Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(12):5-12

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effect of recovery period on reproductive functions in male wistar rats treated with fraction 2 of *Portulaca oleracea*

Oyedeji K. Olusina[1], Adegoke Olajire[2] and Oladosu I. Adebayo[3]

[1]*Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria*
[3]*Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan, Nigeria*

ABSTRACT

Portulaca oleracea is a fleshy annual herb which is distributed throughout the temperate and tropical areas of the world. The chromatographic fraction 2 of this plant has been reported to have deleterious effects on reproductive parameters in male rats. Air-dried specimen of Portulaca oleracea (3.2 kg) 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 bioassays. Twenty male rats (120-150 g) 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 and allowed a recovery (withdrawal) period of 50 days after which plasma testosterone level was assayed using Enzyme-Linked Immunosorbent Assay (ELISA) and semen analysis was done microscopically. There were significant ($p<0.05$) decrease in sperm motility of fraction 2 (1 mg/kg, 2 mg/kg, 3 mg/kg) recovery groups relative to control. Also, there were significant ($p<0.05$) decrease in sperm counts of fraction 2 (1 mg/kg, 3 mg/kg) recovery groups relative to the control. It can therefore be concluded that the deleterious effects induced by chromatographic fraction 2 of Portulaca oleracea on the reproductive parameters in male rats were probably not totally reversible.

Keywords: Fraction 2, Recovery, Sperm motility, Sperm counts, 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 "Esan omode" 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 chromatographic fraction 2 of this plant has been reported to have deleterious effects on the reproductive parameters in male rats [8], this study therefore aims at investigating the effect of recovery period on the reproductive profiles in male rats treated with fraction 2 of *Portulaca oleracea*.

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, 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 *Portulacaoleracea* 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 silical gel and placed in the oven at a reduced temperature (45 – 50 ^{0}C) overnight and then subjected to open column chromatography on silical 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 parameters in male rats were evaluated.

Acute Toxicity Test of Chromatographic Fraction
The acute toxicity test of chromatographic fraction 2 of *Portulaca oleracea* was evaluated inmice 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 daysand allowed a recovery (withdrawal) period of 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 101) after the last day of the recovery period, blood samples were collected from all the animals through the medial cantus for the determination of plasma testosterone levels. All the animals were later sacrificed by overdose of diethyl ether and the testes were removed along with the epididymides 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) [10].

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 [11].

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 [11]. 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 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-saline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the Improved Neubauer hemocytometer 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 or changes in behavior were observed in all the treated and control groups of rats.

There were significant ($p<0.05$) increases in plasma testosterone levels of fraction 2 (1 mg/kg, 2 mg/kg, 3 mg/kg) recovery groups relative to the control (Figure 1).

There were significant ($p<0.05$) reductions in sperm motility of fraction 2 (1 mg/kg, 2 mg/kg, 3 mg/kg) recovery groups relative to the control. There were insignificant ($p>0.05$) changesin sperm viability of fraction 2 (2 mg/kg, 3 mg/kg) recovery groups relative to the control, while there was a significant ($p<0.05$) decrease in sperm viability of fraction 2 (1 mg/kg) recovery group relative to the control. There were significant ($p<0.05$) increase in the percentage of abnormal sperm cells of fraction 2 (1 mg/kg, 2 mg/kg, 3 mg/kg) recovery groups relative to the control (Figure 2).

There were significant ($p<0.05$) reductions in sperm counts of fraction 2 (1 mg/kg, 3 mg/kg) recovery groups relative to the control, while there was no significant ($p>0.05$) change in sperm counts of fraction 2 (2 mg/kg) recovery group relative to the control (Figure 3).

