ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 5, pp.022-029, June, 2010

RESEARCH ARTICLE

EFFECT OF BENZENE LEAF EXTRACT OF *Ocimum sanctum* ON TESTIS AND SPERMATOGENIC PATTERN IN ALBINO RATS

Ravindranath H. Aladakatti [2*], Mukhtar Ahmed[1,3] R. Nazeer Ahamed[1] and Mukhtar Ahmed G. Ghodesawar[4]

[1]Department of Post-Graduate Studies and Research in Zoology, Karnatak University, Dharwad-580003, India,
[2]Central Animal Facility, Indian Institute of Science, Bangalore-560012, India,
[3] Research Centre, Electron Microscope Unit, # 5 College of Science, King Saud University, Post Box 2455, Riyadh 11451, Kingdom of Saudi Arabia,
[4]Department of Zoology, Anjuman Arts, Sciences & Commerce College, Bijapur-586101, India

ARTICLE INFO

Article History:
Received 9th April, 2010
Received in revised from
12th May, 2010
Accepted 28th May, 2010
Published online 1st, June, 2010

Key words:
Ocimum sanctum,
Testis,
Germ cells,
Sertoli cells and Albino rats

ABSTRACT

In the present study, an attempt has been made to assess whether the effect of benzene extract of *Ocimum sanctum* leaves on the spermatogenic pattern and ultrastructure changes in testis of albino rats. Wistar strain male albino rats were orally administered 250 mg/kg body weight of *O.sanctum* leaves followed by maintaining suitable controls for 48 days. Results indicate that spermatogenesis was arrested either at the primary spermatocytes or the spermatogonial stages. Damaged seminiferous tubules and abundance of vacuoles of varying size were observed. Total count and diameter of spermatogonia, spermatocytes, spermatids and Leydig cells were reduced. Ultrastructural analysis revealed that intercellular spaces primarily, though not exclusively in the Sertoli cell cytoplasm, degeneration started within the nucleus of spermatocytes and from acrosomal granule or Golgi complex of round spermatids. Bridges between Sertoli cells–spermatocytes or spermatids were disturbed and most of mitochondria of cytoplasm were hypertrophied. Within the cytoplasm degenerating spermatids were with less electron dense matrix and commencement of vacuolization. The results suggest that the effect of benzene extract of *O.sanctum* leaves on the rat testis may be due to curtailing of androgen supply within the testis through its antispermatogenic and antiandrogenic property.

INTRODUCTION

Currently, one of the social problems regarding world health is the stability of population growth. Even though research on male reproduction has been extensive in the last years, little effort has been made to develop male contraception. However, several liable sites that could be used in male contraception have been proposed. Due to the strict sequence and nature of the events that determine male gamete maturity, interference in any step would have serious consequences on the eventual sperm fertilizing capability. A method of interfering in the male reproductive process, without affecting libido, spermatogenesis or the genomic integrity of the sperm cell would be attractive. Apart from research for finding harmless chemical drugs as effective oral contraceptives in the western countries, the crude vegetal drugs used by tribal people are being closely looked into for their possible efficiency to find out safe and effective oral drugs for controlling human fertility.

***Corresponding author:** Dr. Ravindranath H. Aladakatti, ravindranath@caf.iisc.ernet.in; Tel: (+91 080)22932457, Fax: (+91 080)23606569

Many of the plants which are common in India are reported to possess antifertility activity as spermicidal, abortifacient or antiandrogenic in nature (Akbarsha and Averal, 1996; Aladakatti and Nazeer Ahamed, 2005b; Girini et al., 2005; Lohiya et al., 2006; Gupta and Kachhawa, 2007; and Chauhan and Agarwal, 2008). Therefore, it has become necessary to use biologically active botanical substances or fertility-regulating agents of plant origin which are ecofriendly in approach and interfere with the natural patterns of reproduction (Dixit,1992).

Ocimum sanctum Linn. (Laiatae family) commonly called tulsi has been recognized for its unique properties. It is an important medicinal plant which grows all over India and in different parts of the world (Rajeshwari, 1992; Chopra et al., 1993; and Gupta et al., 2002). In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *O.sanctum*, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. The *O. sanctum* L. has also

been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions. In addition, the leaves of *O.sanctum* significantly altered significantly altered the sperm count, motility, velocity and fructose contained in the cauda epididymis (Mukhtar Ahmed et al., 2002), reduce the mating behaviour of both male and female albino rats (Kantak and Gogate,1992; Khanna et al., 1998; and Sardessai et al., 1999).

Recent studies shown that benzene extract of *Ocimum sanctum* leaves induces the ultrastructural changes in the epithelial cells of the cauda epididymis, its subsequent recovery, after withdrawal of treatment, in the process of spermatogenesis and fertility of male albino rats (Mukhtar Ahmed et al.,2008; and 2009a) and morphological changes in the rat cauda epididymal sperms upon graded dose treatment (Mukhtar Ahmed et al.,2009b).Though, various experimental studies reported by using this plant source particularly on male gamete and reproductive system, still there is the paucity of information about the influence of benzene extract of *O.sanctum* leaves on spermatogenic pattern and ultrastructure of testis albino rats. Hence, the present study was aimed to elucidate the effect of benzene extract of *O.sanctum* leaves on the histometry and ultrastructural changes in the testis of albino rats.

MATERIAL AND METHODS

Preparation of test material

Fresh *O.sanctum* leaves were collected and dried in shade. A voucher specimen (Zoo/herb/File No.47-Acc.No.22) was deposited at Zoology Department, Karnatak University, Dharwad, India. The dried leaves were coarsely powdered and subjected to soxheltation process to get the benzene extract. Extract thus obtained was allowed to dry and stored in a dessicator at 4ºC. The benzene extract is then mixed with propylene glycol as required and administered orally (gavage) to the experimental animals (WHO,1983).

