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Effects Of Crude Extracts Of *Portulaca Oleracea* On Male Reproductive Functions In Albino Rats

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Abstract: *Portulaca oleraces is a shrub which is grown as a vegetable in many parts of the world. Many studies have reported the hypoxic nerve tissue protective, anti- inflammatory and wound-healing effects of its extracts, but there is scanty information on its effect on reproduction.*

This study was designed to investigate the effect of its aqueous and methanolic extracts on reproductive parameters in male albino rats.

Aqueous and methanolic extracts are designated as AEPO and MEPO respectively. AEPO and MEPO were prepared using cold maceration. The extracts at various doses (25 mg/g, 50mg/kg and 75 mg/kg) were orally administered for 50 days. Distilled water (0.5ml) served as the control. Plasma testosterone levels were assayed using Enzyme-linted I(mmunosorbent Assay (ELISA) and semen analysis was done microscopically, histology of the testes was also done. Data were analysed using ANOVA at $p< 0.05$.

Treatment of rats with all the doses (except 25 mg/kg and 50 mg/kg AEPO) caused significant ($p < 0.05$) decrease in testosterone levels. Treatment of rats with all the doses (except 25 mg/kg AEPO) caused significant ($p < 0.05$) reduction in % progressive sperm motility, while treatment with all the doses cause significant ($p < 0.05$) increase in % abnormal sperm cells. The extracts (AEPO and MEPO) also caused reductions of germinal epithelial cells.

It can therefore be suggested that Portulaca oleracea has deleterious effect on the reproductive functions in male albino rats

Key words: *Portulaca oleracea, Testosterone, sperm count, Albino rats, Sperm motility*

I. Introduction

Portulaca oleracea belongs to the family of Portulacaceae. It is commonly called Purslane in English language, babbajibji in Hausa language and esan omode or papasan in Yoruba language. It is a fleshy annual herb, much-branched and attaining 30 cm long (Burkill, 1997).

It is used medicinally in Ghana for heart-palpitations (Johnson, 1997). The plant is used as a diuretic in Nigeria (Ainslie, 1973). A tisane of the plant is drunk in Trinidad as a vermifuge (Wong, 1976).

At some areas near Benin City (Nigeria), the plant, along with other ingredients is taken as an aid to the development of the foetus (Vermmer, 1976).

It has been reported that aqueous and methanolic extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations (Oyedeji *et al,* 2007).

The extracts of *Portulaca oleracea* have been reported to have protective effects on hypoxic nerve tissue (Wang *et al,* 2007), anti-inflamatory effects (Xiang *et al,* 2005) and wound-healing activity (Rashed *et al,* 2003). Parry *et al* (1987) also reported the skeletal muscle relaxant effect of the plant.

This study aims at investigate the effects of aqueous and methanolic extracts of *Portulaca oleracea* on male reproductive functions in albino rats.

II. Materials And Methods

Experimental Animals

Adult male albino rats weighing between 150 g and 250 g bred in the Pre-clinical Animal House of the College of Medicine, University of Ibadan were used. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water; and were acclimatized for two weeks to laboratory conditions before the commencement of the experiments.

Plant Material

Fresh specimens of *Portulaca oleraceae* 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.

Preparation of the Extracts

Large quantity of the 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 pulverished using laboratory mortar and pestle, and then divided into two samples A and B.

(i) Aqueous Extract of *Portulaca oleracea* (AEPO)

Weighted Portions (431.33g) of sample A were macerated and extracted with distilled water (1:2 wt/vol) for 72 hours at room temperature (26 – 28^{O}C). The resulting solution was then filtered using a wire-gazed 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 staring material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

(ii) Methanolic Extract of *Portulaca olearacea* (MEPO)

Weighted portions (420.52g) of sample B were macerated and extracted with 70% methanol (1:2 wt/vol) for 72 hours at room temperature (26 – 28^{O}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 five 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 gramme of AEPO and MEPO were dissolved in 100ml of distilled water to give a concentration of 0.1g/ml.

The dosages of AEPO and MEPO administered in these studies were in accordance with those reported by Miladi-Gorgi *et al*. (2004).

