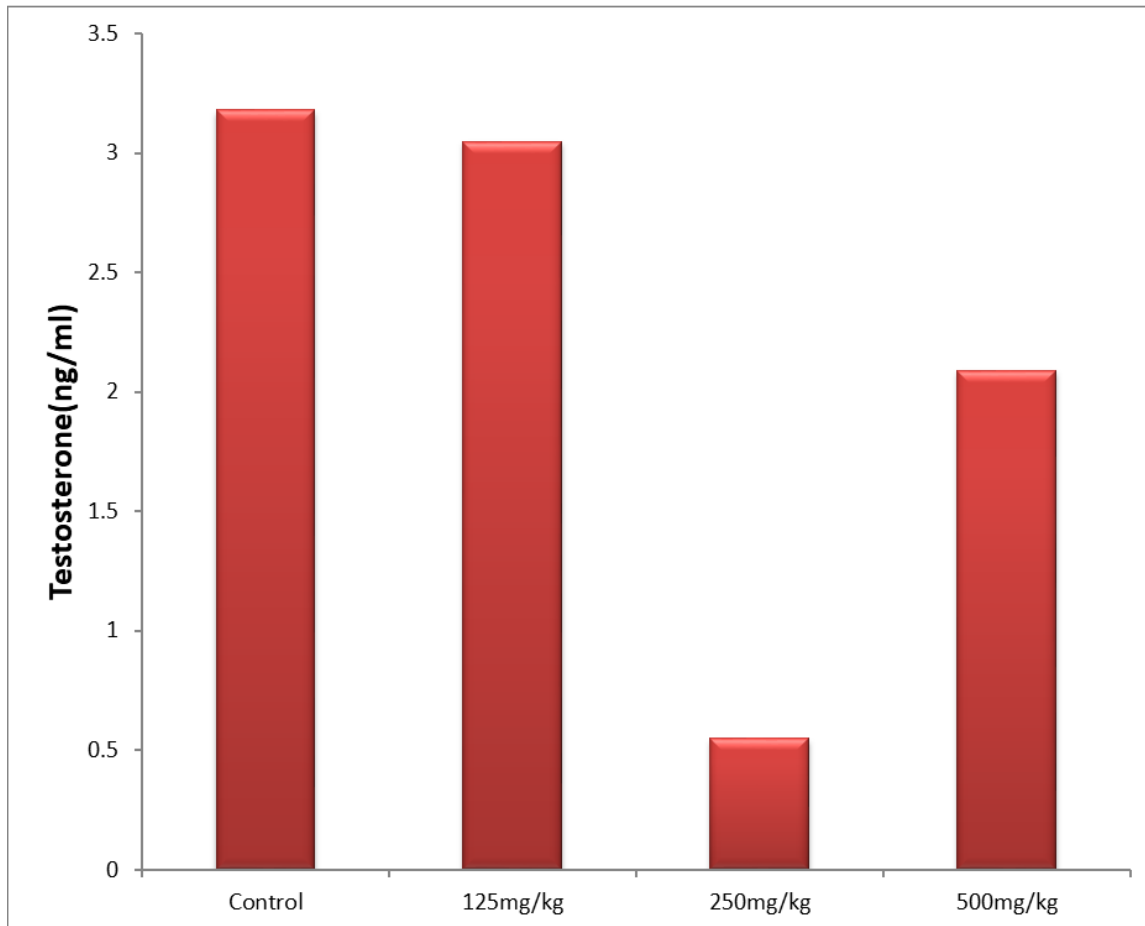
Walker's (2009) findings that testosterone regulates a man's fertility, germ cell growth, and sperm production are supported by these findings. In response to gonadotropins from the anterior pituitary gland, the testes release a considerable quantity of testosterone, which is necessary for spermatogenesis (Zhang et al., 2003). The testes generate testosterone, which in turn interacts with sertoli cells to promote spermatozoa development (Sharpe et al., 1990).