Experimental Animals

Colony bred healthy adult male albino rats (Wistar strain) weighing 190-200g were utilized for experiments. All animals were proven fertility and obtained from the rat colony maintained in the department. They were housed at a temperature of 26 ± 2^0 C and exposed to 13-14 h of daylight and maintained on a standard rat pellet diet (Gold Mohar, Hindustan Level Ltd., Hyderabad) and water was given *ad libitum*. The animals were acclimatized to the laboratory conditions before conducting experiments and the care of the laboratory animals was taken as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) regulations.

Study protocol

The control group (n=5) were administered 1 ml propylene glycol/rat/ day orally for 48 days and test group (n=5), that received benzene extract of *O.sanctum* leaves (250mg/kg/day) orally for 48 consequence days. The effective dose of 250mg/kg body weight has been arrived at after preliminary studies on dose and duration of 48 days is concerned to the spermatogenic cycle of rat in response studies in our laboratory and reported elsewhere (Mukhtar Ahmed et al.,2002). Twenty-four hours after the last dose, the control and treated animals were sacrificed by cervical dislocation. The testes were dissected out, blotted free of mucus and weighed to the nearest milligram. For histological study, the testes were fixed in aqueous Bowin's fluid for 24 hrs.washed thoroughly in 70% alcohol, cleared in benzene and embedded in paraffin wax. Sections of 5µm thickness were obtained and stained in haematoxylin (Delafield's) and eosin. For histometrical studies, the calibrated ocular micrometer (Erma, Japan) was used. From each testis 20 sections randomly were used in each group to record the histometrical data.

Preparation of Testis for ultra study

Preparation of rat spermatozoa for ultra study was performed as described elsewhere (Aladakatti and Nazeer Ahamed, 2005b). Briefly, the testes were removed, rapidly fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4; 0.1 M) for 4 hr at 4°C, washed in phosphate buffer and post-fixed in 1% osmium tetraoxide in phosphate buffer (pH 7.4; 0.1 M) for 6 hr. The fixed testis was washed several times in distilled water, stained *en bloc* in 2% aqueous uranyl acetate for 6 hr, dehydrated in acetone series, infiltered in epon-araldite mixture for 10 hr and embedded in the same media in a beam capsule. The blocks were cut in LKB Bromma ultramicrotome. Semithin sections of 1 µm thickness were stained with toludine blue for identification of stages. Ultrathin sections were cut at 100-300 A°, mounted on copper grids and stained with 1% aqueous uranyl acetate and lead citrate (Reynolds,1963). The stained sections were scanned in Jeol-TEM 100 C X II electron microscope for ultrastructural observations.

Statistical analysis

The data were statistically analysed and expressed as Mean ± Standard error (Ostle,1988). The comparison of data for statistically significant differences was done using student's 't' test and a probability level of $P \leq 0.001$ was considered as significant.

RESULTS

Histology of Testis

The testis of control rats exhibited different stages in seminiferous elements comprising of germ cells, Sertoli cells and interstitial cells which are normal in their appearance. Towards the lumen, the primary spermatocytes, secondary spermatocytes, early spermatids and elongated spermatids were associated with Sertoli cells. Towards the lumen, arrangement of mature spermatozoa could be observed (Fig.1). Morphometric data is presented in tables 2-4.

The rats treated with benzene extract of *O.sanctum* leaves showed atrophic tubules and spermatogenesis was very much suppressed, arrested in majority of the tubules. The tunica propria was disintegrated. Basement membrane was thin and disrupted. Spermatogenesis was arrested either at the primary spermatocytes or the spermatogonial stages. The Sertoli cells showed vacuolization and cell debris due to cytolysis. The intercellular spacing becomes wider, Leydig cells were reduced in number or the interstitium contains mostly fibroblasts (Fig.5). The increase in the seminiferous tubules per microscopic field was highly significant (Table.1, $P \leq 0.001$). The diameter of seminiferous tubules decreased significantly (Table. 2, $P \leq 0.001$). There was highly significant decrease in the total count of spermatogonia, spermatocytes, spermatids,

Table 1. Effect of treatment of benzene extract of *Ocimum sanctum* leaves on total count of seminiferous tubules, germ cells, Leydig cells and Sertoli cells in the testis of albino rats (values are expressed in SEM of five animals)

Group	Treatment	Number of					
		Seminiferous tubules in microscopic field (10 x)	Spermatogonia	Spermatocytes	Spermatids	Leydig cells	Sertoli cells
I	Control (1 ml propylene Glycol)	17.70 ± 0.26	116.90 ± 2.49	557.65 ± 38.41	967.45 ± 8.39	37.50 ± 0.79	22.70 ± 0.68
II	250 mg/kg body weight of benzene extract *O.sanctum* leaves	22.25 ± 0.56***	91.05 ± 1.64***	310.35 ± 2.51***	759.95 ± 3.51***	25.80 ± 1.01***	15.35 ± 0.70***

Data are mean ± S.E.M. of 10 replicates.
*** Significant difference at P ≤ 0.001 level, when compared with control group.

Table 2. Effect of treatment of benzene extract of *Ocimum sanctum* leaves on diameter of seminiferous tubules, germ cells (µm) in the testis of albino rats (values are expressed in SEM of five animals)

Group	Treatment	10 x	100 x		
		Seminiferous tubules (µm)	Spermatogonia (µm)	Spermatocytes (µm)	Spermatids (µm)
I	Control (1 ml propylene Glycol)	262.79 ± 3.79	11.20 ± 0.21	9.30 ± 0.29	9.15 ± 0.29
II	250 mg/kg body weight of benzene extract *O.sanctum* leaves	237.50 ± 1.18***	5.95 ± 0.18***	5.05 ± 0.21***	4.90 ± 0.19***

Data are mean ± S.E.M. of 10 replicates.
*** Significant difference at P ≤ 0.001 level, when compared with control group.

