

Effect of Ethanolic Extract of Ginger on the Micro Anatomy of the Testis of Adult Wistar Rats

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Abstract

Ginger rhizome (*Zingiber Officinale Rosco*, family: *Zingiberaceae*) is used medicinally, it is known to have both nutritional and medicinal value. This study is to determine the effect of ethanolic extra of the rhizome of ginger on the microanatomy of the testis. Twenty five male Wistar rats were selected and randomly divided into control group and experimental group. The control group was further divided into two groups (A & B) with group A as normal control and group B as vehicle control. Group A took distilled water and feed and group B took distilled water, feed and 2mls of olive oil (as vehicle). The experimental group was divided into three groups (C, D & E) and received 100mg/kg, 250mg/kg and 500mg/kg daily according to their body weight for 14days in the 15th day the animals of all groups were sacrificed and the testes harvested and processed for histological observations. Histological sections of the experimental groups showed distortion of germinal and supporting cells, disintegration of matured sperm cells in the lumen, distortion of seminiferous tubules, widened interstitial spaces indicating shrinkage of tubules and destruction of leydig cells while sections of control groups showed normal histological architecture of the testes with all cells arranged orderly. There was significant damage to the testis in general. Hence, the usage of ginger by the males should be moderate, as high consumption may have some side effects on male fertility.

Keywords: Wistar rat, Ginger, Microanatomy, Testis

1. Introduction

Ginger is the underground rhizome of the ginger plant with a firm striated texture. The flesh of the ginger rhizome can be yellow, white or red color, depending upon the variety. It is cover with a brownish skin that may either be thick or thin, depending upon whether the plant was harvested when it was mature or young. It lends its name *zingiber officinale* to its genus and family (*zingibera ceae*) (Akoachere, 2002), other notable members of this plants family are turmeric, cardamom and galangal.

The plant is commonly known as ginger lily and is found in so many places including Nigeria with different names given to it. The English name ginger comes from French: *gingembre*, Old English: *gingifere*, *Medie Val* Latin: *gingiber*, Greek: *zingiberis*. In Malaysia ginger is called *halia*, in Burna, it is called *gyin*, in Arabic ginger is called *zanjabil*. It is known as “*Jinja*” in igbo land, “*Kakizawa*”, in Hausa, “*atale*” in Yoruba “*Jinja*” in Efik and Ibibio land in Nigeria (Iwu, 1983). Angiophone Cameroon calls it *Gingembre*.

Infertility is one of the major health problems in animals (Amann *et al*, 1986) approximately 30 % of infertilities are due to a male factor (Isidori *et al.*, 2006; Carlsen *et al.*, 1992). Drug treatment, chemotherapy, toxins and environmental factors can have harmful effect on spermatogenesis and sperm normal production (Amann *et al*, 1986). Medicinal plant for the treatment of diseases has a long a tradition. The Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China and India. The first written reports was in 600 B.C. with the Caraka Samhita of India and the early notes of the Eastern Zhou dynasty of China that became systematized around 400 B.C. (Detlef *et al.*, 1999). Some medicinal plants contain both useful and harmful substances which could either promote or retard the health of an individual. Base on this it is paramount to screen and rescreen medicinal plants for the purpose of identifying the active ingredients as well as harmful and non harmful. Ginger (*Zingiber officinal*) family: *Zingiberaceae*, is used worldwide as a spice (Sekiwa *et al.*, 2000), sweet, pungent, heating appetizer has been used in traditional oriental medicines for long time.

Its extract and major pungent principles have been shown to exhibit a variety of biological activities (Ghayur *et al.*, 2005; Wei *et al.*, 2005). Ginger is believed to help the common cold, flulike symptoms, headaches, painful menstrual periods (Bone *et al.*, 1990; Grontved *et al.*, 1988); arthritis (Grant, *et al.*, 2000) reduces symptoms in patients with nausea of pregnancy, motion sickness and postoperative nausea and vomiting (Grontved *et al.*, 1988, Phillips, *et al.* 1993). In Nigeria the use of ginger as medicine is vast, it is also used for spicing almost all kinds of food including tea, and it is one of the major ingredients of “zobo” a local drink in Nigeria. The powdered form in combination with garlic is used for the treatment of dysentery, rheumatism, high blood pressure, body pains and eye related problems. Base on its broad usage it is therefore important to investigate on the possible effects of ginger on the male reproductive system.

2. Materials and method

2.1. Breeding of animals

Twenty five male Wistar rats weighing between 90-130 grams were obtained from the university of Calabar animal house and were housed in the animal of the department of human Anatomy, college of basic medical science university of Calabar, Calabar.