Experimental Design

Thirty-five adult male rats were used. The rats were divided into seven groups with each group consisting of five rats.

The AEPO and MEPO were administered orally per day to the first six groups of rats for fifty days as follows:

Group I received 25 mg/kg of AEPO
Group II received 50 mg/kg of AEPO
Group III received 75 mg/kg of AEPO
Group IV received 25 mg/kg of MEPO
Group V received 50 mg/kg of MEPO
Group VI received 75 mg/kg of MEPO
Group VII received 0.5 ml of distilled water as the control group.

Twenty-four hours (day 51) after the last dosing of the animals, blood samples were collected for hormonal assay and the animals were then euthenised by cervical dislocation for semen analysis. Histological preparation of the testes was also carried out.

Body Weight

Body weight was monitored on weekly basis.

Collection of Blood Sample

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 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) (Mhammad-Reza, 2005).

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 calculated (Laing, 1979).

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-salline 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.

Testicular Histology

After weighing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70% alchol. The tissues were then cut in slabs of about 0.5cm transversely and the tissues were dehydrated by passing through different grades of alchol: 70% alchol for 2 hours, 95% alchol for 2 hours, 100% alchol for 2 hours, 100% alchol for 2 hours and finally 100% alchol for 2 hours. The tissues were then cleared to remove the alchol, the clearing was done for 6 hours using xylene. The tissues were then infiltered in molten Paraffin wax for 2 hours in an oven at 57^{O}C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5µm). The satisfactory ribbons were picked up from a water bath (50^{O}-55^{O}C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70%, 90% and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications

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 $p < 0.05$.

III. Results

Effect on Body Weight

The administration of all the treatment doses of AEPO and MEPO for 50 days produced no significant ($P > 0.05$) changes in the body weight of the treated rats relative is the control as shown in Figure 1.

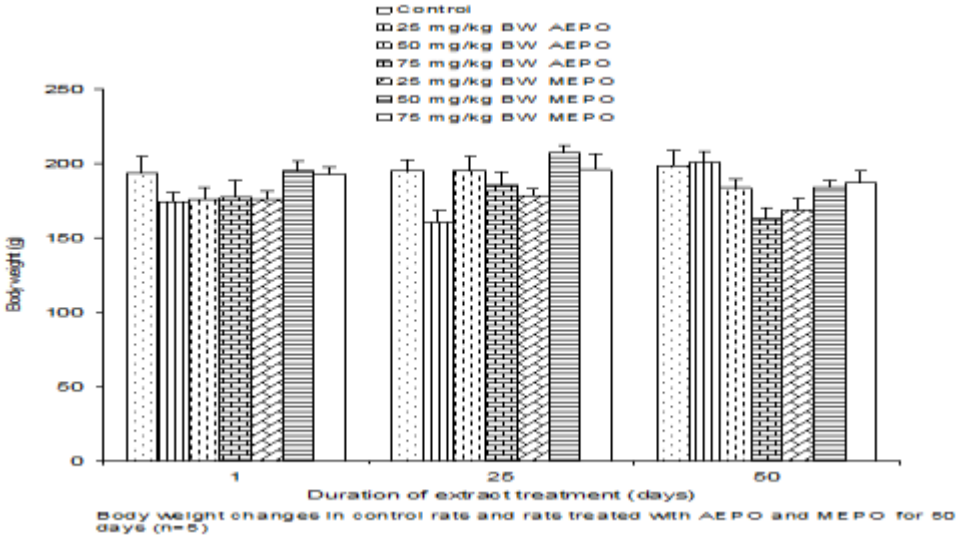
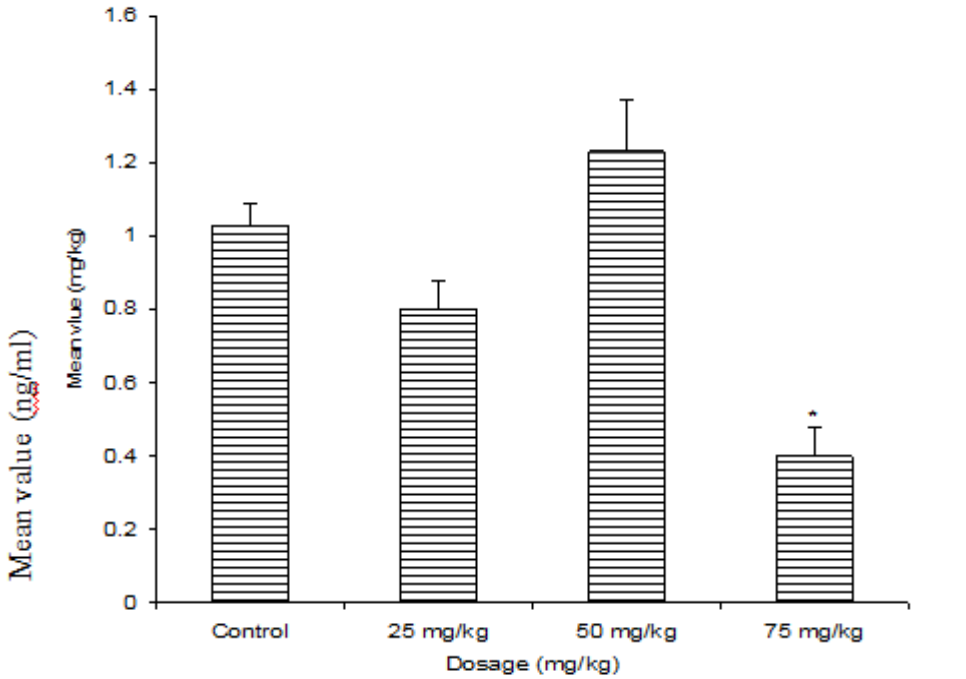