Table 3. Effect of treatment of benzene extract of *Ocimum sanctum* leaves on nuclear diameter (µm) of the germ cells in the testis of albino rats (values are expressed in SEM of five animals)

Group	Treatment	100 x			
		Spermatogonia (µm)	Spermatocytes (µm)	Spermatids (µm)	Leydig cells (µm)
I	Control (1 ml propylene Glycol)	10.40 ± 0.21	7.75 ± 0.20	7.05 ± 0.09	7.95 ± 0.20
II	250 mg/kg body weight of benzene extract *O.sanctum* leaves	5.75 ± 0.20***	3.95 ± 0.15***	4.00 ± 0.19***	3.80 ± 0.17***

Data are mean ± S.E.M. of 10 replicates.
*** Significant difference at P ≤ 0.001 level, when compared with control group.

Leydig cells, and Sertoli cells (Table.1, $P \leq 0.001$). There was highly significant decrease in the cell and nuclear diameter of spermatogonia, spermatocytes, spermatids and the nuclear diameter of Leydig cells (Tables. 2 & 3, $P \leq 0.001$).

Ultrastructure of rat testis

The seminiferous tubules of the control animals contained Sertoli cells and germ cells in different phases of development from spermatogonia to spermatids, consisting of various stages of the cycle of the seminiferous epithelium. The Sertoli cell cytoplasm extended from the basal lamina of the seminiferous tubule to the lumen and surrounded the germ cells. The basal areas of the Sertoli cells contained euchromatic, irregularly shaped nucleus, surrounded by a layer of fine filaments. The cytoplasm of the Sertoli cells showed several profiles of Golgi apparatus, large number of polyribosomes, numerous mitochondria containing tubulovesicular cristae. Membrane bound granules resembling the lysosomes were observed. Adjacent Sertoli cells were connected to the germ cells by intercellular bridges (Figs.2 – 4).

The dark spermatogonia and spermatocytes with a round or oval nucleus and patchy chromatin materials were observed adjacent to the basal lamina and connected to Sertoli cells by intercellular bridges. Golgi apparatus, granular and agranular endoplasmic reticulum were distributed randomly in these germ cells (Fig.2). Early spermatid of cap phase and early acrosomic phase of spermatids had specific mitochondria in the form of periphery in cytoplasm and exhibited the condensed structure, which is characteristic of mitochondria at these stages of germ cells. Acrosomic phase of spermatids exhibited well defined major components of the Golgi complex (Fig.3).The different types of spermatids showed normal structure with well defined nucleus, manchette

