WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 5, 1667-1687. Research Article ISSN 2277– 7105

REVERSIBLE ACTION OF *JUSSIAEA REPENS* (L) INDUCED ALTERATIONS OF HISTOARCHITECHTURE *VIS-À-VIS* FUNCTIONS IN TESTICULAR TISSUES OF RAT

Subhasish Ghosal[1], Indrani Chakraborty[2] and Nirmal Pradhan*[3]

[1]Presidency University, Department of Biological Sciences, 86/1 College Street, Kolkata 700073,WB, India.

[2]Krsishnagar Govt.College, Department of Physiology, Nadia, WB, India.

[3]Hooghly Mohsin College, Hooghly, WB, India.

Article Received on
28 Feb 2015,

Revised on 24 March 2015,
Accepted on 018 April 2015

***Correspondence for Author**
Dr. Nirmal Pradhan
Hooghly Mohsin College,
Hooghly, WB, India.

ABSTRACT

Jussiaea repens is a non-toxic antigonadal herb, which has been reported to cause alteration of testicular functions, morphology of spermatozoa and reduced fertility rate. The present study was designed to investigate whether the crude aqueous extract of *Jussiaea repens* can affect the normal histoarchitechture of testicular tissue and vis-à-vis functions when administered orally and also to study whether the effect was reversible or not after withdrawal of treatment. Results showed that when crude aqueous extract of *J.repens* (except root) was fed orally at a dose of 200 mg/kg b.wt/day for a period of 28 days, caused marked alterations in histology of testis, reduction in seminiferous tubular and Leydig cell nuclear diameter. Spermatogenic stage count showed the arrest of spermatogenesis at stage VII followed by significant reduction in number of sertoli cells and spermatogenic cells i.e., SpgA, pLSc, mPSc, 7Sd and elongated spermatids. The H&E staining and SEM studies of treated testis showed reduction in interstitial tissue with leydig cells, tubular lumen, mature spermatids, thickness of basal lamina with irregular outline and profuse intraepithelial vacuolation. Acridine orange staining of treated testis showed disintegrated DNA with partial damage of spermatozoa in seminiferous tubule. The testicular sialic acid, phospholipid and GSH showed significant reduction but MDA was increased insignificantly in treated group where it was unaltered in serum. Serum level of testosterone, LH, FSH and testicular testosterone were greatly reduced in treated group. All these effects were restored towards normal after withdrawal of treatment. So, from these studies it may be concluded that

J.repens, by altering the male reproductive hormone level, affected the normal histoarchitechture of testicular tissue vis-à-vis functions and the effects were reversible.

KEY WORDS: Histoarchitechture, Seminiferous tubule, Sertoli cells, Leydig cells, Testosterone, Spermatogenesis.

INTRODUCTION

The function of male reproductive system is depicted by histological structure of testicular tissues, which is under the control of Pituitary-Gonadotrophin axis. So, altered hormonal profile, alter the histoarchitechture of testicular tissues and in turn affects the normal testicular functions like spermatogenesis and steroidogenesis.

Jussiaea repens L (synonym – *Ludwigia adscendens L.)*, a water creeping prime rose of onagraceae family. Its stem and leaves float on the surface of water and flowers consist of five white petals with yellow at the base. It has great medicinal values as reported by others as anti-diabetic, anti-inflammatory, hepatoprotective, antibacterial and other such remedial activities.[1-5] Recently we have reported this plant as a non-toxic antigonadal herb.[6] Oral administration of this plant extract (except root) for a period of two spermatogenic cycle, affects the sperm quality, sperm morphology which were reflected through significant reduction in fertility rate.[7] Our studies also show that antifertile activity of this herb on male reproductive system is due to alterations of biochemical parameters at serum and testicular level and thus it acts as herbal male antifertility agent.[5] Reproductive malfunctioning, is one of the consequences of the altered histological structure of the reproductive tissues and also altered reproductive hormone profile, which ultimately disrupt the testicular functions. So present study was designed to see whether the treatment of crude aqueous extract of *Jussiaea repens* affect the histoarchitecture of testes and the reproductive hormone profile which are mainly responsible for maintenance of structure-function relationship of male reproductive system and also to study whether its actions are reversible or not.

MATERIALS AND METHODS

Plant material

The plant, *Jussiaea repens L*, was collected from wetlands of North 24 Pgs, West Bengal, as reported earlier[6] and authenticated by taxonomist of Central National Herbarium (Kolkata), Botanical Survey of India (BSI), Shibpur, Howrah, having voucher specimen number NP-01

dated 25.03.2011. The voucher specimen was deposited in the Botanical Survey of India (BSI) for future reference.

Preparation of extract

The plant extract (except root) was prepared as reported earlier.[6] Briefly, the dried powder sample (400gm) of *J. repens* was extracted in 4 L boiled distilled water at 50°C for 30 minutes and filtered accordingly using clean muslin cloth, ordinary filter paper and then by Whatman No.1 filter paper. The resulting filtrate was concentrated using rotary evaporator and further dried at 40°C, then stored at 4˚C for further use.

Animal selection and maintenance

24 adult male albino rats (*Rattus norvegicus L.*) of Wistar strain weighing 130g ±10 were selected for the experiment. The animals were acclimatized to laboratory environment for a period of one week before starting the experiment. The animals were maintained under standard laboratory conditions (12 hrs light : 12 hrs dark, 25±2°C and relative humidity 40-60%) with free access to standard diet[8] and water *ad libitum*. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) (Ref. no. PU 796/03/ac/CPSEA) guided by CPCSEA, Govt. of India.

Animal treatment

Animals were divided randomly into three groups having 8 animals in each and were treated as –

Group I: Control, fed distilled water (0.5 ml/ 100 g body wt/day) for 28 days.

Group II: Treated, fed 0.5ml aqueous extract (200mg/kg body weight/day) for 28 days.

Group III: Recovery, fed 0.5ml aqueous extract (200mg/kg body weight/day) for 28 days and kept without treatment for next 28 days.

The daily dose was prepared by suspending the extract in 0.5 ml of sterile distilled water and administered to each animal orally by oral gavage needle. The initial body weight of each animal was recorded before administration of the extract and subsequently weighed twice weekly throughout the experiment and the dose was adjusted accordingly.