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

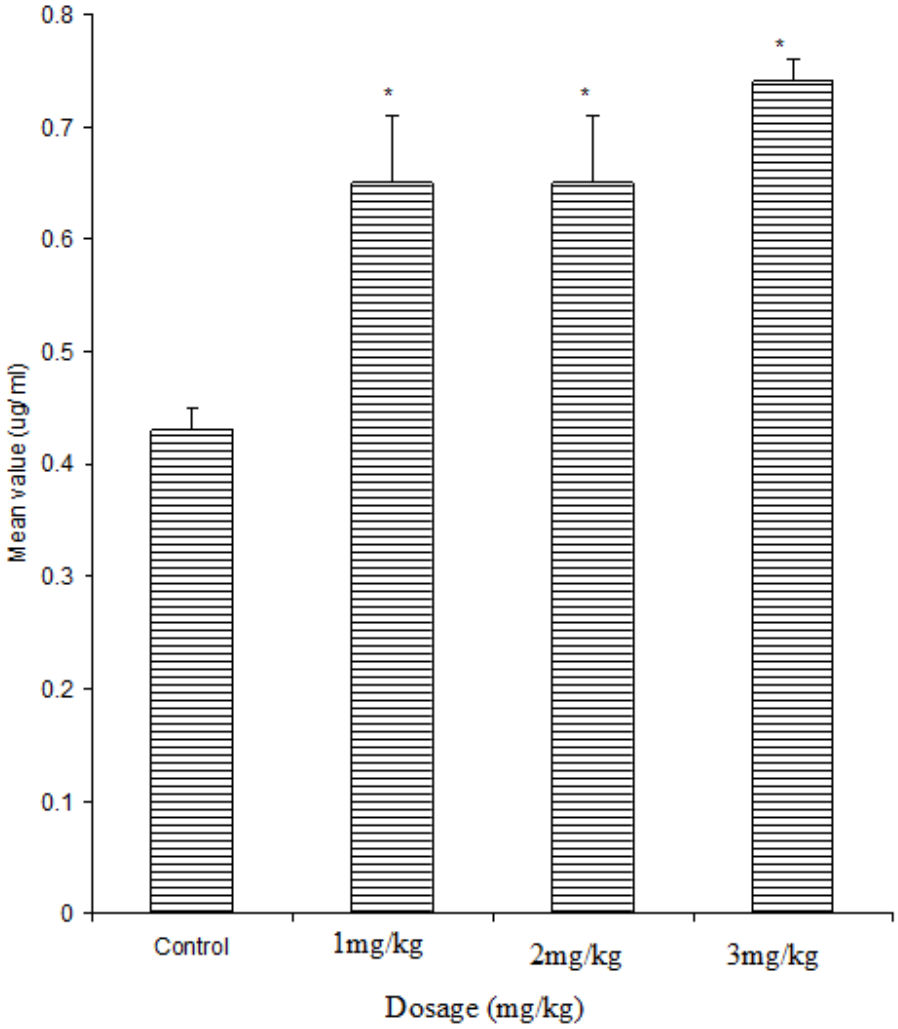
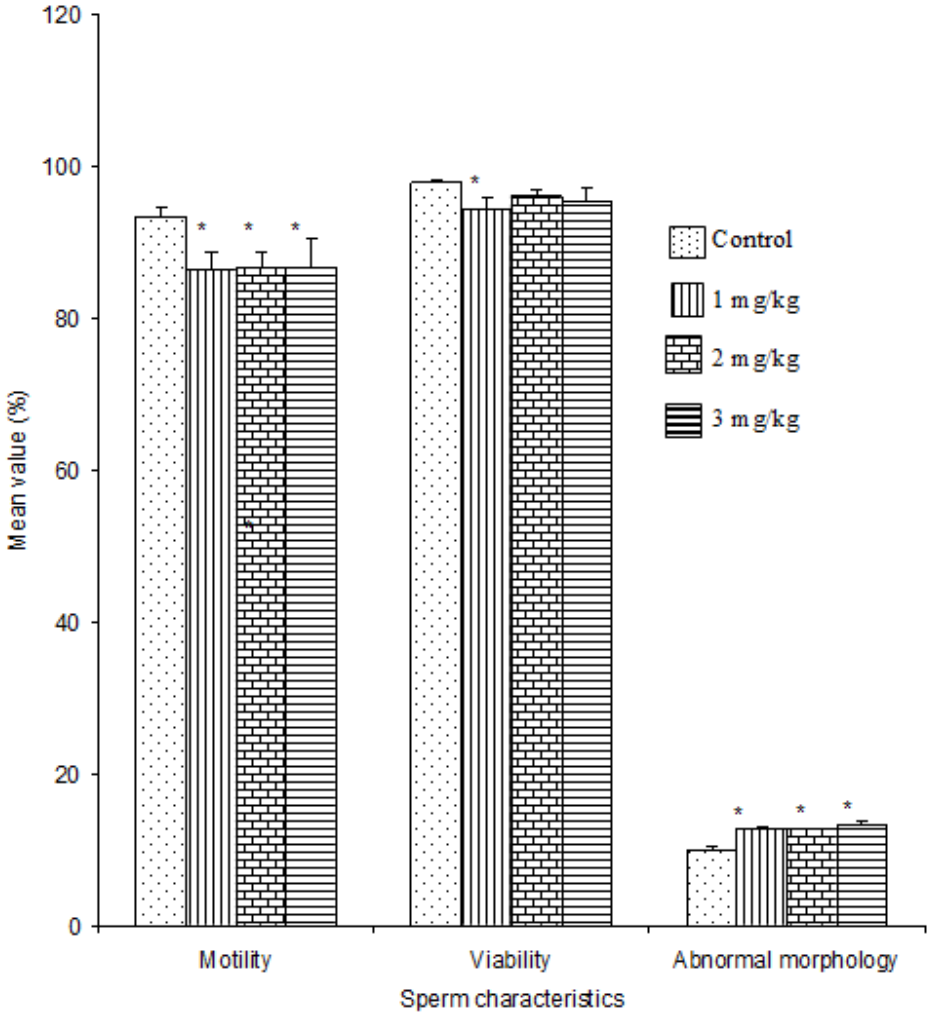