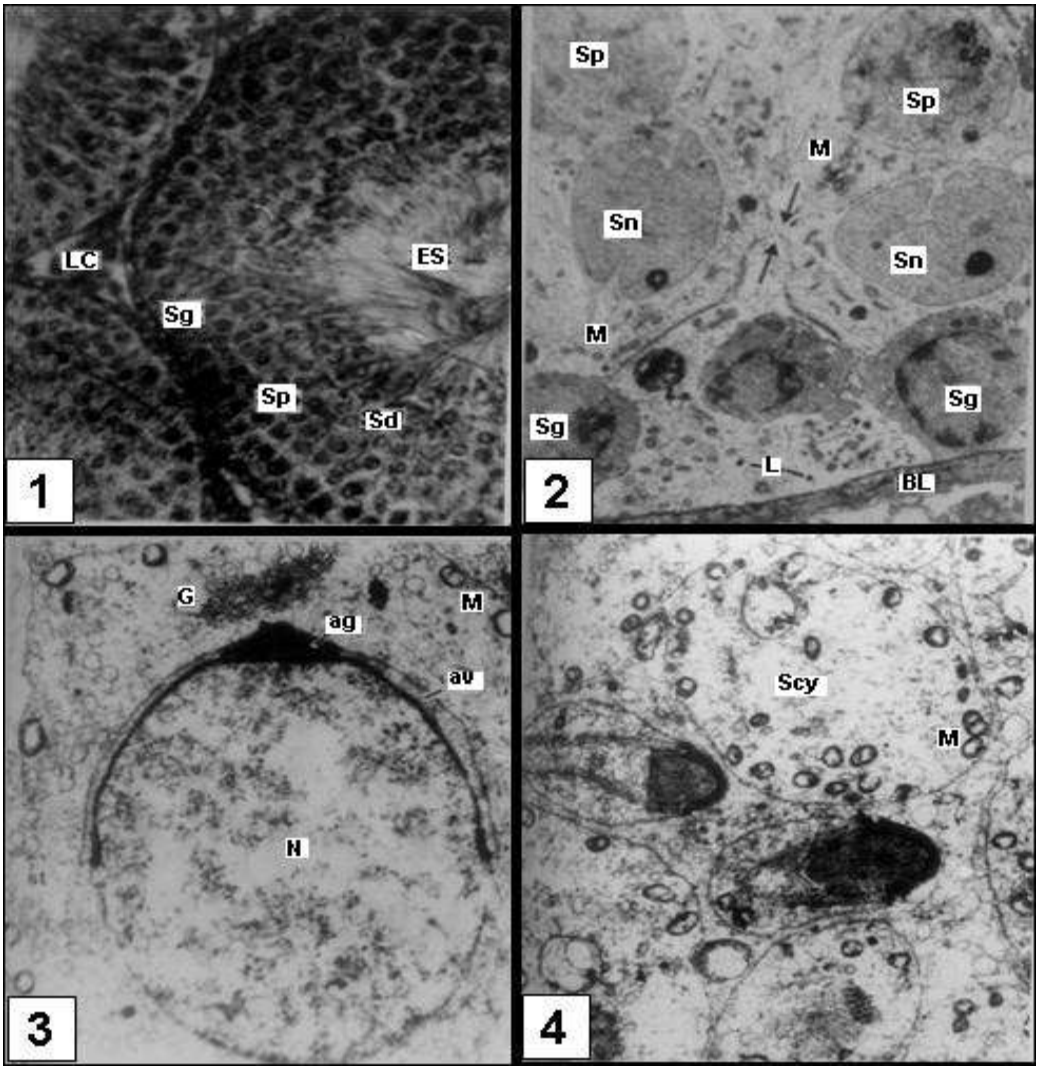

Fig.1: Section of the seminiferous tubules of control rat exhibiting normal spermatogenesis with normal features consisting of spermatogonia (Sg), spermatocytes (Sp), spermatids (Sd), elongated spermatids(ES) and interstitial elements(LC) X 400. **Figures.2-4.** Electron micrographs of control rat testis**. Fig.2.**Spermatogonia (Sg) are rest upon the basal lamina (BL). Spermatocytes (Sp) connected to Sertoli cells by intercellular bridges (arrow heads).Sertoli cell nuclei (Sn) at the basal region are in irregular shape with euchromatic and surrounded by a layer of fine filaments. Mitochondria (M) of cytoplasm of Sertoli cells and germ cells exhibited in abundance. Few lysosome like bodies (L) are also seen X 4800. **Fig.3.** Acrosomic phase of spermatid exhibited normal appearance of acrosomal vesicle (av), acrosomal granule (ag) and Golgi zone (G). Smooth coated tubules (st) are seen in the cytoplasm of spermatid X 2,700. **Fig.4.** Late stage spermatid showing nuclei, acrosomes, manchettes and other cytoplasmic elements. Mitochondria (M) of Sertoli cell cytoplasm (Scy) are seen scattered and associations between Sertoli cell and spermatids are well defined X 8,400.

with microtubules, acrosomic vesicle, Golgi complex, acrosomal granules and Golgi zone (Fig.4).

In the animals treated with benzene extract of *O.sanctum* leaves, the seminiferous tubules showed vacuolization, intercellular spaces primarily, though not exclusively in the Sertoli cells (Figs.6 – 8). At the region of basal lamina, spermatogonia and Sertoli cell nuclei could be recognized with normal structures. Intraepithelial vacuoles were found to consist of intercellular spaces and intracellular vacuoles in the cytoplasm of the Sertoli cells (Fig.6). Degeneration started within the nucleus of spermatocytes and from acrosomal granule or Golgi complex of round spermatids. Bridges between Sertoli cells– spermatocytes or spermatids were disturbed and most of mitochondria of cytoplasm were disturbed or hypertrophied. Within the cytoplasm, a giant chromatoid body was seen. In acrosomic phase of spermatids, the Golgi bodies with few Golgi vesicles are concentrated at the supranuclear region in the highly vacuolated cytoplasm, which exhibiting characterization of vacuolization and mitochondria in the cytoplasm exhibited characteristics of hypertrophy. Other cell organelles in the cytoplasm were absent (Fig.7).Degenerating spermatids were with less electron dense matrix and commencement of vacuolization (Fig.8).

DISCUSSION

Histology of Testis

In the present study, the antispermatogenic activity of benzene extract of *O.sanctum* leaves is reflected in the arrest of spermatogenesis. It has been reported that reducing testicular weight and maturational arrest of the primary spermatocyte manifest androgen deficiency (Saito et al., 2000).The morphometric analysis confirms the adverse effect on the spermatocytes, spermatids and Leydig cells. The degeneration of Leydig cells reflects the depletion of androgen levels and absence of germinal cells