Testosterone guarantees spermatogenesis process since it promotes meiotic development (Reece, 2015). It is important to remember that spermatogenesis stops at the meiosis stage if neither testosterone nor the androgen receptor produced by the sertoli cells is present (Walker, 2011).

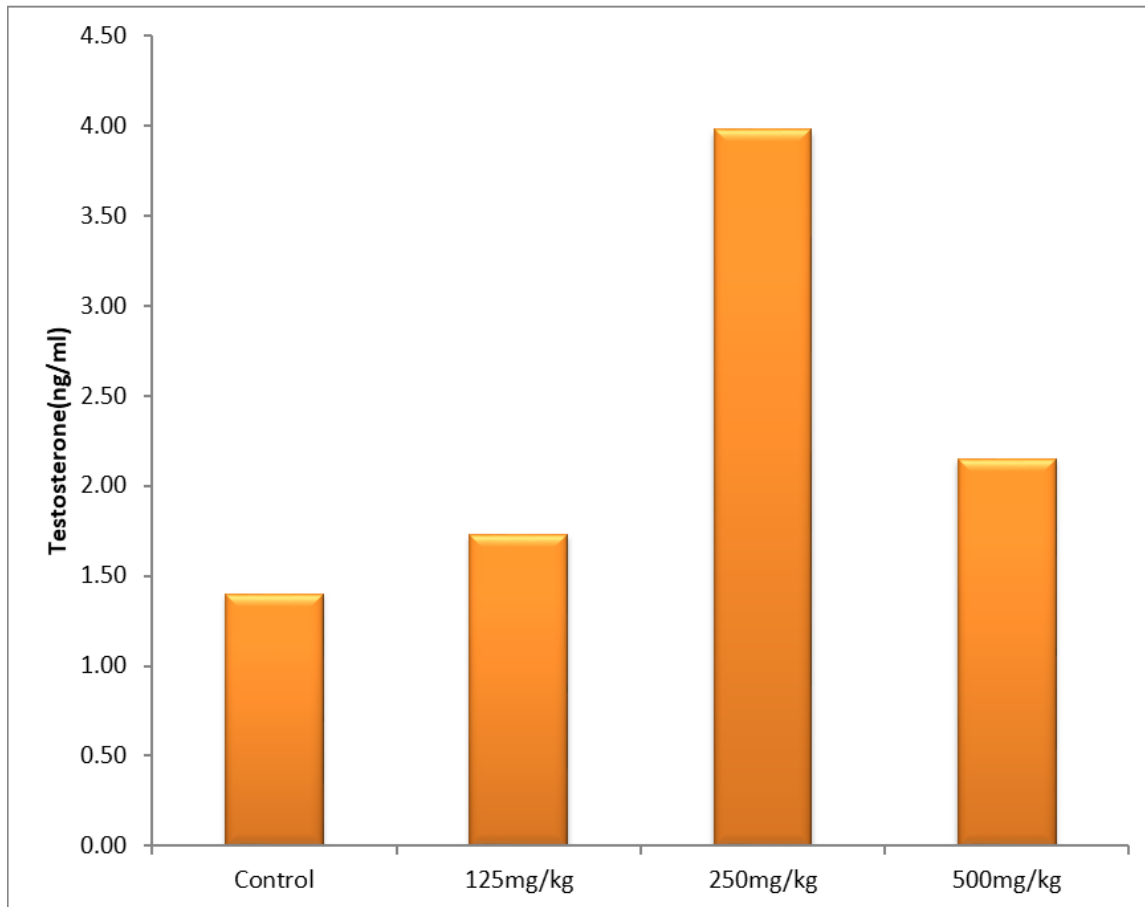
The extracts had no abnormal effect on sperm cell morphology rather caused an increase though non-significant of normal sperm cells. This also points out it's pro-fertility effect.