The rats were picked randomly into (5) five groups with each group containing (5) five rats. The rats were housed in wooden cages; the roof of each cage was covered with a wire gauge to allow for proper ventilation. The floor of the cage was lined with sand dust which helped to prevent sores on the feet of the rats and was also bedding for the animals. The beddings were changed daily, the cage cleaned twice a week. The cages were also well labeled to indicate each group.

The animals were fed with growers mash obtained from vital feed mill Nigeria limited. The animals were fed with flat stainless steel plate in order to minimize spillage and mixture with the saw dust. Water was given in a plastic water bottle with plastic straws which helped prevent water spillage in the cage and granted easy access to water for the animals. The lighting condition in the animal house was kept normal at 12 hours day light and 12 hours dark. The environmental temperature ranged from about 28°C to 32°C. The animals were allowed to habituate for two weeks after which they were re-weighed and weighed between 125-200 grams.

2.2. Drugs preparation

Ginger rhizome (*Zingiber officinale* Roscoe) was purchased from the local market at Calabar south known as watt market. The roots were identified and tested by the botanist in botany department, university of Calabar, Calabar Nigeria.

The fresh ginger rhizome weighting 4.5kg was washed cleaned and cut into small pieces and dried for two weeks and was blended into powder form using an electric blender 2000g (2kg) of ginger powder was macerated completely in 500ml of 99.9% ethanol and shaken vigorously. It was allowed to stay for 48hours at room temperature and was stirred at intervals, after which the mixture was filtered using small spores stainless sieve after which filter paper and funnel was used to filter the mixture again. The filtrate was collected in a tray and was airified for (5) five days to ensure complete evaporation of ethanol. At the end of 5 days the ginger paste was collected with a spatula from the tray into a container and was measured using an electronic weighing balance 50 grams of ginger paste was obtained and dissolved using 100ml of extra virgin olive oil and was kept in a cool dry place.

2.3. Administration

Twenty five matured Wistar male rats was used for this experiment and were divided into five (5) groups with each group having five (5) rats each and the groups were labeled A, B, C, D and E. group C, D and E served as experimental groups while group A served as normal control group and group B as olive oil control.

The various doses of ethanolic extract of *zingiber officinale* was administered to the animals in the experiment at groups C, D and E orally at 100mg/kg, 250mg/kg and 500mg/kg per body weight in each group using oralpharyngeal tube while animals in control group (A) were given normal laboratory diet and distilled water only and group B served as vehicle control group and was administered 2ml of virgin olive oil. This was done for two weeks (14 days). The table 1 gives a summary of various dosages administered to the experimental groups.

3. Result

3.1. Morphological observation

There were no much changes morphological, there was increase in body weight of the animals and growth was normal in both the control and experimental groups and there feeding habits were normal and the same.

3.2. Histological observation

The histological study of the testis was done using haematoxylin and eosin method and the observations carried out on various groups are as follows:

Control group A:- Sections showed normal histology of the testis, the seminiferous tubules lined with germinal epithelium, the supporting cell (sertoli cells) and the spermatogenic cells in their various stage of development. Also seen are the interstitial spaces below the basal lamina with the leydig cells. (Plate 1)

Vehicle control Group B: This group received feed and distilled water and 2ml of olive oil. Sections also showed normal histology of the testis as the normal control group (Plate 2)

Low Dose Group C: This group received between 0.10ml – 0.15ml of ginger extract and their sections showed slight degeneration of germinal cells and mature spermatozoa from the lumen. (Plate 3)

Medium Dose Group D: This group received between 0.31ml – 0.38mls of the extract and their histological sections showed degeneration of cells (germinal and supporting) degeneration of matured spermatozoa from the lumen, shrinkage of seminiferous tubules, more interstitial space with effect on leydig cells and distortion of cell membrane (Plate 4)

High Dose Group E: This group received between 0.5ml – 1ml and their histology sections showed more degree in degeneration of cells (germinal and supporting) much destruction of matured spermatozoa from the lumen, distortion of cell membrane of the seminiferous tubules, shrinkage of the tubules and degeneration of leydig cells (Plate 5).

4. Conclusion

Testis is the male gonad that produces spermatozoa in a cascade of process collectively referred to as spermatogenesis. This process is under the influence of testosterone, Luteinizing Hormone and Follicle Stimulating Hormone. The testis is suspended in the scrotum by the spermatic cord (Oluyemi *et al.*, 2007; Akpantah *et al.*, 2003). Ginger is a rhizome that is used as a spice or medicine and can be used fresh, dried and powdered or as a juice or oil. Ginger has been used medicinally and as a culinary spice and dates as far back as 5000 years with origin in Asia and was used especially in India and China.