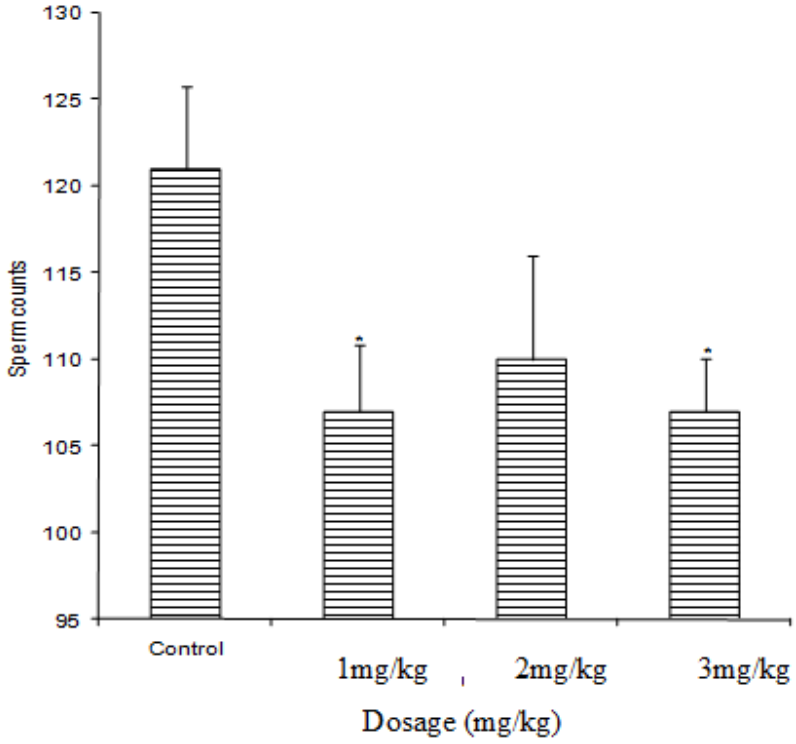
Figure 2: Spermogram showing the effect of 50 days recovery period from 50 days treatment of rats with fraction 2on sperm characteristics (n=5, *p<0.05)



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

There were significant increases in plasma testosterone levels after the recovery period. This increase in testosterone levels could probably be due to self-induced activation of androgen receptors by testosterone, since testosterone has been known to have the capability to activate the androgen receptor by itself. This increase in testosterone level 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 rats [12]. It could also be related to the hypersensitization of the hormone receptors during the recovery period. It was earlier reported [8] that there were significant reductions in testosterone levels after 50 days treatment with fraction 2,this increase in testosterone level after the recovery period probably indicates that the effect of the fraction was reversible at hormonal level. Contrary result was reported by [13] in *Ricinus communis* extract treated rats.