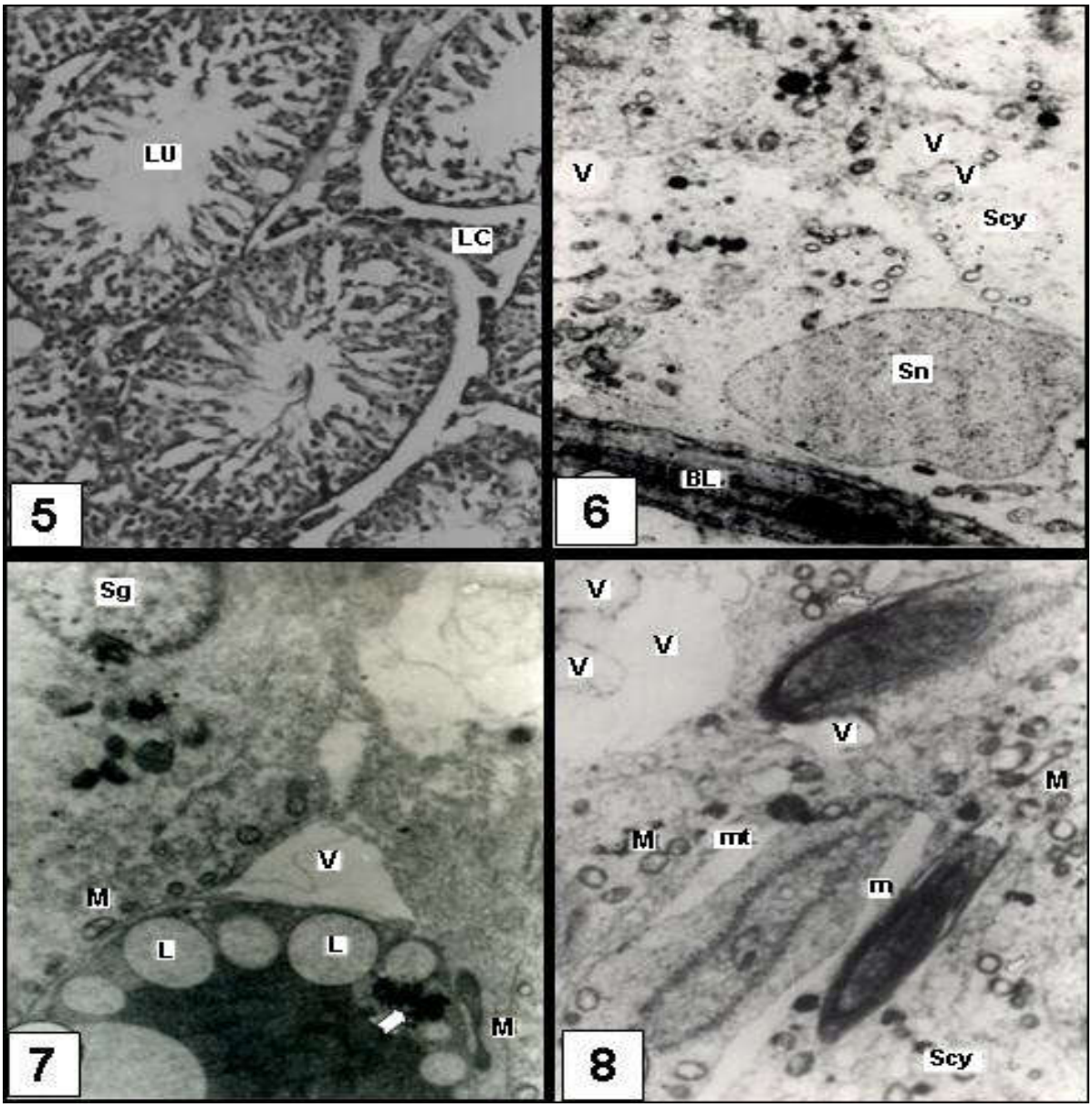

Fig.5. Seminiferous tubules of the rat treated with *O.sanctum* leaves exhibiting severe effects on the tubules and reduction in their si Disruption of seminiferous epithelia is evident. Spermatogenesis stopped at the primary spermatocytes stage. Interstitial spaces increased a atrophy of Leydig cells (LC), which are sparsely distributed. The germ cells show over all decrease in cytoplasmic ground substance follow by vacuolation at the basal lamina and towards the lumen (LU) X 400. **Figures.6-8. Electron micrographs of benzene extract of *O.sanctu* leaves treated rat testis. Fig. 6.** Vacuolization (V) in the seminiferous epithelium, but not in the Sertoli cells. Basal lamina is not affect Sertoli cell nuclei (Sn) rest on the basal lamina and normal in appearance. Mitochondria (M) in the cytoplasm are hypertrophied X 4,800. **Fig** There is a complete _disturbance in the spermatogonia (Sg). The lysosomes (L) like bodies are evident in the section. Vacuolization (V) l started throughout the cytoplasm of the cell. Cell debris is evident. The microtubules are deposited in one corner of the cell (⌂).The c organelles are totally disturbed X 7,800.**Fig.8.**There is commencement of degeneration of microtubules (mt, hallow arrows). The vacuolizati (V) is evident in the seminiferous tubules. In the Sertoli cell cytoplasm (Scy) the lysosome like bodies are more. Normal appearance manchette (m) in elongated spermatid. There is commencement of vacuolization in between the manchette. Mitochondria (M) are disorganiz or hypertrophied in the Sertoli cell cytoplasm X 8,000.

i.e., spermatocytes, spermatids and support the observations/ hypothesis/ view, since these stages are completely androgen dependent (Beardsley and O'Donnell, 2003). The adverse effect of benzene extract of *O.sanctum* leaves on the rat testis including tubular atrophy, along with the abnormal histological appearance of the seminiferous epithelium and the Leydig cells are may be due to curtailing of androgen supply within the testis or it may be a direct effect of this plant extract on the tissue. Similar observations related to the unique nature of changes in the seminiferous tubule treated with different parts of plant extracts have been assayed so far, in the perspective of male antifertility. To quote a few, *Azadirachta indica* (Aladakatti and Nazeer Ahamed, 2006; an edible-tuber crop of *Tropaeolum tuberosum* (Cárdenas-Valencia et al., 2008); *Peganum harmala* (El-Dwairi and Banihani, 2007); methanol extract of *Dendrophthoe falcate* (Gupta and Kachhawa, 2007); aqueous extract of *Chromolaena odoratum* (Yakubu et al., 2007); and ethanolic extract of *Aegle marmelos* leaves (Chauhan and Agarwal, 2008) have been studied in this category.

Ultrastructure of Testis

It has been suggested that androgens produced by the interstitium may play some role in spermiogenesis and subsequent maintenance of spermatogenesis in adult

animals. A few studies have been attempted to relate experimentally induced morphological changes in germ cells, Sertoli cells and Leydig cells in common laboratory animals to their function in regulating spermatogenesis (Saito et al., 2000; Beardsley and O'Donnell, 2003). Testosterone has been shown to be essential for spermatogenesis completion, because it stimulates the conversion of round spermatids into elongated spermatids of the spermatogenetic cycle. Androgen deficiency disturbs the spermiation process (Saito et al., 2000) by altering spermatid-Sertoli cell junctions, which results in premature detachment of round spermatids from Sertoli cells and seminal epithelium (Beardsley and O'Donnell, 2003). Decreased testosterone levels have been associated with alterations in Sertoli and Leydig (androgen target) cells (Yang et al., 2006). Treatment with leaf powder of *Azadirachta indica* (Aladakatti and Nazeer Ahamed, 2005a,b); raw crude garlic feeding (Hammami et al., 2008) and purified compounds of the seeds of *Carica papaya* (Lohiya et al., 2005) on ultrastructure of the rat testis revealed that the vacuolization in the Sertoli cells and germ cells, loss of cytoplasmic characteristics in the Sertoli cells, nuclear degeneration and mitochondrial vacuolization in spermatocytes and spermatids, a decrease in nuclear density with evagination of the nuclear envelope and some times ruptures of the plasmatic membrane. Changes in the Sertoli cells consisted of cytoplasm vacuolization in which some lysosomes contained degraded material (Aladakatti and Nazeer Ahamed, 2005a).