Figure 1: Body weight changes in control rats and rats treated with AEPO and MEPO for 50 days (n =5)

Effect on hormonal Levels

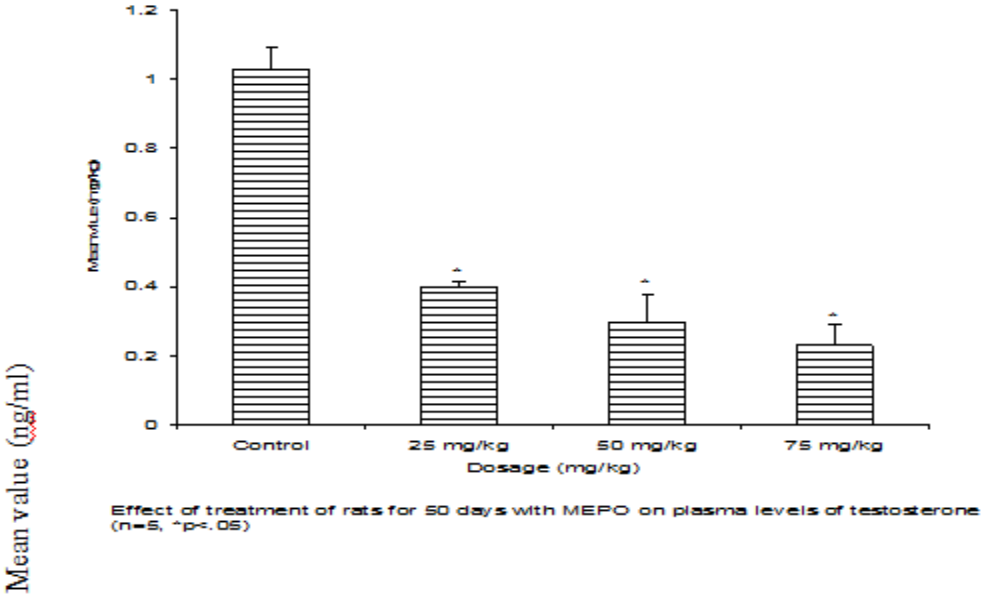
Treatment of rats with all the dose of AEPO and MEPO (except 25 mglkg and 50 mglkg AEPO) for 50 days produced significant ($P < 0.05$) decrease in testosterone levels relative is the control as shown in Figure 2 and 3.

Figure 2: Effect of treatment of rats for 50 days with AEPO on serum levels of testosterone (n=5, *P<0.05).