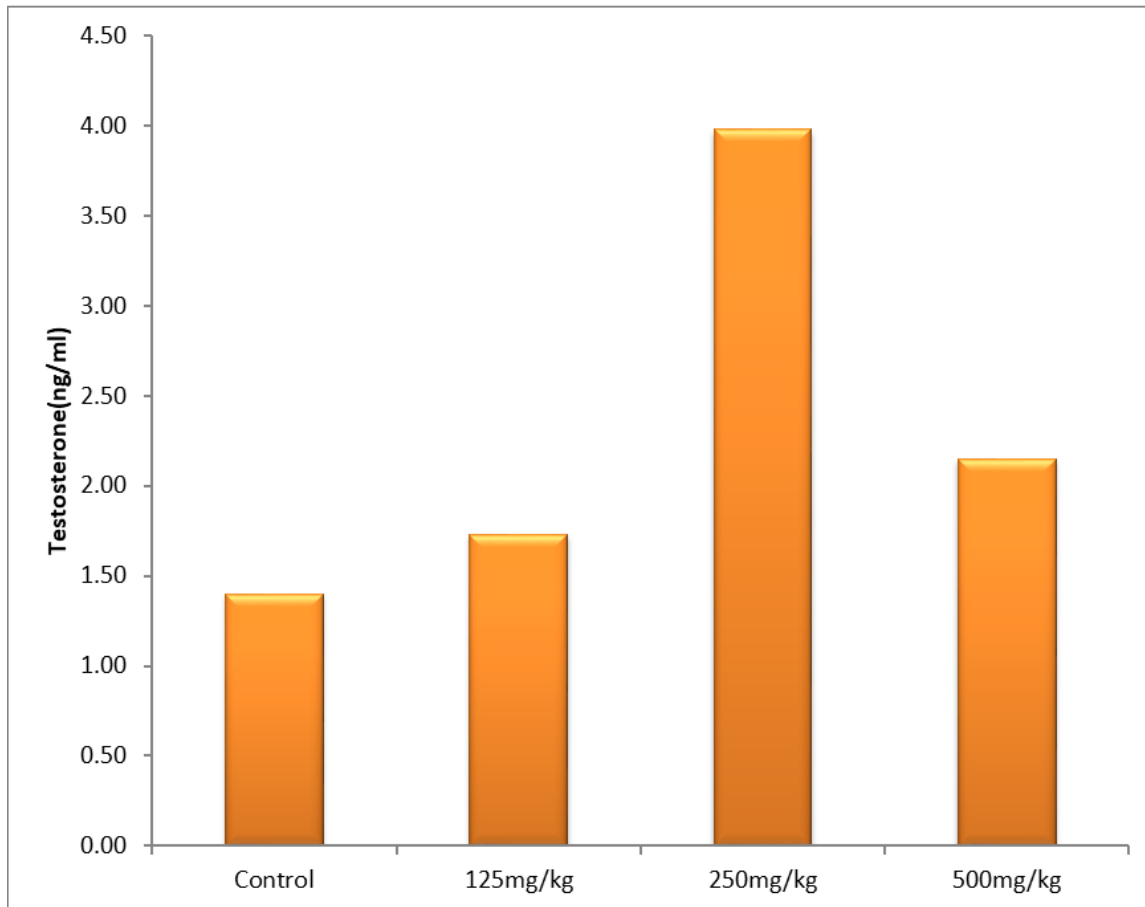
Effect of *Portulaca oleracea* on serum testosterone on day 15