Studies from Aladakatti and Nazeer Ahamed (2005a, b) had shown that *Azadirachta indica* leaf powder treatment causes disruption of intercellular bridges between germ cell - germ cells, germ cells - Sertoli cells or Sertoli cells - Sertoli cells in rats due to its antiandrogenic nature. In view of the dynamic role of androgen in the initiation and maintenance of spermatids, it is believed that the degenerative changes observed in the spermatids may be due to deprivation of androgen (Saito et al., 2000; Beardsley and O'Donnell, 2003; and Yang et al., 2006). In the present study, benzene extract of *O.sanctum* leaves treatment causes disruption of intercellular bridges between germ cell - germ cells or germ cells - Sertoli cells probably due to the antiandrogenic properties of benzene extract of *O.sanctum* leaves. It is known that the function of bridge partitioning complexes has not been established. Collectively, the data demonstrated that bridges are not static structures, but are modified at specific phases of development, especially during spermiogenesis. The morphological changes observed do not provide definitive information on bridge function, but their initial description serves as a basis for the companion study, which specifically address the function of certain bridge components (Russell and Griswold, 1993).

It is well known that Sertoli cells interact directly with germ cells and perform a number of functions critical to spermatogenesis, including compartmentalization of the seminiferous tubule, physical and metabolic support of germ cells, an secretion of numerous factors that promote germ cell viability and differentiation (Russell and Griswold, 1993). The presence of agranular and stacks of granular endoplasmic reticulum, numerous mitochondria and multiple Golgi bodies in the basal part of the Sertoli cell cytoplasm are characteristics to Sertoli cells that are metabolically active in protein and steroid biosynthesis (Pudney, 1986). Proteins of Sertoli cells, mainly the androgen binding protein (ABP), required or achieving a specific step in germ cell maturation are secreted at the highest rate in the testis during spermatid elongation and spermiation. In addition, ABP is being synthesized by the Sertoli cells, transported up to the adluminal compartment and transferred to the germ cells (Gerard et al., 1994). The granular endoplasmic reticulum of the Sertoli cells that surrounds the heads of late spermatids has been interpreted as morphological evidence for steroidogenic activity (Pudney, 1986).

The concurrent appearance of numerous vacuoles in this study represents a morphological indicator of Sertoli cell damage. Support for this idea has been provided by the studies of short term hypophysectomized rats (Ghosh et al., 1991; Kerr et al., 1993) and leaf powder of *Azadirachta indica* (Aladakatti and Nazeer Ahamed, 2006). Ultrastructural analysis showed that most vacuoles were within the cytoplasm of the Sertoli cells, although occasional extra cellular spaces were observed. The appearance of vacuoles in the basal regions of the experimentally disrupted seminiferous epithelium, specifically within the cytoplasm of the Sertoli cells, suggests that they arise independent of germ cell degeneration. Examples of this phenomenon occur in the experimentally antiviral drug ribavirin and carbendazim treated rat testis (Nakai et al., 1995; Narayana et al., 2005) and vacuolization of the Sertoli cells can occur in the absence of (degenerating) germ cells of the testis following withdrawal of testosterone (Kerr et al., 1993). Dacarbazine treatment in rats provoked degeneration of Sertoli cell cytoplasm (Ganesh Kumara et al., 2006) and leaf powder of *Azadirachta indica* treatment causes abundance of vacuoles of varying size within the seminiferous tubules and the germ cells showed overall decrease in cytoplasmic ground substance (Aladakatti and Nazeer Ahamed, 2005a,b; and 2006).

In this study, treatment with benzene extract of *O.sanctum* leaves treatment resulted in vacuolization of Sertoli cell cytoplasm and loss of cytoplasmic organelles, suggesting loss of metabolic activity. Thus, degeneration and maturational arrest of germ cells particularly spermatocytes and spermatids observed here could be attributed to the Sertoli cell factors, responsible for germ cell maturation (Lohiya et al., 2005). The Sertoli cell lesions consisting of clear watery cytoplasmic vacuoles of varying size and distensions of Sertoli - Sertoli cell junction and adjacent germ cells are observed in treated group. The mechanism of formation of vacuoles is not known, but studies suggested that cytoplasmic vacuoles appear to arise from swellings of elements of agranular reticulum and of subsurface cisternae. Vacuolization appears to be a non specific reaction of Sertoli cell to injury (Kerr et al., 1993). And as yet, there is no evidence on whether occurrence of intraepithelial vacuolization alone is related to a specific stage or stages of the spermatogenic cycle. Hence, in the present study, it may be suggested that the antiandrogenic property of benzene extract of *O.sanctum* leaves probably affects the Sertoli cells via deprivating the target of FSH and testosterone

which simultaneously exert their effect on Sertoli cells which in turn results in stimulation of intratubular factors essential for survival of germ cells. This view / hypothesis is strengthened by the evidence suggesting that FSH and androgen receptors are found almost exclusively in the Sertoli cells (Kierszenbaum , 1994; Sharpe, 1994).

Ultra sections of testis of treated group demonstrated the lumen contained multinucleate giant cells with several nuclei containing marginalized chromatin usually arranged around the periphery of a mass. Spermatogenesis is an orderly process of transformation of spermatogonia through a series of stages into the round spermatids, which involves cell division through mitosis as well as meiosis. The occurrence of multinucleate giant cells towards the lumen may suggest that during mitosis of the spermatogonia and meiosis of the spermatocytes, subsequent to division of the nucleus the cells fail to undergo cytokinesis (De Krester and Kerr, 1994). The multinucleate giant cells produced due to the action of benzene extract of *O.sanctum* leaves are comparable to the symplasts generated by *Andrographis paniculata* leaf extract (Akbarsha and Murugaian, 2000) and Ursolic acid (Akbarsha et al*.,* 1998). Symplasts are generated due to drug induced opening up of the intercellular bridges of the male germ cells clones (Weber et al*.,* 1985). The stage-specific nature of benzene extract of *O.sanctum* leaves in inducing symplast formation deserves further investigation.