On29th. day (24 hours after the last dose of treatment and 18 hours after fasting), animals from control and treated groups, where after next 28 days (at 57 th. day) the animals from recovery group were sacrificed by diethyl ether anaesthesia. Blood samples were collected

for Serum preparation by centrifugation and reproductive organs i.e. testes of each animal were dissected out, freed from adherent tissues. One testis was stored at - 20°C for different biochemical assay and other was taken for histological studies.

Methods of biochemical estimations

Estimation of testicular sialic acid[9]

Testicular tissue was homogenized in PBS buffer (50 mg / ml) (0.1M, PH 7.4). Homogenate was hydrolysed with 0.1 N H_2SO_4 and centrifuged at 3000 rpm for 10 min. The supernatant was incubated at 80°C for 1 hour. 0.5ml of the hydrolysed supernatant was taken in a test tube and 0.2ml of sodium periodate solution (0.2 M sodium periodate in 9M phosphoric acid) was added and mixed thoroughly by shaking. Tubes were cooled at 20°C for 20 min and 1ml of sodium arsenite solution (10% sodium arsenite in 0.1 N H_2SO_4 with 0.5 M sodium sulfate) was added to this mixture.The brown colour was disappeared after shaking. Then 3ml of thibarbituric acid solution (0.6% TBA in 0.5 M sodium sulfate) was added and the mixture was heated in boiling water bath for 15 minutes. After cooling, 4.5ml of cyclohexanone was added with thorough shaking for 15 seconds to develop pink colour by cyclohexanone. The mixture was centrifuged to get a clear pink layer of cyclohexanone. This supernatant was pipeted out and intensity of colour was measured in spectrophotometer at 550 nm. The sialic acid content of the sample was determined from the standard curve of sialic acid and was expressed in mg/gm of tissue.

Estimation of serum and testicular total lipid and phospholipid

Testicular tissues were extracted according to the procedure of Folch *et al.* [10] 250 mg of tissue was extracted with 6 ml 3:1 (V/V) alcohol-ether mixture and kept at 65°C in a water bath for two hours, cooled and centrifuged at 3000 rpm for 30 min. The supernatant was decanted. 6 ml 3:1 alcohol-ether mixture was added to the residue and repeated twice following same procedure. The total volume of decanted filtrate was used for the estimation of total lipids, phospholipids (PL).

Estimation of total lipid[11]

Three test tubes were labelled as test, blank and standard. 0.1 ml serum, 0.1 ml tissue extract, 0.1 ml distilled water and 0.1 ml working standard (1000 mg/dl, dissolving 1.0 gm of olive oil in 100 ml chloroform) were taken respectively. 2 ml concentrated H_2S0_4 was added to each tube and they were heated in boiling water bath for 10 minutes, Cooled and 2.5 ml phospho-vanillin reagent [Mixed 35ml of vanillin reagent (Dissolved 0.6 g of vanillin with

100 ml water) and 60 ml of concentrated phosphoric acid was mixed with constant stirring in addition to 5.0 ml water, which was stored in a brown bottle at room temperature] was added to all tubes and incubated at 37°C for 15 minutes. Lipids react with vanillin in presence of sulphuric acid and phosphoric acid to form a pink colored complex. The optical densities was measured at 540 nm in a spectrophotometer. Result was expressed as mg /dl of serum and mg /gm of tissue.

Estimation of phospholipid[12]

0.5 ml serum / tissue extract was mixed with 0.2 ml 0.1M ammonium ferrothiocyanate solution (27.03 gm of ferric chloride hexahydrate and 30.40 gm of ammonium thiocyanate was dissolved in 1000 ml distilled water) and 2.5 ml chloroform. The contents of the tubes were vortexed vigorously on cyclo mixer for 15 sec. and centrifuged at 1000 rpm for 5 min. The lower layer was removed by using syringe with long needle. The absorbance of test solution and standard (1mg phosphotidylcholine was dissolved in 1 ml of chloroform) was taken at 485 nm against blank. Result was expressed as mg /dl of serum and mg /g of tissue.

Estimation of serum and testicular reduced glutathione (GSH) and MDA

About 250 mg of testicular tissue was homogenized in 4 ml of PBS buffer (0.1M, PH 7.4). Homogenates were centrifuged at 9000 rpm for 20 min at 4°C and the resultant supernatant was used for the determination of GSH and MDA.

Estimation of reduced glutathione (GSH)[13]

0.2ml serum/1.0 ml tissue supernatant was precipitated with 1.0 ml 10% TCA. The mixture was allowed to stand for 30 min. at room temperature and centrifuged at 2500 rpm for 15 min. From it 0.5 ml supernatant was taken and mixed with 2.0 ml of DTNB (60 mg DTNB was dissolved in 100ml of 0.2M sodium phosphate, pH 8.0) and 0.5 ml phosphate buffer (0.2 M, pH 8.0). The absorbance was read within 5 min. just after addition of DTNB. O.D of unknown and standard (10 mg of reduced glutathione dissolved in 100 ml of distilled water) were read against blank at 412 nm in a spectrophotometer and result was expressed in mg GSH /g tissue.

Estimation of malondialdehyde(MDA)[14]

0.2ml serum / 1.0ml tissue supernatant was added to 2ml of TCA-TBA- HCL (1:1:1) reagent (thioarbituric acid 0.37%, 0.24N HCL and 15% TCA), boiled at 100℃ for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10

minutes. The supernatant was removed and the absorbance read at 532 nm against a blank in spectrophotometer. MDA was calculated using standard MDA (0.2 mg of 1,1,3,3 Tetrahydroxy-propane dissolved in 100 ml of distilled water) and expressed in mg/ gm tissue.

Testicular histology

For the histological studies, testes were removed and immediately fixed in Bouins solution for 18 hours. After fixation picric acid was removed through three changes in 70% ethanol (0ne hour each), then the tissues were dehydrated using graded concentrations of ethanol (80 %, 95 %, 100 % alcohol twice at 1 hour each). Tissues were cleared in xylene by transferring into equal volumes of absolute alcohol and xylene (1:1 v/v) for twice at 1 hour each. The tissues were then infiltered in molten Paraffin wax in an oven at 58°C. Three changes of molten paraffin wax at one-hour interval, finally embedded and blocked out using L shaped metal molder. Serial sections were cut from the mid portion of testis using the rotary microtome at 5 µ thickness, mounted on slides and were stained using Hematoxilin-Eosin method. Each section, finally mounted in DPX with cover slip, and examined under the microscope.