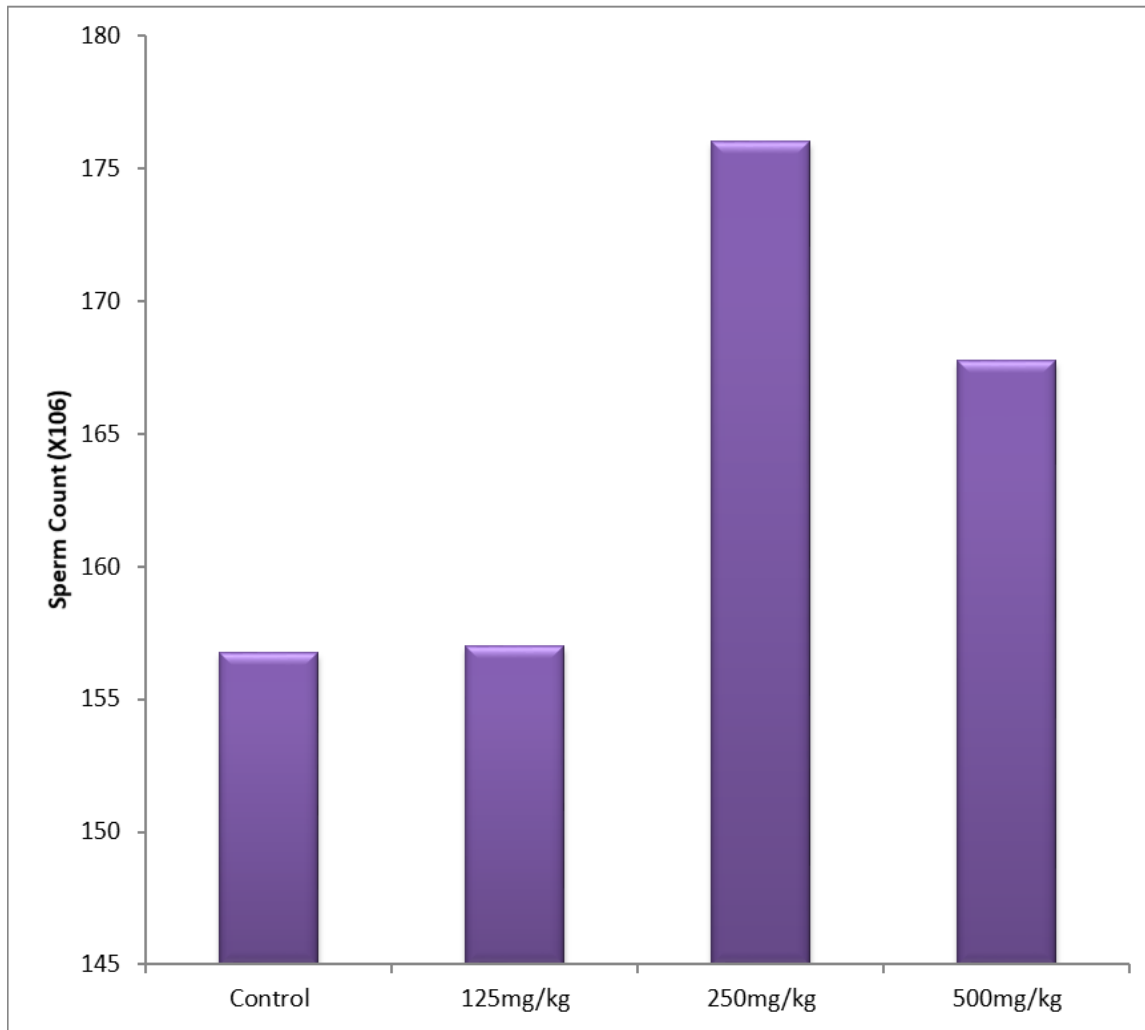
Effect of *Portulaca oleracea* on serum testosterone on day 30