Ginger is considered a safe herbal medicine with only few and insignificant side effects (Dawson *et al.*; 1992) but not a great deal is known regarding its metabolism and metabolites. In tissue distribution study, it was shown that ginger was distributed to all examined tissue (brain, heart, lungs, spleen, liver, kidney, stomach and small intestine) with highest concentrations in the gastrointestinal tract. The concentration of ginger was higher in tissues than in plasma (Jaing, Wang, and Mi; 2008).

Medicinally or as herbal medicine, fresh ginger is used as a remedy for abdominal distension, cough, vomiting and nausea, sweat promotion (cold) and reducing poisonous effect of other herbs. The steamed or dried ginger is used to treat abdominal pain, lumbago and diarrhea, cholera, hemorrhage, rheumatism and toothache. (Awang 1992; Bone 1997; 2002).

Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities (Ahmed *et al.*, 2000) found that ginger significantly lowered lipid per oxidation by maintaining the activities of the antioxidant enzymes—super oxide dismutase, catalase and glutathione peroxides in rats. Cellular damage in the semen is the result of an improper balance between Reactive Oxygen Species (ROS) generation and scavenging activities. Excessive ROS production that exceeds critical levels can overwhelm all antioxidants defence strategies of spermatozoa and seminal plasma causing oxidative stress (Lamminard *et al.*, 1997; Sikka, 1996).

According to Ghayur and Giliani, (2006) pre- incubation of the rabbit's aortic strip with ginger significantly reduced the calcium chloride. This demonstrated that ginger has a calcium channel blocking effect. These results are parallel to those of (Bolton, 1979; Mecca and Love, 1992) who reported that a vasodilator component mediated by the aqueous ginger extract was due to calcium channel blockade, as it relaxed the high K^+ -induced contractions specifically as well as shifted the Ca_2^+ dose-response curves to the right as reported by (Godfraind *et al.*, 1986; Karaki *et al.*, 1997).

Drugs are known to produce adverse effects when used frequently and over a long period and my observation from this research work indicates that long term ingestion of ginger may result in significant disintegration of germinal, supporting and matured spermatozoa cells, shrinkage of seminiferous tubules, distortion of seminiferous epithelium, leydig cells and widen intestinal spaces. The extract is dose dependent.

In the study conducted by Arash *et al.*, (2009), he demonstrated that ginger possess an antioxidant and androgenic activity in doses of 50 mg/kg/rat and 100mg/kg/rat and have a useful effects on spermatogenesis and sperm parameters in rats. This is not in line with my present study on the effect of ethanolic extract of *Zingiber officinale* (ginger) on the histology of the testis which revealed that the long term ingestion of ginger results in disintegration of germinal, supporting and matured spermatozoa cells. This is indicative that ginger may have toxic effect on the histology of the rats' testis.

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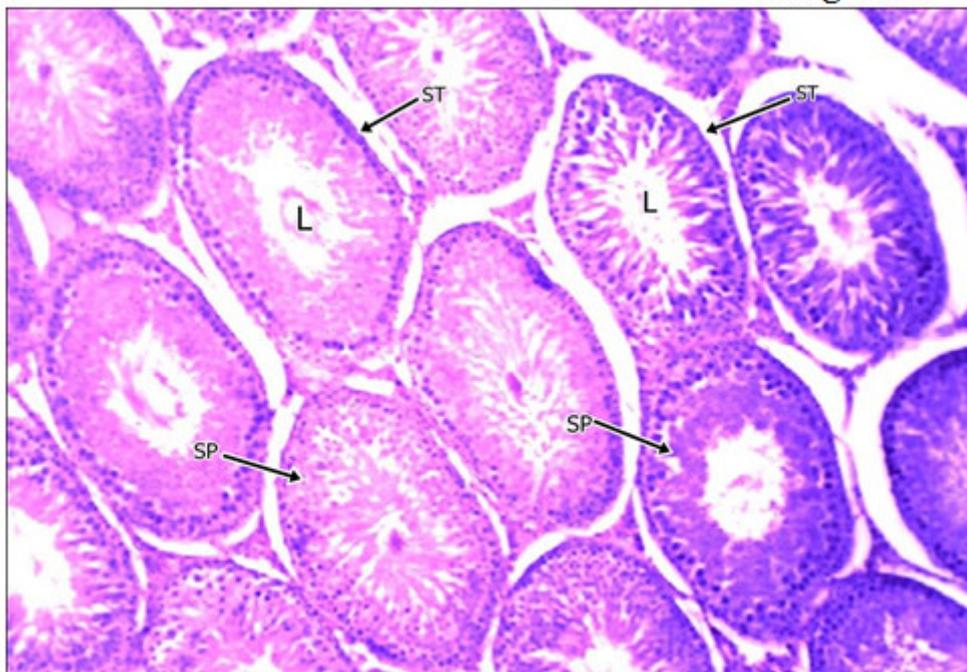
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Table 1: Various dosages administered to the experimental group