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



There were significant reductions in sperm motility after the recovery period. This could probably be due to the non-total renal clearance of this fraction leading to its accumulation in the ECF with a resultant manifestation of its biological activity probably by permeating 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 [14] and thus creating a different microenvironment in the inner part of the wall of the seminiferous tubules from the outer part [15]. It has earlier been reported [8] that treatment of rats for 50 days with fraction 2 caused significant reductions in sperm motility.

There wasa significant decrease in sperm viability as well as significant increases in the percentage of morphologically abnormal sperm cells after the recovery period. This could probably be due to the non-total renal clearance of this fraction leading to its accumulation in the ECF with a resultant manifestation of its biological activity probably by intervening with the spermatogenic process in the seminiferous tubules [16, 17]. It has earlier been reported [8] that treatment of rats for 50 days with fraction 2 caused significant reductions in sperm viability as well as significant increases in the percentage of morphologically abnormal sperm cells.

Sperm count is considered to be an important parameter to assess the effect of chemicals on spermatogenesis [18]. There were significant reductions in sperm counts after the recovery period. This could probably be due to the non-total renal clearance of this fraction leading to its accumulation in the ECF with a resultant manifestation of its biological activity probably by alteration of the microenvironment of the seminiferous tubules as earlier explained. It has earlier been reported [8] that treatment of rats for 50 days with fraction 2 caused significant reductions in sperm counts.

CONCLUSION

It can therefore be concluded that the deleterious effects induced by chromatographic fraction 2 of *Portulaca oleracea* on the reproductive profiles in male rats were probably not totally reversible.

REFERENCES

[1] HM Burkill. The Useful Plants of West Tropical Africa, Vol.4, The Whitefriars Press Limited, Tonbridge, Kent TN9 IQR, Great Britain, **1997**.
[2] A Zargari. Medicinal Plants Tehran: University Press,**1987**, 218-220.
[3] K Gholamreza;Z Toktam;N Azam, *Iranian J. Basic Med. Sci*., **2008**, 10 (4), 229-322.
[4] K Chan;MW Islam;M Kamil, *J. Ethnopharmacol*.,**2000**, 73, 445-451.
[5] KO Oyedeji;FS Oluwole; S Ademola, *Sci. Foc*., **2007**, 12, 14 –18.
[6] R Radhakrishnan;MNM Zakaria;MW Islam, *J. Ethnopharmacol*.,**2001**,76, 171-176.
[7] KO Oyedeji;AF Bolarinwa, *Afr. J. Biomed. Res*.,**2012**, 15, 41-47.
[8] KO Oyedeji. Effects of *Portulaca oleracea* Extracts on Hematological, Serum Biochemical and Reproductive Parameters in Albino Rats, A Ph.D Thesis Submitted to the Department of Physiology, College of Medicine, University of Ibadan, Nigeria, **2010**.
[9] LC Miller;ML Tainter, *J. Pharmacol*.,**1994**, 24, 839-840.
[10] P Mohammad – Reza; D Farzaneh; TK Taherch *et al*.,*Achv. Iranian Med*.,**2005**,8, 211-216.
[11] Laing JA. Fertility and Infertility in Domestic Animals, Third edition, Bailliere Tindall, a Division of Cassell Ltd, **1979**.
[12] DM De Krester. The Testis. In: Austin and Short (Eds.), A Hormonal Control of Reproduction, 2nd ed., **1987**, 76-90.
[13] Y Raji;AK Oloyo;AO Morakinyo,*Asian J. Androl*.,**2006**, 8, 115-121.
[14] RJ Baldessarini. In Drugs and the Treatment of Psychiatriod Disorders, The Pharmacological Basis of Therapeutics, Goodman and Gilman, Macmillan Pub. Co. Inc.,**1980**, 301 - 417.
[15] W Bloom, DW Fawcett. Male Reproductive System. Textbook of Histology, Saunders Company, Philadelphia,**1975**.
[16] KW William. Hormones and Hormone Antagonists, In: Remington, The Science and Practice of Pharmacy, 20th edition,**2000**, 1390 – 1391.
[17] WC Bowman, MJ Rand.The Reproductive System and Drugs Affecting the Reproductive Systems. Textbook of Pharmacology, 2nd edition, **1985**, 1 - 8.
[18] PS Reddy;T Pushpalatha;PS Reddy,*Toxicol. Lett*.,**2006**, 166, 53 – 59.