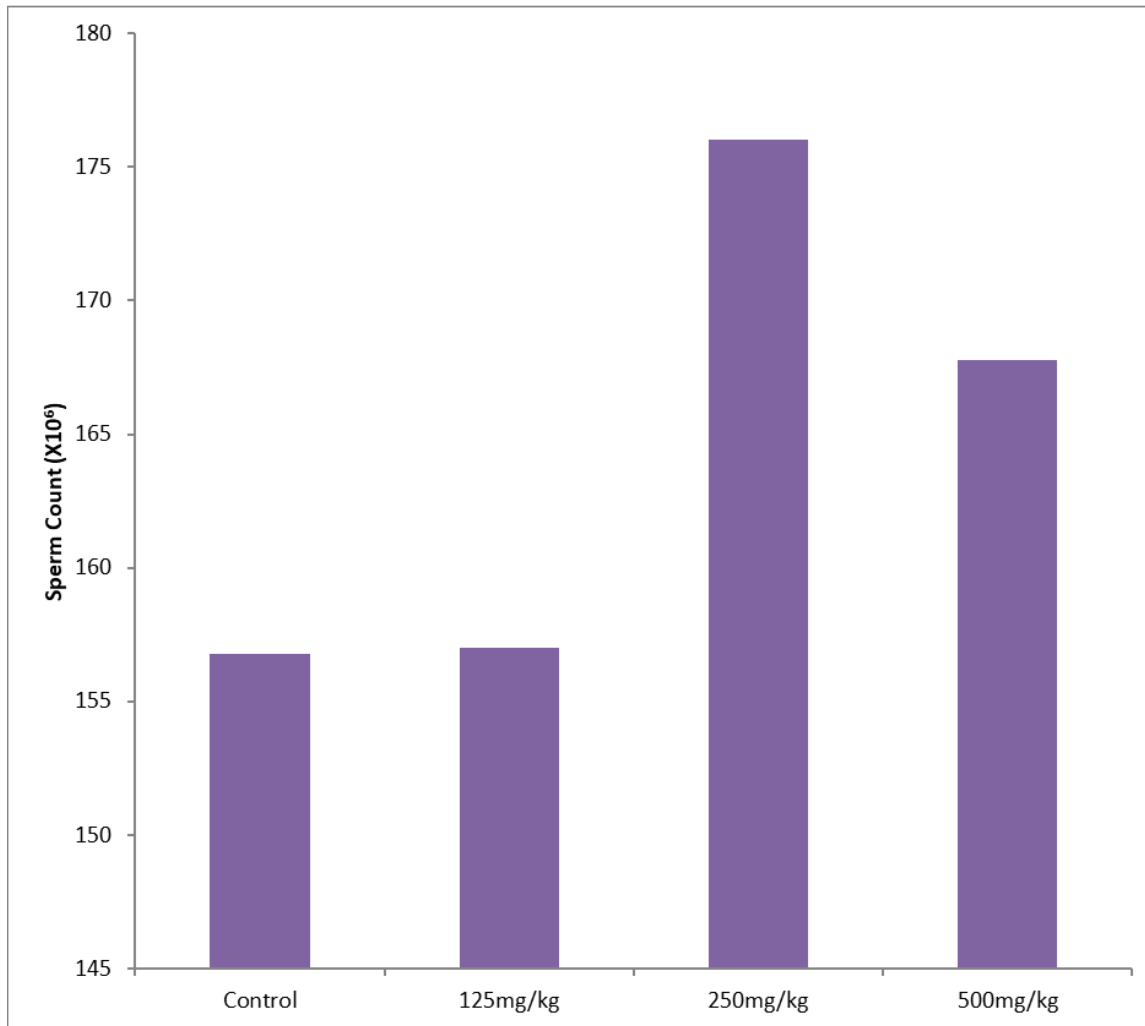
Effect of *Portulaca oleracea* on serum testosterone on day 45