GROUP C (LOW)	GROUP D (MEDIUM)	GROUP E(HIGH)
L1 = 0.15ml	M1 = 0.31ml	3H1 = 1.0ml
L2 = 0.13ml	M2 = 0.38ml	H2 = 0.5ml
L3 = 0.15ml	M3 = 0.38ml	H3 = 0.63ml
L4 = 0.13ml	M4 = 0.31ml	H4 = 1.0ml
L5 = 0.10ml	M5 = 0.38ml	H5 = 0.88ml

Mag x100



Mag x 400

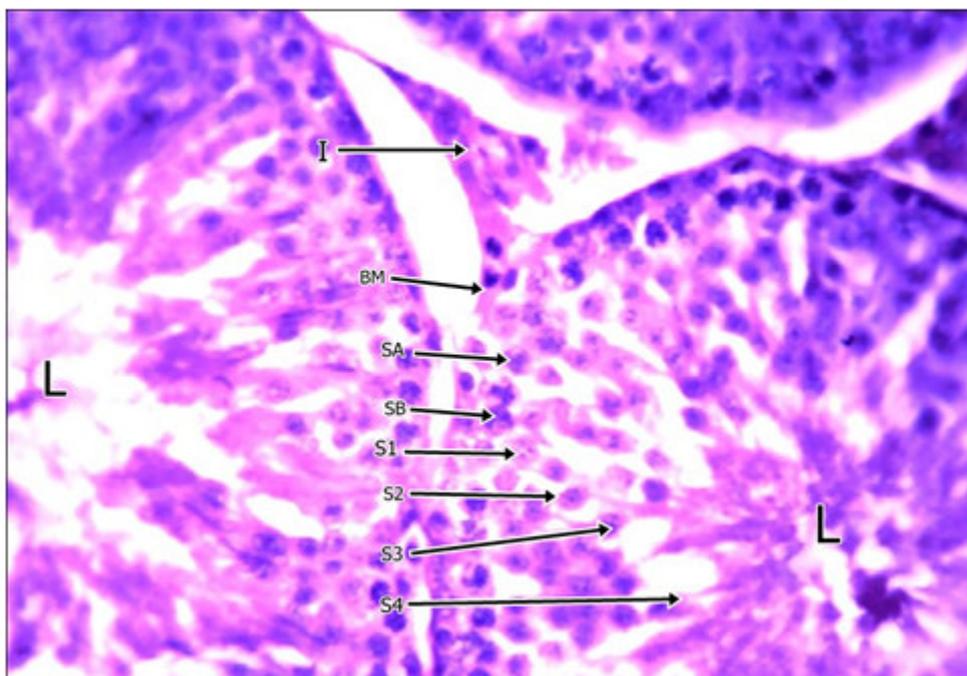
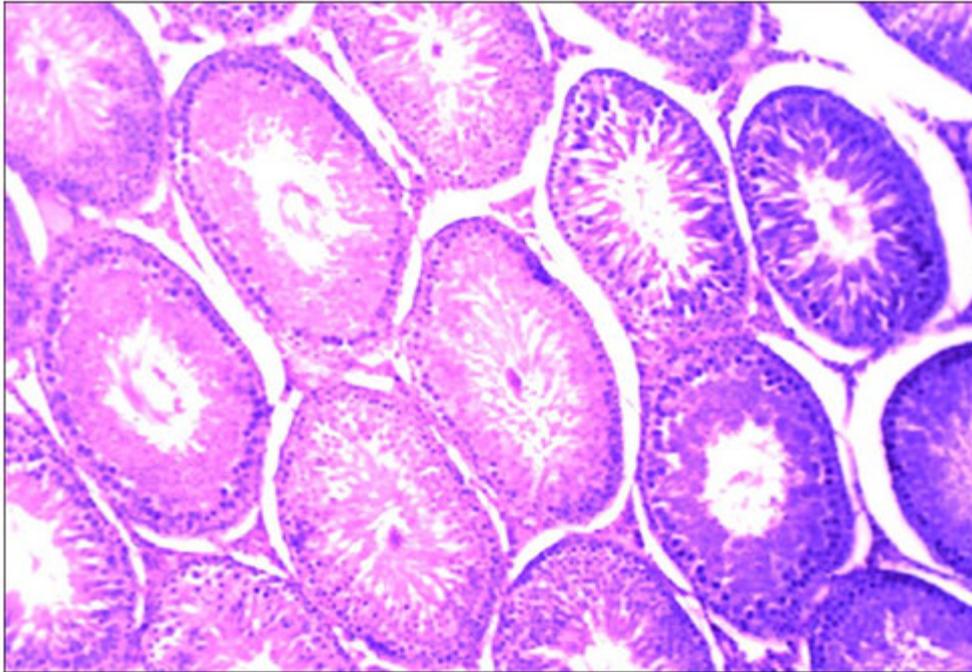