In conclusion, the present histological and ultrastructural investigations give a clue that the benzene extract of *O.sanctum* leaves possibly affect the spermatogenesis through its antispermatogenic and antiandrogenic property directly or indirectly. In the light of the adenohypophysial control of the Leydig cell (De Krester and Kerr, 1994), it could be hypothesized that the impact of benzene extract of *O.sanctum* leaves is primarily on the adenohypophysis and the changes in the Leydig cell with direct manifestation. It has been suggested that any toxic effect on the gonadotrophs of the adenohypophysis would lead to depletion of gonadotrophins, which in turn would lead only to regression of the Leydig cell (Akbarsha et al*.,* 1995).

Acknowledgement

This research was supported by Department of Zoology, Karnatak University, India and Union Grant Commission under SAP and COSIST program, New Delhi, India. We also acknowledge National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India for their kind help in electron microphotograph.

REFERENCES

Akbarsha, M.A, Murugaian, P. 2000. Aspects of the Male Reproductive Toxicity/Male Antifertility Property of *Andrographolide* in Albino Rats: Effect on the Testis and the Cauda Epididymidal Spermatozoa. *Phytother Res,* 14: 432–435.

Akbarsha, M.A. and Averal. H.I. 1996.Epididymis as a target organ for the toxic effect of vincristine: light microscopic changes in the epididymis epithelial cell types. *Biomedical letters.,* 54: 133-146.

Akbarsha, M.A., Palanisamy, M., Murugaian, P. and Latha, P.N.L.1998. Ursolic acid generates symplasts in rat spermatogenic clones. *Phytother. Res,* 12: 32- 36.

Akbarsha, M.A., Stanley, A. and Averal, H.I. 1995. Effect of vincristine Leydig cell and accessory reproductive organs. *Curr Sci,* 68:1053-1057.

Aladakatti, R..H. and Nazeer Ahamed, R. 2005a. Changes in Sertoli cells induced by *Azadirachta indica* A Juss leaves in albino rats. *J Basic Clin Physiol Pharmacol,* 16: 67-80.

Aladakatti, R..H. and Nazeer Ahamed, R. 2005b. Ultrastructural changes in Leydig cell and cauda epididymal spermatozoa induced by *Azadirachta indica* leaves in albino rats. *Phytother Res,* 19: 756-766.

Aladakatti, R..H. and Nazeer Ahamed, R.2006 *Azadirachta indica* A.Juss induced changes in spermatogenic pattern in albino rats. *J Nat Remed*, 6: 62-72.

Beardsley, A. and O'Donnell, L. 2003. Characterization of normal spermiation and spermiation failure induced by hormone suppression in adult rats. *Biol Reprod,*68:1299–307.

Cárdenas-Valencia, I., Nieto, J., Gasco, M., Gonzales, C., Rubio, J., Portella, J. and Gonzales, G.F. 2008. *Tropaeolum tuberosum (*Mashua) reduces testicular function: effect of different treatment times. *Andrologia,* 40: 352-357.

Chauhan, A. and Agarwal, M. 2008. Reversible changes in the antifertility induced by *Aegle marmelos* in male albino rats. *Syst Biol Reprod Med,* 54; 240-246.

Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapoor, L.D.1993. Indigenous drugs of India (Published by UN Dhar, Pvt. Ltd., Calcutta).

De Krester, D.N. and Kerr, J.B. 1994. The cytology of the testis. In The Physiology of Reproduction, ed. by E Knobil and J Neill, Raven Press, New York, p. 1177-1290.

Dixit,VP. 1992. Plant products/non-steroidal compounds affecting fertility in the Indian desert gerbil, Meriones hurrianae Jerdon. In: Prakash I & Ghosh PK (Editors), Rodents in Indian Agriculture. Vol. 1. Scientific Publishers, Jodhpur, India, p.595-604.

El-Dwairi, Q.A. and Banihani, S.M. 2007. Histo-functional effects of *Peganum harmala* on male rat's spermatogenesis and fertility. *Neuro Endocrinol Letter,* 28: 305-310.

Ganesh Kumara, S., Narayana, K., Bairy, K.L., Urban, J.A., D'Souza., Vijaya Paul Samuel. and Gopalakrishna, K. 2006. Dacarbazine induces genotoxic and cytotoxic germ cell damage with concomitant decrease in testosterone and increase in lactate dehydrogenase concentration in the testis. *Mutation Research,* 607: 240–252.

Gerard, H., Gerard, A., EnNya, A., Felden, F. and Gueant, J.L. 1994. Spermatogenic cells do internalize Sertoli androgen-binding protein: a transmission electron microscopy autoradiograhic study in the rat. *Endocrinology,* 134: 1515–27.

Ghosh, S., Sinha Hikim, A.P.and Russell, L.D. 1991. Further observations of stage specific effects seen after short-term hypophysectomy in the rat. *Tissue Cell,* 23: 613-630.

Girini, M.M., Ahamed, R.N. and Aladakatti, R.H. 2005. Effect of graded doses of *Momordica charantia* seed

extract on rat sperm: scanning electron microscope study. *J Basic Clin Physiol Pharmacol,* 16; 53-66.

Gupta, R.S. and Kachhawa, J.B. 2007. Evaluation of contraceptive activity of methanol extract of *Dendrophthoe falcata* stem in male albino rats. *J Ethnopharmacol,* 112: 215-218.

Gupta, S.K., Prakash, J. and Srivastava, S. 2002. Validation of claim of Tulsi, *Ocimum sanctum Linn* as a medicinal plant. *Indian J Experimental Biology,* 40: 765–773.