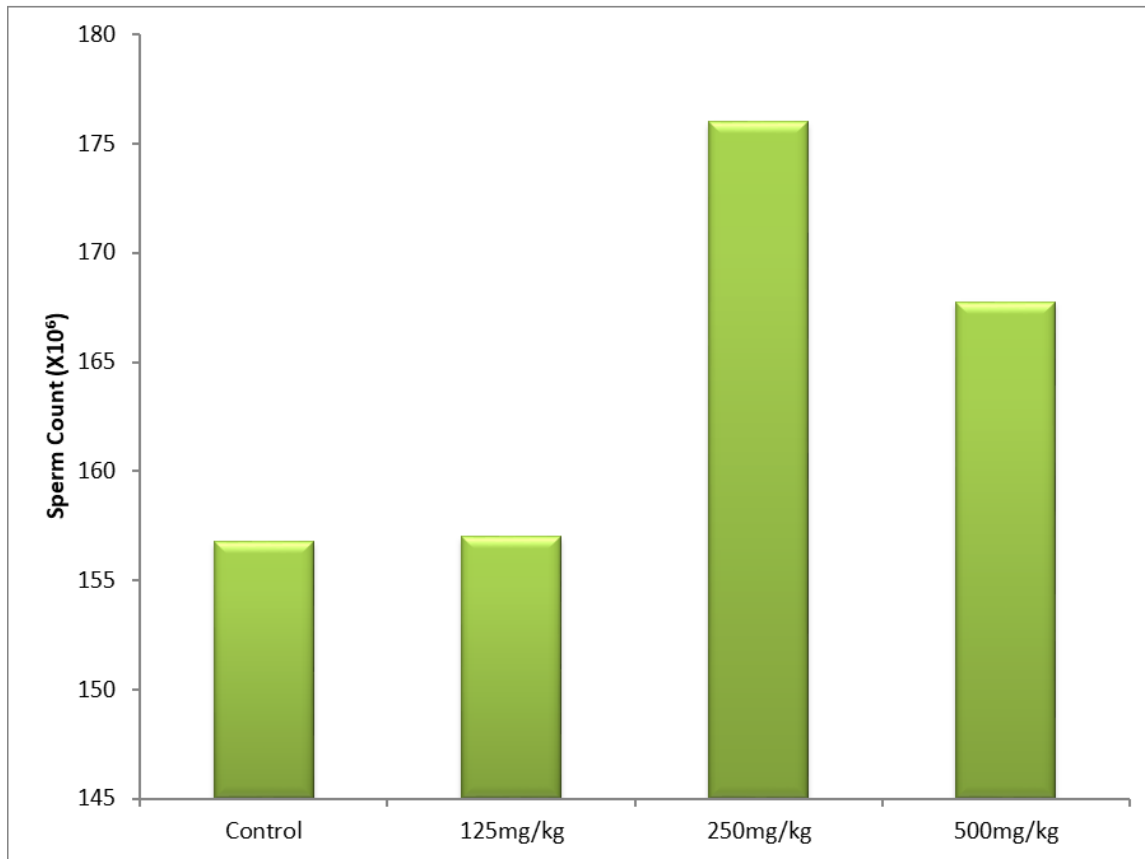
Effect of *Portulaca Oleracea* on serum testosterone on day 60



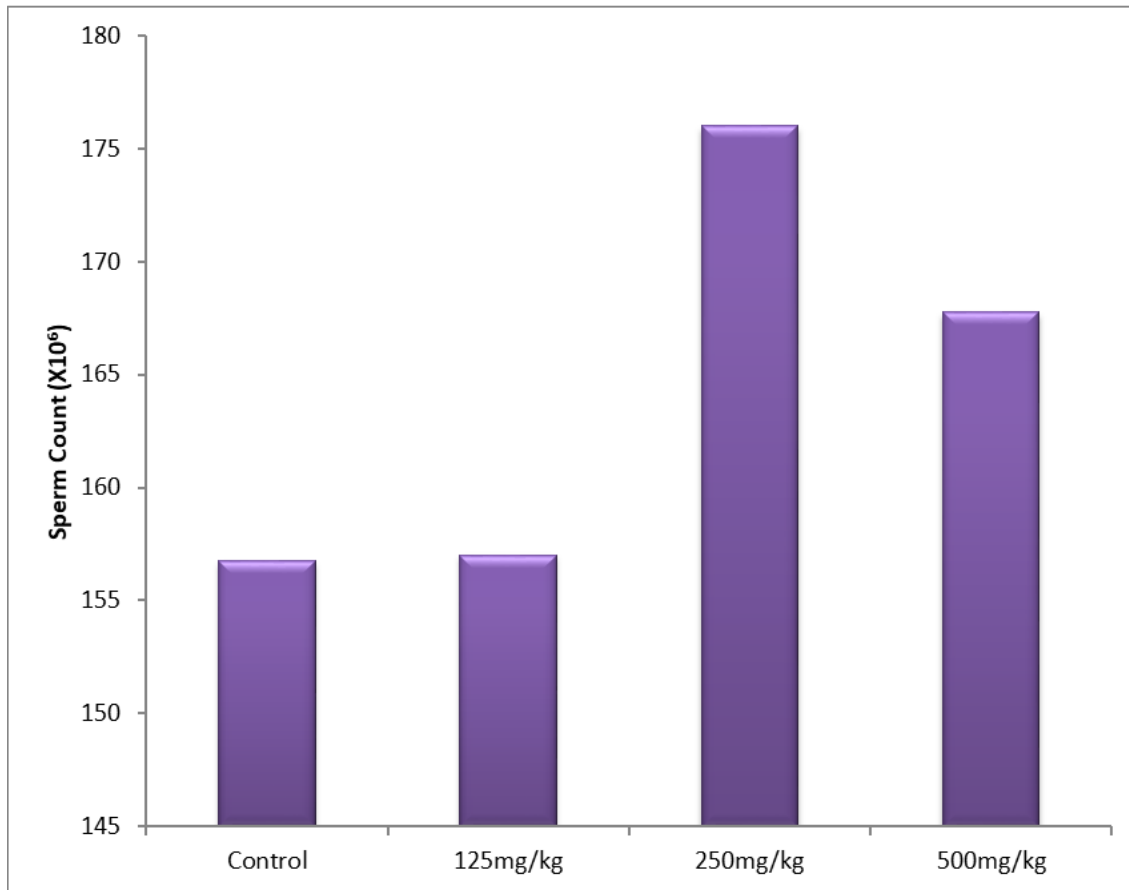
Effect of *Portulaca oleracea* extract on Sperm count on day 15