Few sections were randomly stained with acridine orange[5] and studied under a fluorescent microscope.

Spermatogenic cells in the testis at stage VII were counted according to the method of Leblond and Clermont [15] to evaluate spermatogenesis. Nearly, 20 round seminiferous tubule were randomly selected. The number of germ cells i.e. type A spermatogonia, preleptotene spermatocytes, midpachytene spermatocytes, round spermatids and elongated spermatids was counted in seminiferous tubular cross sections of each rat testis. All the counts (crude count) of the germ cells were converted to true counts by the Abercrombie formula, as true counts = (crude count × section thickness) / (section thickness + nuclear diameter of germ cells).[16] Mean number of these cells in each group was compared with other groups. The seminiferous tubular diameter, sertoli cell and leydig cell nuclear diameter were measured using imaje J software with 40X magnification.

Scanning electron microscopy (SEM) study

Testicular tissues were fixed with 2.5% glutaraldehyde in phosphate buffer (PH 7.4) for 4 h. Then were washed and dehydrated in ascending grades of ethyl alcohol (50%- 70%- 80%-

90%- 100%).The tissues were then further dehydrated in amyl acetate for 30 minutes and dried at critical point using liquid carbon dioxide in BALTEK CPD030 critical point dryer. Testis samples were mounted on an aluminium stub and then coated with gold. The prepared samples of testis were examined under the scanning electron microscope and photographs were taken under S-530 Hitachi SEM.

Estimation of serum testosterone, LH and FSH

Blood samples were taken from the cardiac puncture, centrifuged at 2500 rpm in room temperature for 15 min. to separate the serum and stored at - 20°C and immediately assayed for the measurement of testosterone by ELISA Method [17] and Luteinizing hormone (LH), follicle stimulating hormone (FSH) by CLIA method [18,19] using standard assay kits. Assay procedures were followed as specified by manufacturers instruction. The ELISA (AcccuBind™ ELISA) and CLIA (AccuLite™ CLIA) microwell kits were used from Monobind Inc. Lake forest,CA 92630,USA.

Estimation of testicular testosterone

About 100 mg of testicular tissue was homogenized in 1 ml of PBS buffer (PH 7.4). Homogenates were centrifuged at 10000 rpm for 10 min.[20] and the resultant supernatant then kept in - 20°C and were used immediately for determination of testosterone by ELISA Method.[17] Assay procedures were followed as specified by manufacturers instruction .The ELISA (AcccuBind™ ELISA) kits were used from Monobind Inc. Lake forest,CA 92630,USA. Testicular testosterone content was calculated as ng/ml/mg of testis.

Statistical analysis of data

All the recorded values were expressed in mean ± SEM. The treated groups were compared to control using one way ANOVA with post hoc LSD test were performed using SPSS version 16 Software. The value of $p<0.05$ was considered to be statistically significant.

RESULTS

The results of the present study revealed that, oral administration of crude aqueous extract of *Jussiaea repens L.* for consecutive 28 days to male rats caused no significant change in total lipid, phospholipid, GSH and MDA level in serum when compared to control (table-1). In table-2, results from the testicular biochemical parameters in treated group showed significant decrease in sialic acid ($P < 0.01$) and phospholipid ($P < 0.05$) content and also in testicular GSH ($P < 0.05$) activity, but no change was observed in testicular total lipid but

insignificant increase in MDA level in treated groups when compared with control. However, in recovery group (Gr-III), after withdrawal of extract treatment, all the parameters were found almost unaltered in respect to control group.

Table 1: Serum biochemical parameters in different groups.

	Serum total lipid (mg/ml)	Serum phospholipid (mg/ml)	Serum GSH level (µmol /ml)	Serum MDA (nmol/ml)
Group I: Control group	2.0263±0.1530	3.6738±0.2999	0.5146±0.0037	6.2020±0.4272
Group II: treated with aqueous extract (200 mg/kg body weight/day)	2.14967±0.1371	3.9343±0.4589	0.5076±0.0031	6.9465±0.1096
Group III: Recovery group	2.0149±0.1320	3.8388±0.3223	0.5136±0.0020	6.3244±0.3894

Values were expressed as mean ± SEM, N=8, Group II (treated) was compared with Group I (Control) and Group III (Recovery).

Table 2: Testicular biochemical parameters in different groups.

	Testicular Sialic acid (mg / gm tissue)	Testicular total lipid (mg /gm tissue)	Testicular phospholipid (mg/gm tissue)	Testicular GSH (µmol /gm tissue)	Testicular MDA (nmol/ml)
Group I: Control group	2.7200± 0.2284	3.9754±0.3422	36.3248±2.3727	2.1885±0.0970	17.4478±1.1172
Group II: treated with aqueous extract (200 mg/kg body weight/day)	1.9830**±0.914	3.9328±0.4194	30.1988*±1.4831	1.8975*±0.0430	21.4455±1.8231
Group III: Recovery group	2.6950±0.1568	4.0223±0.3086	33.5585±1.3696	2.1025±0.1319	18.5075±1.3375

Values were expressed as mean ± SEM, N=8, *Significant (P < 0.05), **Significant (P<0.01). Group II (treated) was compared with Group I (Control) and Group III (Recovery).