Effect of treatment of rats for 50 days with AEPO on plasma levels of testosterone (n=5, *p<.05)

Figure 3: Effect of treatment of rats for 50 days with MEPO on serum levels of testosterone (n=5, *P<0.05).



Effect on Sperm Characteristics

Treatment of rats for 50 days with all the doses of AEPO and MEPO (except 25 mg/kg AEPO) caused significant ($p < 0.05$) decrease in percentage progressive sperm motility relative to the control as shown in Figure 4. However, treatment with all the does of AEPO and MEPO produced non-significant ($p > 0.05$) reductions in the percentage of viable sperms relative is the control as shown in Figure 4. Treatment of rats with all the treatment doses of AEPO and MEPO caused significant ($p < 0.05$) reductions in sperm counts relative to the control as shown in Figure 5. Treatment of rats with all the doses of AEPO and MEPO caused significant ($p < 0.05$) increase in the percentage of abnormal sperms relative to the control.

Figure 4: Spermogram showing the effect of AEPO on sperm characteristics after treatment of rats for 50 days (n=5, *P<0.05)

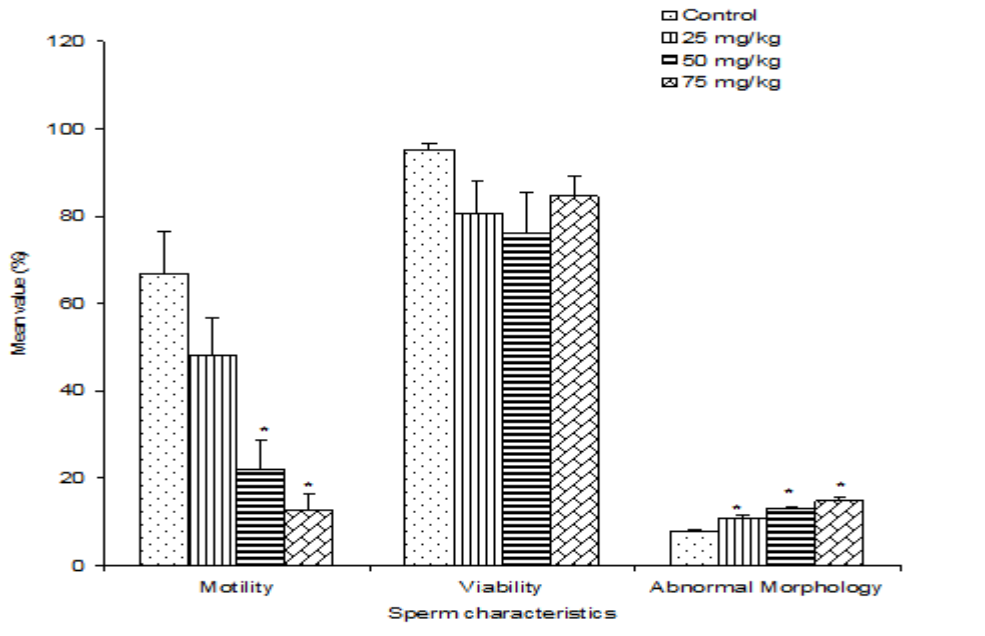


Figure 5: Spermogram showing the effect of AEPO on sperm counts after treatment of rats for 50 days (n=5, *P<0.05)