Plate 1: Photomicrograph of Normal Control testis section, stained with H&E showing: Normal lumen (L) with matured spermatozoa, spermatids (S) Sertoli cell (SC), Basement membrane (BM), Leydig (LE)

Mag x 100



Mag x 400

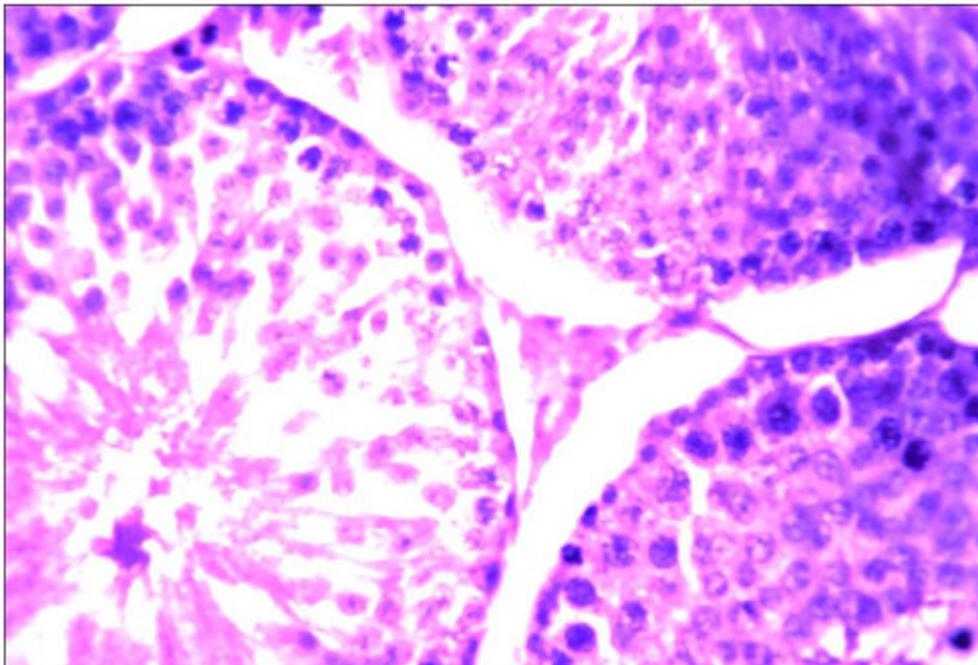
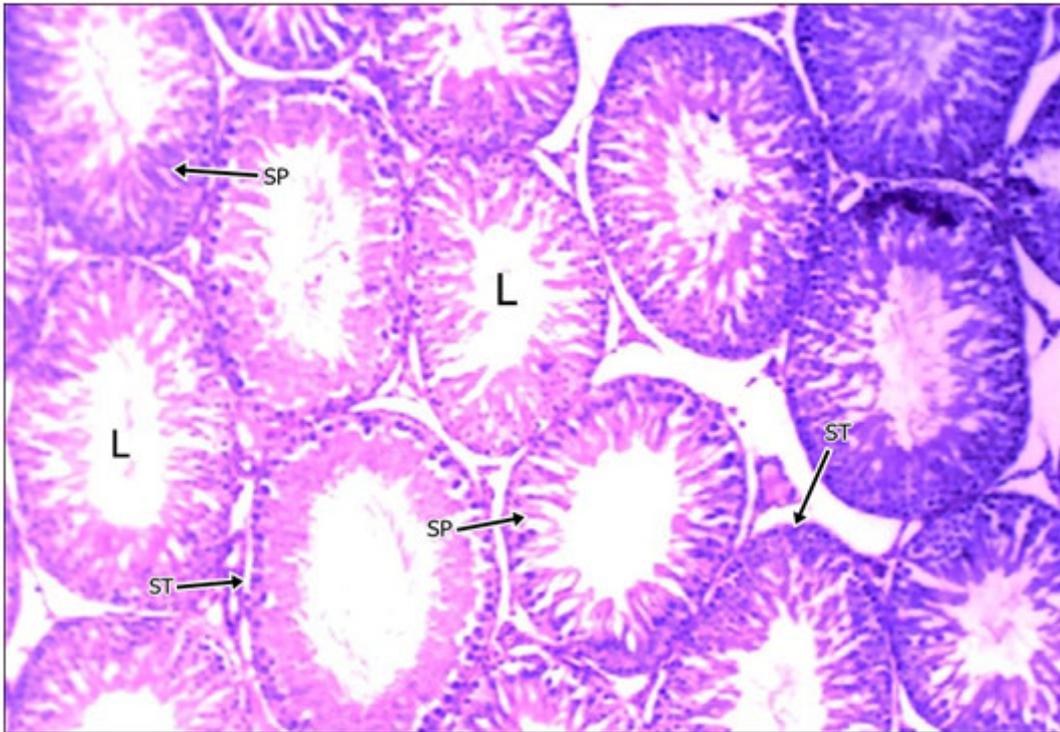