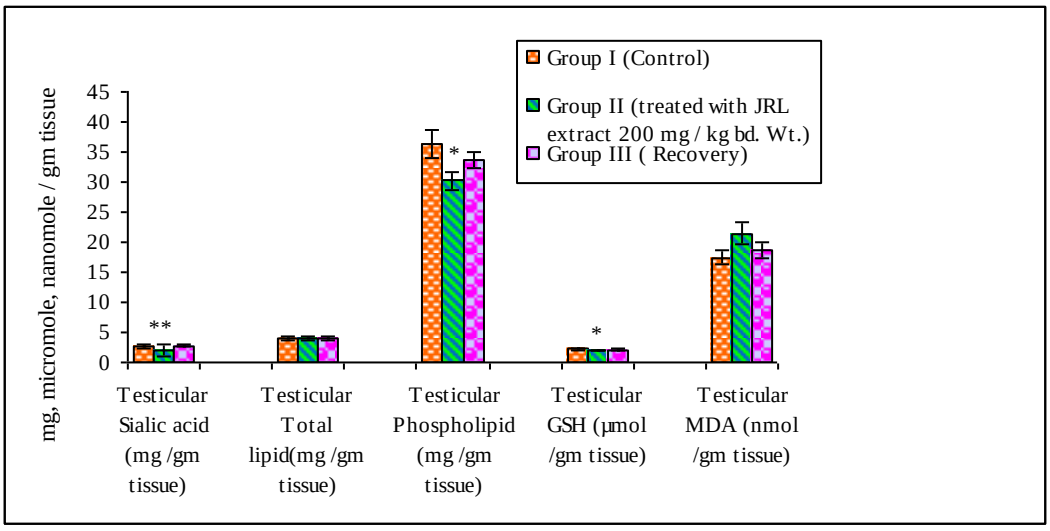

Fig. 1: Effect of *J. repens* (L) extract in testicular biochemical parameters in different groups. Values were expressed as mean ± SEM, n=8, *significant (p < 0.05), **significant (p<0.01).Group II (treated) was compared with group I (control) and group III (recovery).

Histological analysis of H&E stained sections of testes in control group showed the presence of compact seminiferous tubule and well organized germinal cells at various stages of spermatogenesis with clear spermatozoa maturation occurring near the lumen. The interstitial cells of Leydig prominently interspersed between the seminiferous tubules (Fig 3). But the testicular tissue in treated animals (Group II) showed alteration and distortion in both germinal epithelium and seminiferous tubules.

Where Sertoli cells were markedly reduced in number ($P < 0.01$) when compared to control. Vacuoles and few primary spermatocytes with pyknotic nuclei in seminiferous tubules were predominant in treated groups (Fig 4).Seminiferous tubular diameter and Leydig cell Nuclear Diameter of the rats in treated group (Table 3) were significantly decreased ($P < 0.01$) as compared to control and the lumen of the seminiferous tubule contain no or little spermatozoa. In the treated group there was a significant reduction in the total count of spermatogonia A (SpgA), preleptotene spermatocytes (pLSc), mid pachytene spermatocytes (mPSc) ($P < 0.01$) and step 7 spermatid (7Sd) ($P<0.05$) (Table 4) as compare to control. A massive loss of elongated spermatids (ESd) was found in stage VII of the spermatogenic cycle. After 56 days cessation of treatment (after recovery period), however, all testicular cell counts restored towards the normal range (Table 4, Fig. 5).

Table: 3 Seminiferous tubular and Leydig cell nuclear diameter in testis of rats.

	Seminiferous Tubular Diameter (µm)	Leydig cell Nuclear Diameter(µm)
Group I: Control group	255.5450± 2.9911	5.6713±0.3112
Group II: treated with aqueous extract (200 mg/kg body weight/day)	238.4650 **± 2.9046	3.2050 **± 0.2125
Group III: Recovery group	249.3875± 2.2739	5.0088±0.1841

Values were expressed as mean ± SEM, N=8, **Significant ($P<0.01$). Group II (treated) was compared with Group I (Control) and Group III (Recovery).

Table: 4 Cell population dynamics in stage VII seminiferous tubule of rats.

	No. of Sertoli cell	SpgA	pLSc	mPSc	7Sd	Elongated Spermatids
Group I: Control group	2.1922±0.1223	1.6773±0.1032	16.3489±1.7088	23.3278±1.2017	97.4654±5.6737	145.7500±11.1271
Group II: treated with aqueous extract (200 mg/kg body weight/day)	1.4909**±0.1164	1.2357**±0.0668	9.6186**±0.5892	17.0656**±0.9823	79.9604*±5.3678	100.7500*±13.8818
Group III: Recovery group	1.9601±0.1568	1.6292±0.0807	15.4267±1.7883	22.9399±1.2541	96.7943±5.3114	136.3750±12.4124

Values were expressed as mean ± SEM, n=8, *significant ($p < 0.05$), **significant ($p<0.01$). Group II (treated) was compared with group I (control) and group III (recovery).

Table 5: Hormone profiles in serum and testis of rats.

	Serum Testosterone (ng/ml)	Serum LH (mIU /ml)	Serum FSH (mIU /ml)	Testicular Testosterone (ng/100 mg tissue)
Group I: Control group	3.7538± 0.4813	6.2775± 0.7261	9.6500±1.2068	3.538±0.228
Group II: treated with aqueous extract (200 mg/kg body weight/day)	1.9300 **± 0.2035	3.2200 *±0.6358	5.8125 *±0.9687	2.601 **±0.146
Group III: Recovery group	3.1238± 0.3278	4.4625± 0.9578	9.6125± 1.1529	3.356±0.172

Values were expressed as mean ± SEM, N=8, *Significant (P < 0.05), **Significant (P<0.01). Group II (treated) was compared with Group I (Control) and Group III (Recovery).

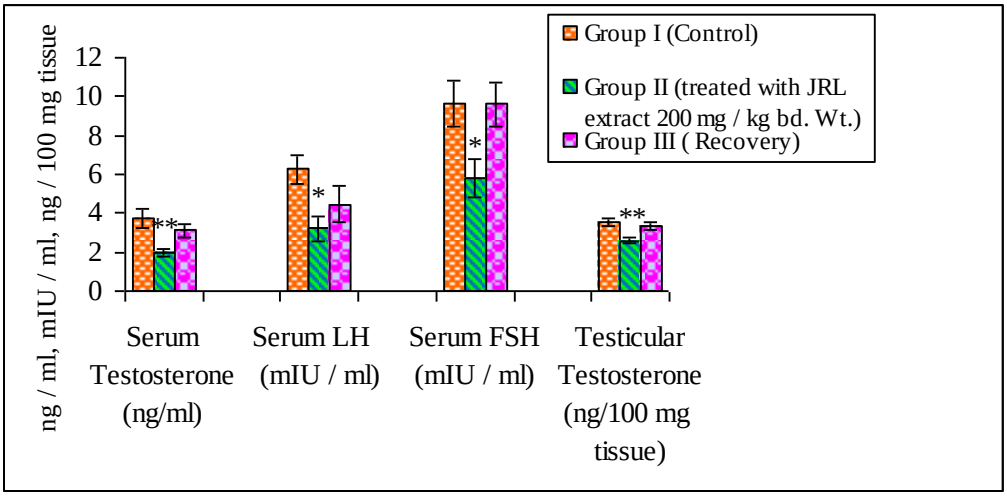

Fig. 2: effect of *J. repens* extract in serum testosterone, LH, FSH and testicular testosterone in control (group I), treated (group II) and recovery (group III) groups. Values were expressed as mean ± sem, n=8, *significant (p < 0.05), **significant (p<0.01). Group II (treated) was compared with group I (control) and group III (recovery).