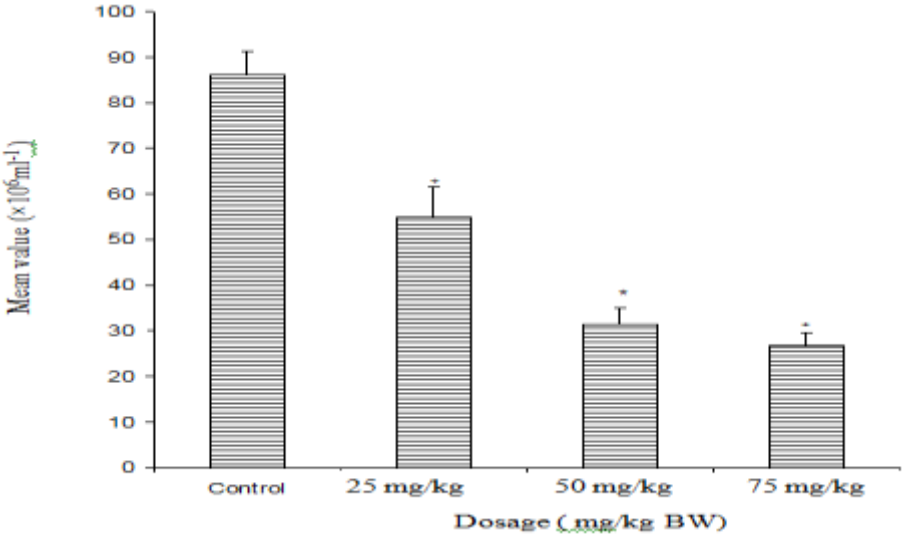


Figure 6: Spermogram showing the effect of MEPO on sperm characteristics after treatment of rats for 50 days (n=5, *P<0.05)

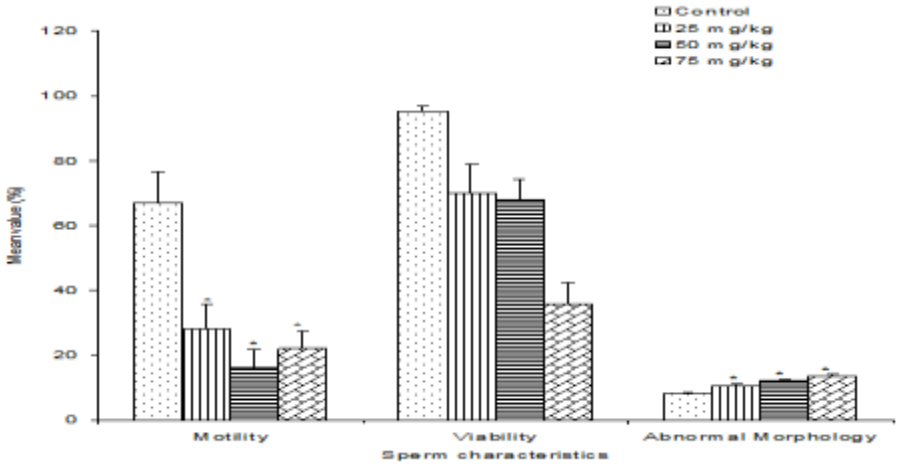
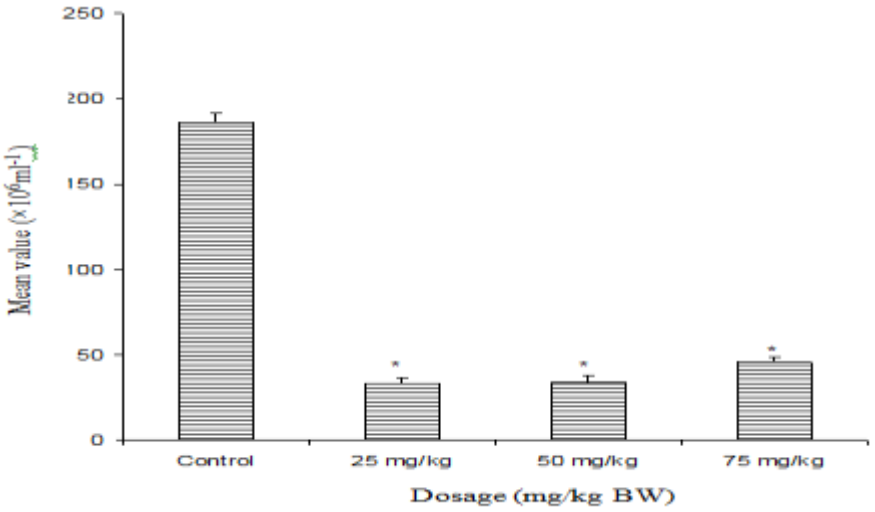


Figure 7: Spermogram showing the effect of MEPO on sperm counts after treatment of rats for 50 days (n=5, *P<0.05)



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Histopathological Findings

Treatment of rats for 50 days with the extracts (AEPO and MEPO) caused acellulars seminiferous tubules as well as Leydig cells hyperplasia while the control rats presented with normal germinal epithelium in the seminiferous tubules as shown in Plates 1 and 2.