Plate 2: Photomicrograph of Vehicle Control testis section, stained with H&E showing: Normal lumen (L) with matured spermatozoa, spermatids (S) Sertoli cell (SC), Basement membrane (BM), Leydig (LE)

Mag x 100



Mag x 400

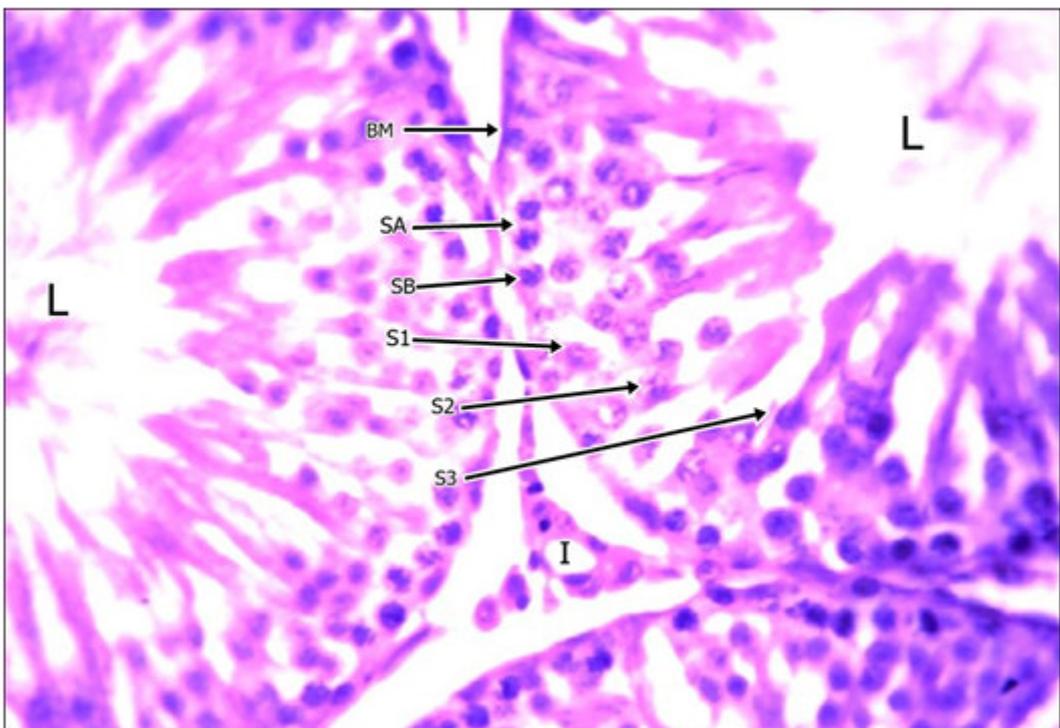
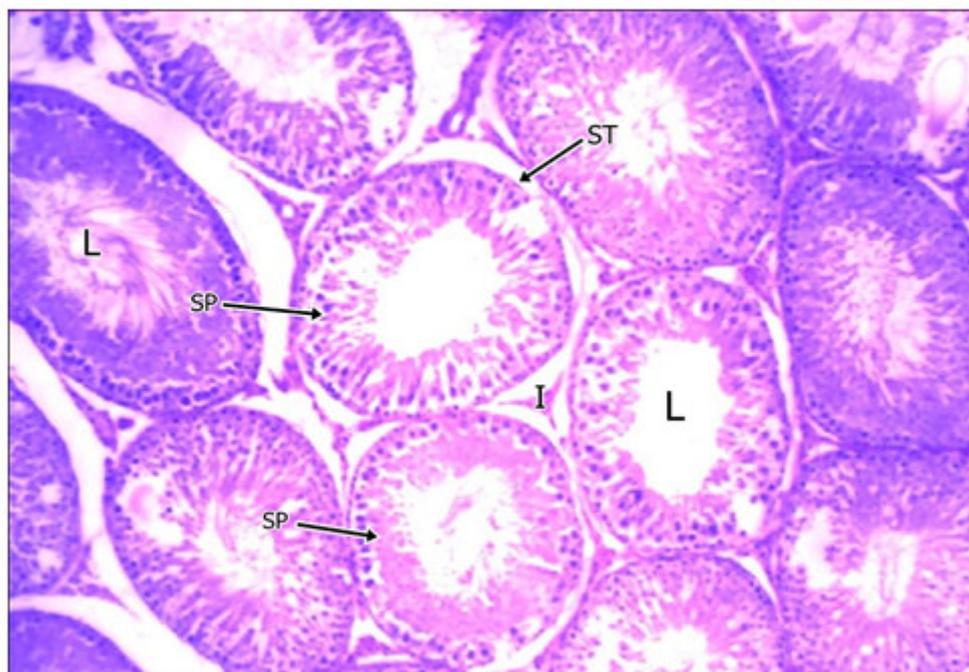