Results presented in table 5, shows a significant reduction in serum and testicular testosterone (P<0.01), FSH and LH (P<0.05) levels of J. repens treated animals which was restored in recovery group towards normal.

Acridine orange staining was performed to assess the sperm DNA integrity in the experimental groups. Fluorescent microscopic study of the sperm cell with normal integrity showed green whereas those with diminished DNA integrity stained orange-red. Histological analysis of Acridine orange stained section of testes in the control group showed normal structure of seminiferous tubules and plenty of spermatozoa (SpZ) in their lumen having normal green fluorescence nuclei (Fig.6) whereas in treated group, the alteration and

distortion of both germinal epithelium and seminiferous tubules with large number of spermatozoa lost their tail in their lumen and having red or orange fluorescence nuclei (Fig.7) which indicate the partial damage of DNA stained with acridine orange (A.O). But after the withdrawal of plant extract, the seminiferous tubules of the recovery group, showed the lumen filled with spermatozoa and their nuclei were stained green (Fig.8) which indicate the normal integrity of DNA.

Scanning electron microscopic (SEM) observation of the testicular sections from the control group showed round seminiferous tubules surrounded by distinct basal lamina (BL) which has regular outlines and interstitial tissue and spaces in between (Fig. 9).The seminiferous tubules having all types of germ cells lying close to each other. The lumen filled with spermatozoa with long tail. SEM sections of the testis from extract treated group showed moderate morphological changes compared to the control animals. Hence, seminiferous tubules found with irregular outline and reduced thickness of basal lamina (BL) with wide interstitial tissue and spaces. Also, irregular distribution of epithelial lining with germ cell and intraepithelial vacuolation (V) and reduced number of spermatozoa were clearly seen in their lumen (Fig.10). Testicular section of recovery group showed a near normal seminiferous tubule with clearly seen germ cells which was improved after withdrawal of plant extract (Fig.11).

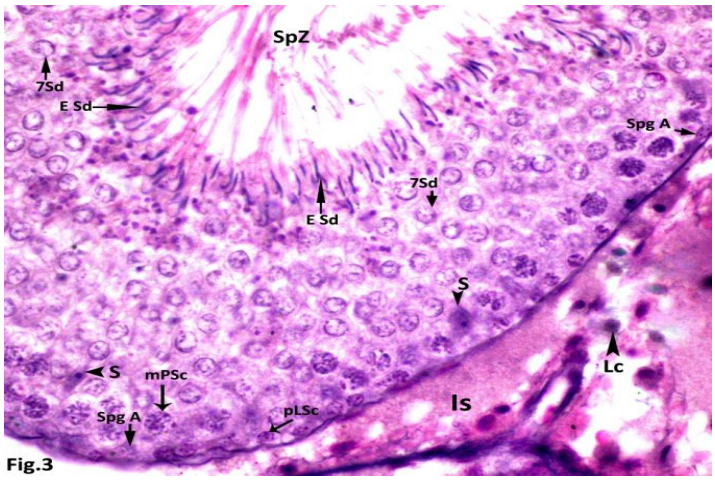

Fig. 3: Photomicrograph of rat testis of control group showing well arranged seminiferous tubules containing different type of spermatogenic cells; spermatogonia A(SpgA), preleptotene spermatocytes (pLSc), midpachytene spermatocytes (mPSc), sertoli cells (S), step 7 spermatids (7Sd), elongated spermatids (ESd) and spermatozoa (SpZ) in their luman also normal architecture of interstitial tissue with leydig cells (Lc). (H&E staining, X400).

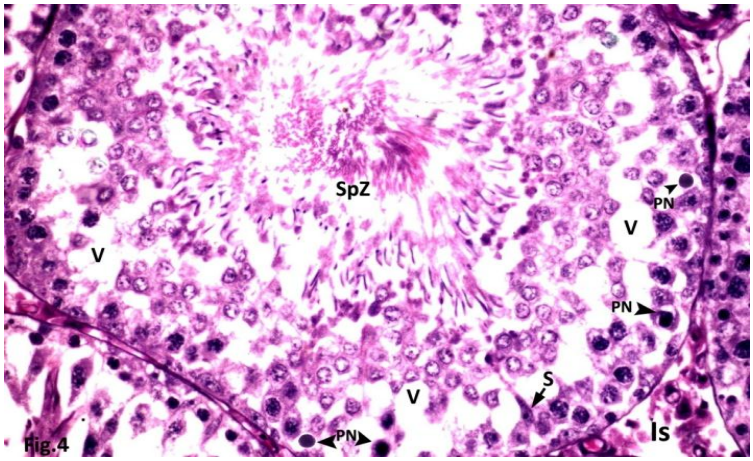

Fig. 4: Photomicrograph of rat testis of treated group showing distorted seminiferous tubules, degeneration of spermatogenic cells, intraepithelial vacuolation (V), pycnotic nuclei (PN) and deformed sertoli cells are clearly seen but reduction in the interstitial tissue with leydig cells. (H&E staining, X400).