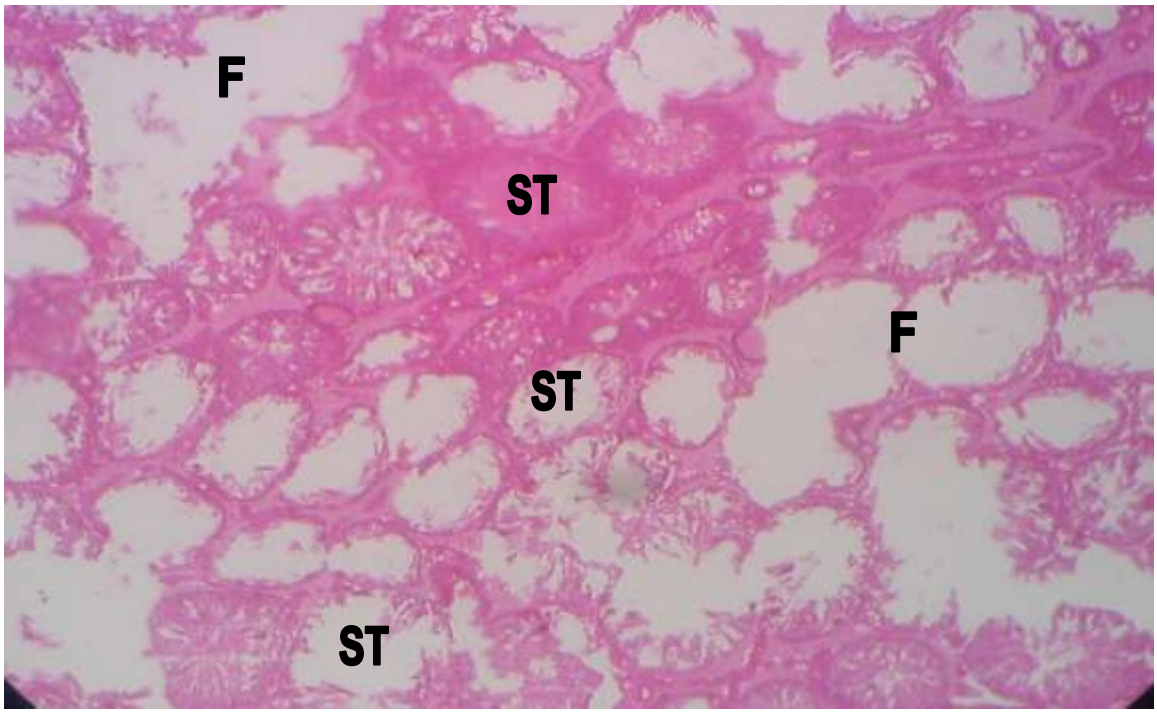

Plate 1: The effect of 75mg/kg MEPO on the testes at x 100 on day 50
Photomicrograph showing acellular and fused (F) seminiferous tubules (ST) with papillary configuration .