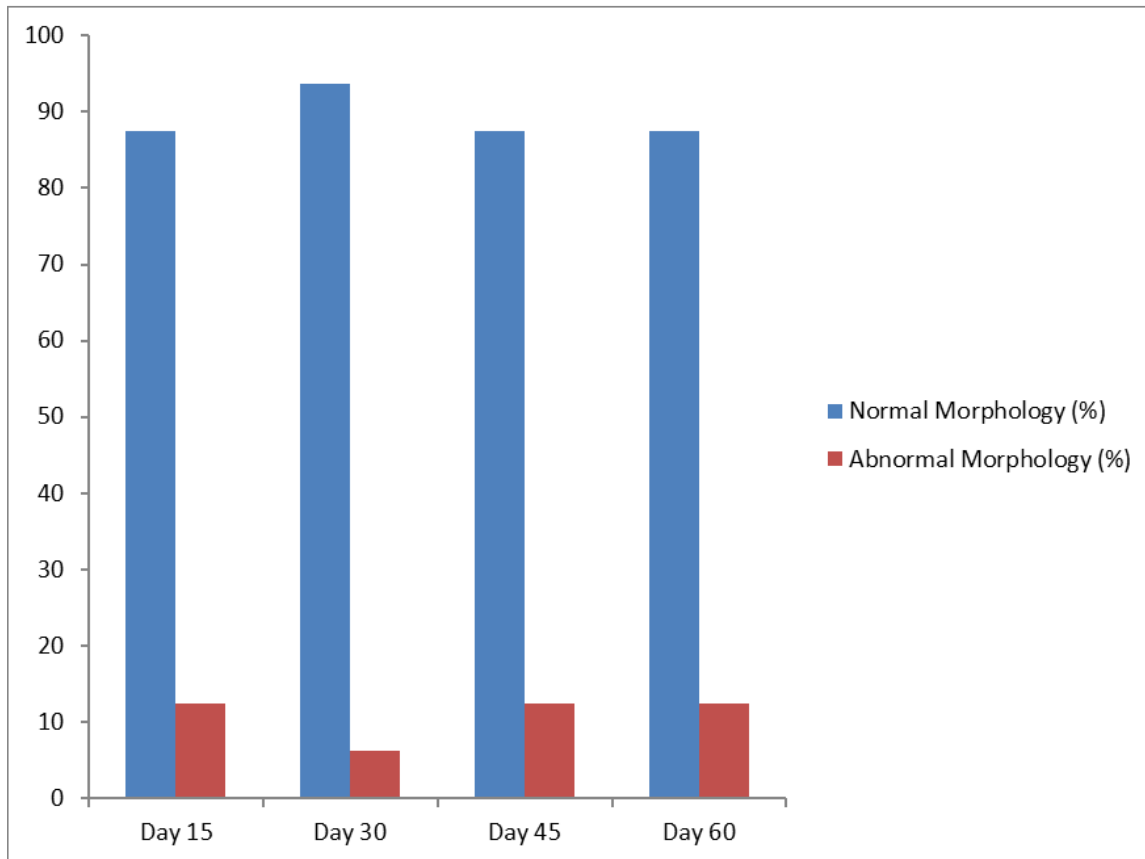
Effect of *Portulaca oleracea* extract on Sperm count on day 30