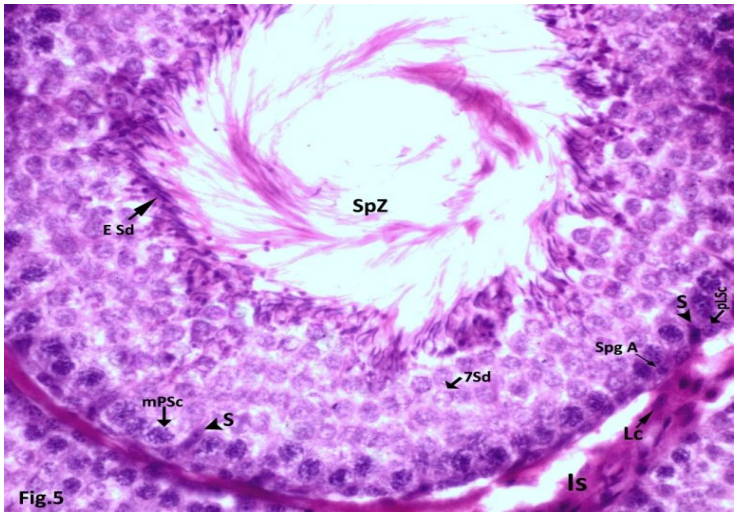

Fig. 5: Photomicrograph of rat testis of recovery group showing the near normal structure of seminiferous tubules and normal distribution of epithelial lining with germ cells. The interstitial tissue (Is) contains leydig cells (Lc) as normal. (H&E staining, X400).

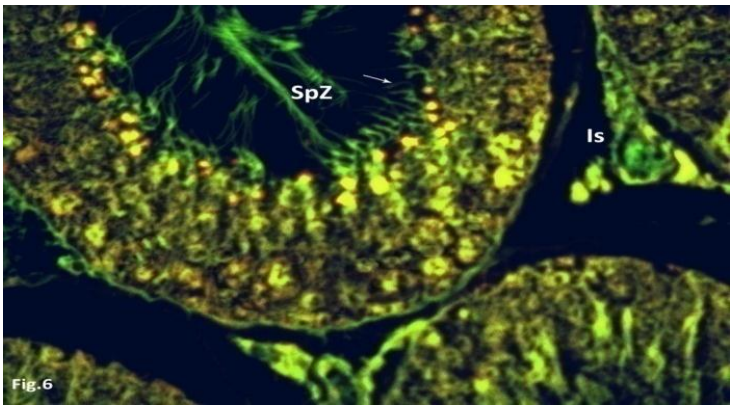

Fig. 6: Photomicrograph of rat testis of control group showing normal structure of seminiferous tubules and plenty of spermatozoa (SpZ) in their lumen having normal green fluorescence nuclei (thin arrow) stained with acridine orange (A.O staining, X400).

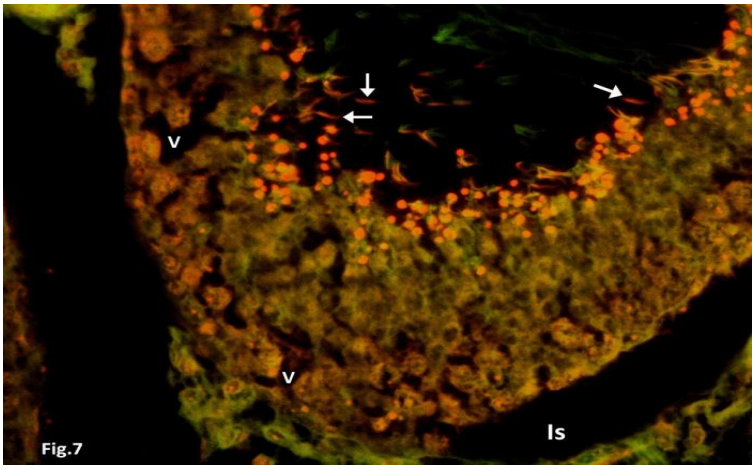

Fig. 7: Photomicrograph of the rat testis of treated group showing deformed structure of seminiferous tubules and irregular distribution of epithelial lining with germ cell and intraepithelial vacuolation (v) are clearly seen and the large number of spermatozoa lost their tail in their lumen and having red or orange fluorescence nuclei (thick arrow) which indicate the partial damage of DNA stained with acridine orange (A.O staining, X400).

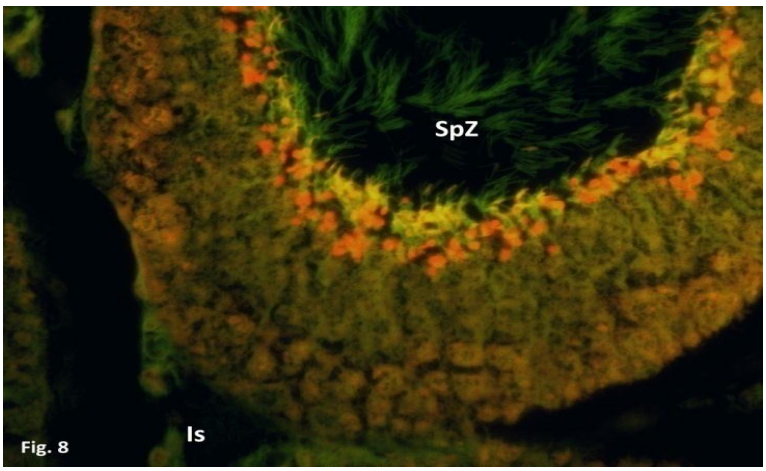

Fig. 8: Photomicrograph of rat testis of recovery group showing normal structure of seminiferous tubules and plenty of spermatozoa (SpZ) in their lumen having almost normal green fluorescence nuclei stained with acridine orange (A.O staining, X400).

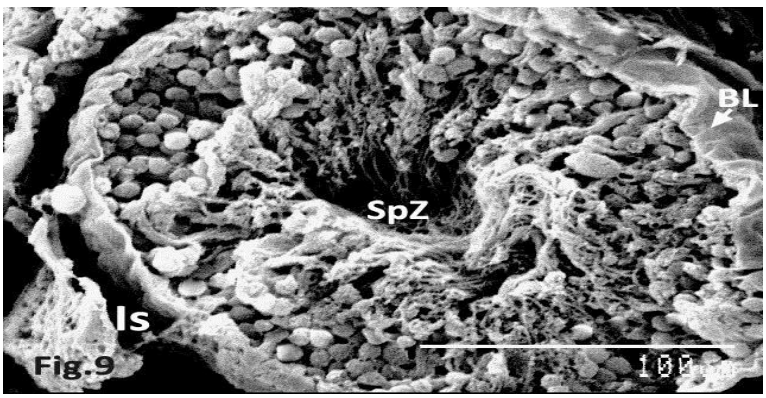

Fig. 9: Scanning electrone micrograph of testicular section of rat of the control group showing normal seminiferous tubule surrounded by basal lamina (BL) and interstitial tissue (Is) and spaces in between. The presence of spermatozoa (SpZ) with long tail in their lumen.