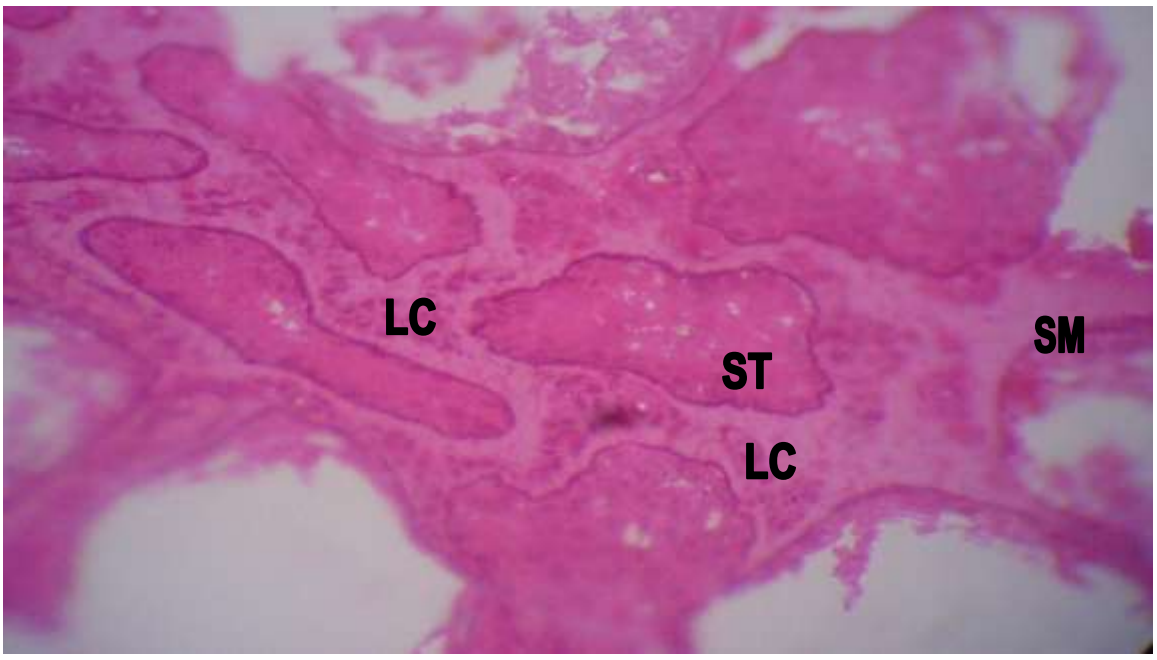

Plate 2: The effect of 75mg/kg MEPO on the testes at x 400 on day 50
Photomicrograph showing marked dense fibrosis of the stroma (SM) with Leydig cells (LC) hyperplasia.

IV. Discussion

The extracts caused non-significant changes in the body weight of rats after treatment for 50 days, this could be due to the absence of androgenic properties in this plant, since it has been reported that androgens possess anabolic activities (Johnson and Everitt, 1988) similar report was given by Gonzales *et al* (2006) in rats treated with *Lepidium meyenii* extracts.

The extracts caused significant decrease in testosterone levels. Similar report was given by Das *et al.* (2009) in rats treated with *Aegle mermelos* extract. This decrease in testosterone levels could indicate that the extract inhibit the mechanism intervening in the process of hormone synthesis in the Leydig cells.

The andrological results show that treatment of rats with the extracts caused significant decrease in sperm motility. Similar report was given by Verma *et al* (2002) in rats treated with *Sarcotemma acidum* extract. This suggests that the extract 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 barrier (Baldessarini, 1980) and thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in the outer part (Bloom and Fawcett, 1975).

There was a statistically non-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 extracts. This could be due to the ability of the extract 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 (William, 2000; Bowman and Rand, 1985).

Sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis (Reddy *et al.*, 2006). Spermatogenesis is influenced by the hypothalamic- adenohypophysial – Leydig cell system relating gonadotrophin releasing hormone, leutinizing hormone and androgen. This implies that the decrease in sperm count caused by the extracts in treated rats could 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 (Christensen, 1975). Similar report was given by Krishnamoorthy *et al.* (2007) in *Terminalia chebula* extract treated rats.

This study has also revealed suppression in sperm production in the extracts treated rats as evidenced by the reduction in the number of spermatogenic cells relative to the control. Similar results were found in *Colebrookia opositifolia* treated rats (Gupta *et al.*, 2001) and *Mentha arvensis* treated mice (Sharma and Jacob, 2001). This reduction in number of spermatogenic cells may be due to insufficient amount of testosterone, since it has been reported that spermatogenesis is activated by testosterone which is synthesized by Leydig cells and act on Sertoli cells and peritubular cells (Sharpe, 1987).

There was an increase in the number of Leydig cells in the extracts treated rats, but there was a decrease in testosterone production by the treated rats, this could indicate that the Leydig cells were deformed or immature since it has been reported that the number of mature Leydig cells has a direct bearing on spermatogenesis (Gupta *et al.*, 2000), and that the deformation of Leydig cells further indicates the inefficiency of these cells to synthesize testosterone (Reddy *et al.*, 1997).

In conclusion, these studies have shown that the crude extracts of *Portulaca oleracea* could have some toxic potentialities on the male reproductive functions of albino rats. However, the effects of the crude extracts of this plant on the 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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