Plate 3: Photomicrograph of low dose (100mg/kg) testis section stained with H&E showing slight disintegration of germinal cells, disintegration of matured spermatozoa in the lumen.

Mag x 100



Mag x 400

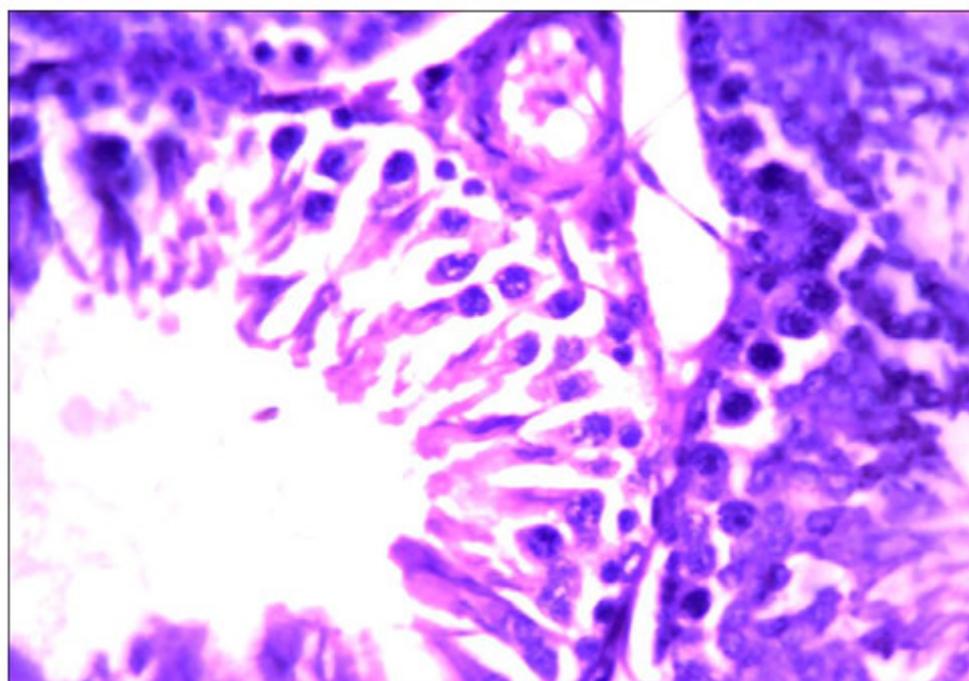
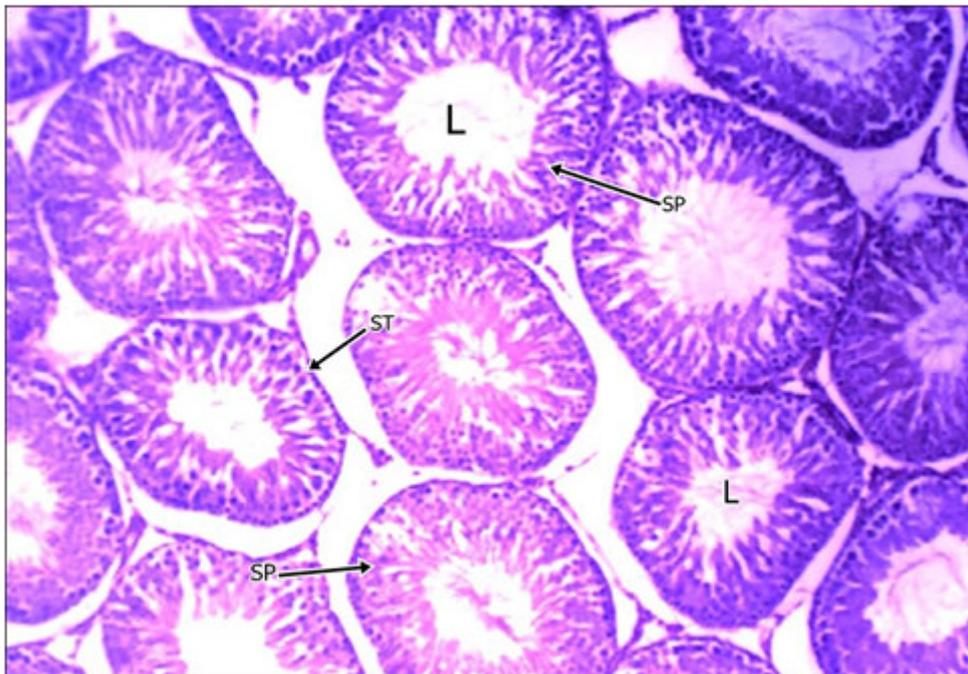