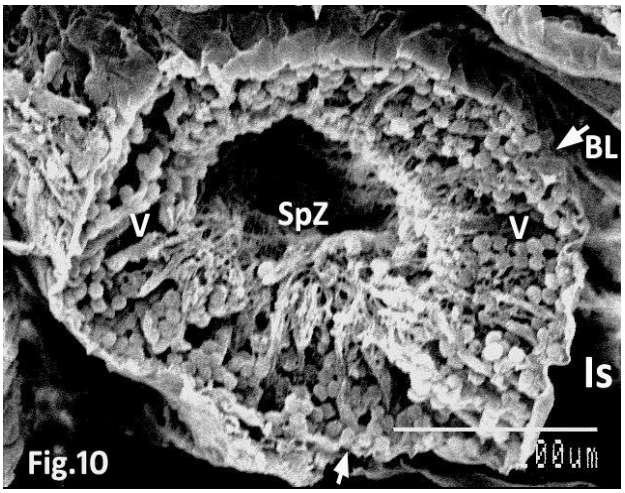

Fig. 10: Scanning electrone micrograph of testicular section of rat of the treated group showing irregular seminiferous tubule and reduced thickness of basal lamina (BL) and wide interstitial tissue and spaces. Irregular distribution of epithelial lining with germ cell and intraepithelial vacuolation (v) are clearly seen and reduced number of spermatozoa their lumen.

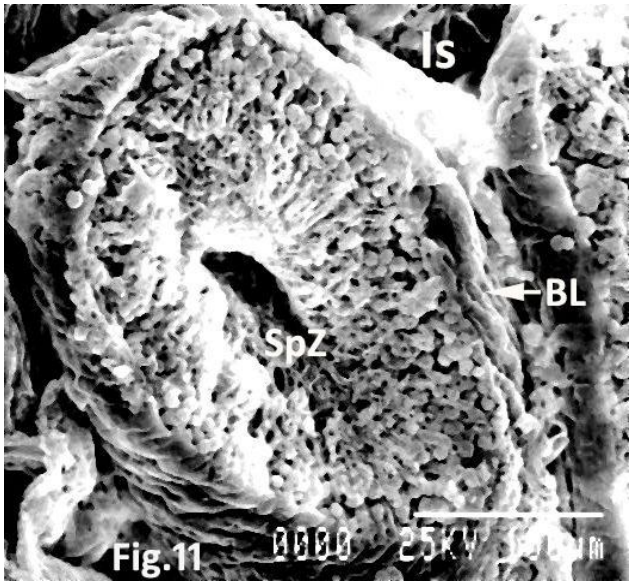

Fig. 11: Scanning electrone micrograph of testicular section of rat of the recovery group showing normal seminiferous tubule surrounded by basal lamina and interstitial tissue and spaces in between. The presence of spermatozoa with long tail in their lumen.

DISCUSSION

Various herbal Plants are in used globally as safe and natural source of medicines for treatment of different diseases including for infertility. Various medicinal plants have significant anti-fertility activity in animal models. But development of effective, safe, cheap

and acceptable male contraceptive is challenging. *Jussiaea repens L* is such a herb that has non toxic antigonadal activity,[6] which reduced the sperm count, motility, viability and morphological alteration of spermatozoa reflected through significant reduction in fertility rate.[7] The present study was designed to explain whether crude aqueous extract of *Jussiaea repens* (JR) affects the histoarchitecture of testes and the reproductive hormone profile which are primarily responsible for maintenance of structure-function relationship of reproductive system and also to explain whether its actions are reversible or not. In our previous study we have reported that crude aqueous extract of JR caused alteration of several biochemical parameters in serum and testicular level, which directly or indirectly inhibits the steroidogenic and spermatogenic pathways and effects are reversible after withdrawal of treatment.[5]

The present study showed that though oral administration of crude aqueous extract of *J.repens* (except root) at a dose of 200mg/ kg b.wt for a period of 28 days, did not altered the total lipid, phospholipid, GSH and MDA level in serum significantly when compared to control (table-1), but the testicular sialic acid, phospholipid content and GSH activity were significantly decreased without altering the testicular total lipid and showed insignificant increase in MDA level when compared with control (Table-2, Fig.1). A significant decrease in the sialic acid content of testes after JR extract treatment in this study may be associated with the reduced androgen production, as supported by others and cause alteration of structural integrity of acrosomal membrane and may lead to inhibition of spermatogenesis.[21-26] In normal physiological conditions, nearly all cellular glutathione is present in its reduced form which is used by the cells as a defence against oxidative stress and as an amino-acid source for spermatogenesis.[27-29] It is also reported that most of the testicular GSH is probably present in the developing germinal cells and it increases during the initiation of spermatogenesis.[30-32] The reduced GSH and insignificant rise of MDA in treated group of our study (Table-2) indicate the utilization of GSH for reduction of oxidative stress. Decrease of GSH in testis may be the indication of impaired spermatogenesis which may be due to protamination disturbance [29] or due to the decreased activity of glucose 6-phophate dehydrogenase and their coenzyme NADPH in testis as we have reported in our previous study after extract treatment. [5] It may be the cause of reduced production of GSH in testis in this present study, as GSH depends on NADPH [33] and G-6-PDH,which are responsible for the production of NADPH.[28] The activity of G-6-PDH is associated with the functions of Leydig cells.[34] So, the decreased G-6-PDH activity

by JR extract, suggesting that it is indirectly responsible for suppression of Leydig cell functions which has reflected on our histological studies.