Hammami, I., Nahdi, A., Mauduit, C., Benahmed, M., Amri, M., Ben Amar, A., Zekri, S., Ahmed, E.M. and Michele, V.E.M. 2008. The inhibitory effects on adult male reproductive functions of crude garlic (*Allium sativum*) feeding. *Asian J Androl,* 10: 593–601.

Kantak, N.M. and Gogate, M.G. 1992. Effect of short term administration of Tulsi (*Ocimum sanctum* Linn.) on reproductive behaviour of adult male rats. *Indian. J.Physiol. Pharmacol,* 36:109-111.

Kerr, J.B., Millar, M., Maddocks, S. and Sharpe, R.M.1993. Stage - dependent changes in spermatogenesis and Sertoli cells in relation to the onset of spermatogenic failure following withdrawal of testosterone. *Anat Rec,* 235: 547-559.

Khanna, S.K., Gupta, S.R. and Grover, J.K. 1998. Effect of long term feeding of Tulsi (*Ocimum sanctum* L.) on reproductive performance of adult albino rat. *Indian J Exp Biol,* 24: 302-304.

Kierszenbaum, A. 1994. Mammalian spermatogenesis in vivo and in vitro: partnership of spermatogenic and somatic cell linkages. *Endocr Rev,* 15: 116 - 134.

Lohiya, N.K., Mishra, P.K., Pathak, N., Manivannan, B., Bhande, S.S., Panneerdoss, S. and Sriram, S. 2005. Efficacy trial on the purified compounds of the seeds of *Carica papaya* for male contraception in albino rat. *Reproductive Toxicology,* 20: 135–148.

Lohiya, N.K., Manivannan, B. and Garg, S.2006. Toxicological investigations on the methanol sub-fraction of the seeds of *Carica papaya* as a male contraceptive in albino rats. *Reprod Toxicol,* 22: 461-468.

Mukhtar Ahmed, Nazeer Ahamed, R. and Aladakatti, R.H. 2009b. Effect of benzene extract of *Ocimum sanctum* leaves on cauda epididymis of albino rats. *J Basic Clin Physiol Pharmacol,* 20: 29-41.

Mukhtar Ahmed., Nazeer Ahamed, R. and Aladakatti, R.H. 2008. Reversible histoarchitecture study of testis and cauda epididymis and changes in cauda epididymal epithelial cell types on treatment with benzene extract of *Ocimum sanctum* leaves in albino rats. *Oriental Pharm Exp Med,* 8: 111-124.

Mukhtar Ahmed., Nazeer Ahamed, R. and Aladakatti, R.H. 2009a. Dose dependent effect of benzene extract of *Ocimum sanctum* leaves on cauda epididymal spermatozoa of albino rats. *Oriental Pharm Exp Med,* 9: 339-349.

Mukhtar Ahmed., Nazeer Ahamed, R., Aladakatti, R.H. and Ghodesawar, M.G. 2002. Reversible antifertility effect of benzene extract of *Ocimum sanctum* leaves on sperm parameters and fructose content in rats. *J Basic & Clinical Physiol Pharmacol,* 13: 51-59.

Nakai, M., Hess, R.A., Netsu, J. and Nasu, T. 1995. Deformation of the rat Sertoli cell by oral administration of carbendazim (methyl 2-benzimidazole carbamate), *J.Androl,* 16: 410–416.

Narayana, K., D'Souza, U.J.A., Prashanthi, N. and Ganesh, K. 2005. The antiviral drug ribavirin reversibly affects the reproductive parameters in the male Wistar rat, *Folia. Morphol,* 64: 65–71.

Ostle, B. 1998. Statistics in Research: Basic Concepts and Techniques for Research Workers, 4th ed. Ames: Iowa State University Press. P.664.

Pudney, J. 1986. Fine structural changes in Sertoli and Leydig cells during the reproductive cycle of the ground squirrel, *Citellus lateralis*. *J Reprod Fertil,* 77: 37–49.

Rajeshwari, S. 1992. *Ocimum sanctum.* The Indian home remedy. In: Current Medical Scene, (Edited and published by S. Rajeshwari, Cipla Ltd., Bombay Central, Bombay).

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol,* 17: 208-212.

Russell LD, Griswold MD. 1993.The Sertoli cell. Clearwater, FL: Cache River Press.

Saito, K., O'Donnell, L., McLachlan, R.I. and Robertson, D.M. 2000. Spermiation failure is a major contributor to early spermatogenic suppression caused by hormone withdrawal in adult rats. *Endocrinology,* 141: 2779–85.

Sardessai, S.R., Borker, A.S. and Abraham, M.E. 1999. Effects of short term administration of Tulsi leaves on sexual behaviour in female rats. *Indian J Physiol. Pharmacol,* 43: 398-400.

Sharpe, R.M. 1994. Regulation of spermatogenesis. In: Knobil E, Neill JD, eds, The Physiology of Reproduction, II Ed., New York, NY, USA, Raven Press, Inc. p. 1363-1434.

Weber, J.E., Russell, L.D. and Voge, A.W. 1985. Preliminary studies of cytochalasin D (CD)-related actin ®lament disruption in rat seminiferous tubules. *J Cell Biol,* 101: 368.

WHO Protocol, LG-06. 1983. Extraction and fractionation for biological and phytochemical studies. *A.P.J.F/I.PA,* p.1001-1083.

Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. 2007. Evaluation of antiandrogenic potentials of aqueous extract of *Chromolaena odoratum* (L.) K. R. leaves in male rats. *Andrologia,* 39: 235-243.

Yang, Z.W., Kong, L.S., Guo, Y., Yin, J.Q. and Mills, N. 2006. Histological changes of the testis and epididymis in adult rats as a result of Leydig cell destruction after ethane dimethane sulfonate treatment: a morphometric study.*Asian J Androl*, 8: 289–299.

******