Plate 4: Photomicrograph of medium dose (250mg/kg) testis section with H&E showing degeneration of germinal and supportive cells, degeneration of spermatozoa in the lumen, slight shrinkage of seminiferous tubules, and distortion of Leydig cell

Mag x 100



Mag x 400

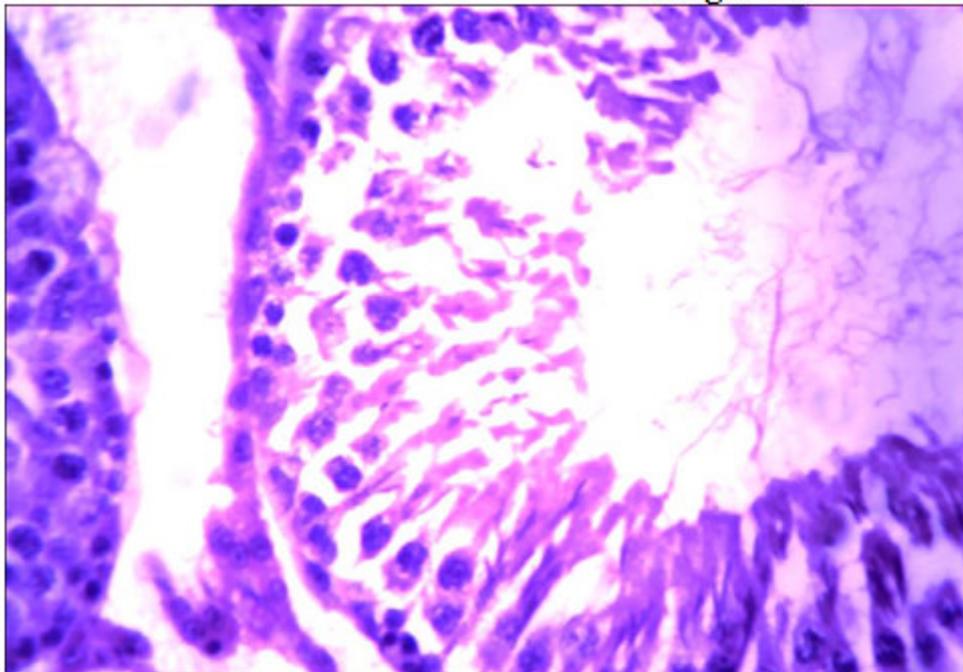


Plate 5: Rat received high dose (500mg/kg) histology of testis show degeneration of germinal and supporting cells, distortion of seminiferous tubules epithelium, shrinkage tubules cells and degeneration of leydig cells.

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