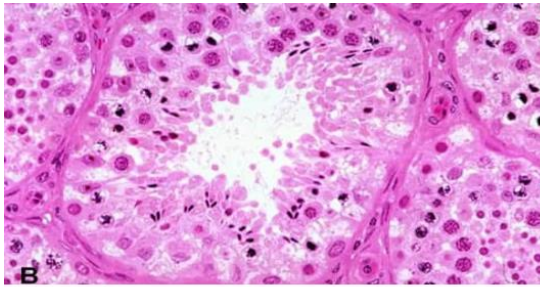
Effect of *Portulaca oleracea* extract on Sperm count on day 45



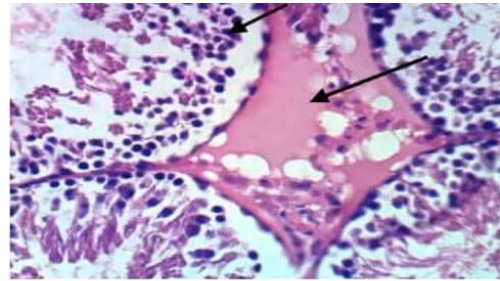
Effect of *Portulaca oleracea* extract on Sperm count on day 60



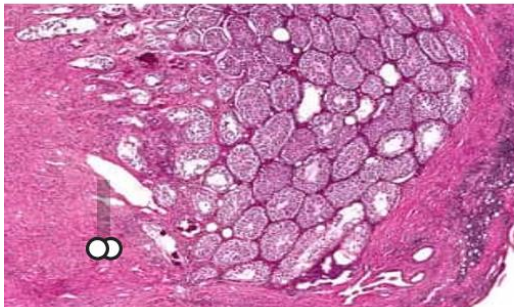
Effect of *Portulaca oleracea* extract on Sperm Morphology



Normal



Necrosis (1 of 4 at 250mg/kg dose)



Fibrosis (1 of 4 at 500mg/kg dose)



Autolysis (1 of 4 at 250mg/kg dose)

Histologic changes seen and normal

CONCLUSION

This study has shown that methanol stem extracts has no acute toxicity

The extracts was observed to cause an increase in testosterone levels especially when administered over a longer duration of time,

The extracts caused an increased in sperm cell counts and an increase in sperm morphology thus has a pro-fertility effects and can therefore be used as a fertility booster.

RECOMMENDATIONS

Further research should be carried out to

- i. determine the mechanism behind the histology change in the testes of the selected few animals who had abnormal histology to be sure it had nothing to do with the stem extracts.
- ii. Identify the mechanism for the increase testosterone on prolong administration.
- iii. Extracts of other parts of the plant should be studied individually for reproductive effect.
- iv. Clinical trials of these extracts should be carried out.

CONTRIBUTION TO KNOWLEDGE

- *Portulaca oleracea*, from this study has been proven apparently safe for consumption
- It was observed to cause an increase in testosterone, sperm cell count and improved fertility thus can be used as fertility booster in men
- It may also reduce abnormal morphology when used at higher doses and at a longer duration thus improving fertility as well.

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