Alterations of histological structure of the testicular tissues, like reduction of seminiferous tubular diameters and Leydig cell nuclear diameters and also spermatogenic cells in Stage VII seminiferous tubule (Table 3) were found in treated group. Stage VII seminiferous tubule has been selected for quantitative study of spermatogenesis, because all varieties of germ cells are present at this stage.[35] Similar results were observed in another study where depletion of GSH alterd spermatogenesis.[36] The Phospholipid increases during maturation of the spermatids in the testis and is closely associated with spermatogenesis,[37] reduction of which may impair spermatogenesis in JR extract treated rats.

In the present study, marked alterations in hormone profile like lower levels of all male reproductive hormones including FSH, LH and testosterone were observed in compare to control (Table-5,Fig.2).These hormonal levels are highly correlated with the change in histoarchitechture of the seminiferous tubules (Figure 4). Normally, FSH stimulates the development of spermatogonia to spermatocytes and also maintains the spermatogenic process.[38-39] Both FSH and LH are necessary for meiosis and development of spermatids.[40] The observed reduction in the number of spermatogonia and other spermatogenic cells (Table - 4) may indicate lowered availability of FSH and LH, which are essential for initiation and maintenance of spermatogenesis.[41-42] Low levels of these hormones decrease endogenous testosterone secretion from the testis depriving development of normal maturation of sperm and also it suppress testicular steroidogenesis and spermatogenesis.[43-44] Similar results are also reported in other study where depletion of FSH, LH and testosterone leads to inhibition of spermatogenesis.[45-46] In the testicular tissue, mean of the seminiferous tubular diameters are used generally to assess histopathologic damage.[47] In our study, the decreased diameter of seminiferous tubule and Leydig cell nucleus also may be due to low gonadotrophins and testosterone which is correlative with other studies.[48-49]

The acridine orange staining is an established cytochemical method for determining sperm DNA integrity.[50] Sperm chromatin condensation and stability can detect the sperm maturation and possible disorders in spermatogenesis.[51] The presence of orange or red color sperm in the lumen of the seminiferous tubule in treated group (Fig.7) indicated that there may be abnormal spermatid maturation due to partial denaturation and loss of binding of DNA to basic protein and hamper the spermatogenesis process.[52-55]

In SEM study, altered basement membrane structure has been associated with severe functional impairment of the testis. The destruction might subsequently affect transportation of different metabolites[42] widening of interstitial spaces and decreased number of Leydig cells in interstitial space is responsible for decreased production of testosterone known to be responsible for normal testicular architecture.[56]

Results of the above studies show that the antispermatogenic activity of *Jussiaea repens* on male reproductive system of rat is mediated through alterations of several biochemical parameters at serum and testicular level and also histoarchitecture of testes and the reproductive hormone profile which directly or indirectly inhibit the steroidogenic and spermatogenic pathways, there by reduced the fertility rate and the effects were reversible after withdrawal of treatment. The reversible action of this plant extract is highly encouraging for using the extract as contraceptive drug. Our present study shows that, all extract induced alterations of histoarchitecture of testes, biochemical and the reproductive hormone profile of serum and testes, which are actually responsible for maintenance of structure-function relationship of reproductive system, affect the normal fertilization processes and returned back towards normal control level after withdrawal of treatment. Our fertility and mating experiments with recovery group also supported the reversible action of this extract.[7]

CONCLUSION

From the present studies it may be concluded that oral administration of crude aqueous extract of *Jussiaea repens* (except root), at a dose of 200mg/kg b.wt/day for 28 days consecutively on male rats, alters the histoarchitechture vis-à-vis functions of testicular tissues by altering the biochemical and hormonal profile of male reproductive system and the effects are reversible on withdrawal of treatment.

ACKNOWLEDGEMENTS

Authors are thankful to the Department of Physiology, Presidency University, for providing infrastructural facilities to carry out this research; USIC Dept. University of Burdwan for fluorescence microscopic and SEM study.

REFERENCES

1. Marzouk MS, Soliman FM, Shehata IA, Rabee M, Fawzy GA. Flavonoids and biological activities of Jussiaea repens. Nat Prod Res, 2007; 2l(5): 436-443.

2. Firoz A, Selim MST, Shilpi JA. Antibacterial activity of Ludwigia adscendens. Fitoterapia, 2005; 76(5): 473-475.
3. Panda A, Misra MK. Ethnomedicinal survey of some wetland plants of South Orissa and their conservation. Ind J Trad Knowl, 2011; 10(2): 296–303.
4. Sunyapridakul L, Chin Ting Sae, Ratdilokpanich Kitti,Poneprasert Sakchai. Chinese Medicinal Plants in northern Thailand, Chiang Mai Medical Bulletin, 1983; 137-157.
5. Chakraborty Indrani, Ghosal Subhasish, Pradhan Nirmal Kr. Jussiaea repens (L) Acts as an Antifertility Agent – A Search for Herbal Male Contraceptive. Int. J. Pharm. Sci. Rev. Res., 2014; 24(2): 288-296.
6. Pradhan NK, Ghosal S, Chakraborty I. Jussiae repens is a nontoxic antigonadal herb—A dose dependent study on male rats. Int J Pharm Bio Sci., 2013; 4(2): 131-143.
7. Ghosal S, Chakraborty I, Pradhan NK. Jussiaea repens (L) induced morphological alterations in epididymal spermatozoa of rat. International Journal of Pharmaceutical Sciences Review and Research, 2013; 22(2): 288-295.
8. Sciences (NCLAS) – A profile, The Indian Council of Medical Research, Published by Shri JN Mathu, New Delhi, 2004; 34(4): 21-28.
9. Warren L. A thiobarbituric acid assay of sialic acid. Journal of Biological chemistry. 1959; 234:1971-1975.
10. Folch J, Lees M, and Sloane-Stanley G H. A simple method for preparation for total pure lipids extracts from brain. Fed. Proc, 1954; 13: 209.
11. Frings C S, and Dunn RT. A colorimetric method for determination of total serum lipid based on the su1fo-phosphovanillin reaction. Amer J. Clin. Pathol., 1970; 53: 89.
12. Stewart JC. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. Anal Biochem 1980;104 (1):10-4.
13. Ellman G L. Tissue sulfhydryl groups. Arch Biochem Biophys, 1959; 82: 70-77.
14. Buege JA. and Aust SD. Microsomal Lipid Perox-idation. Methods Enzymol, 1978; 52: 302-310
15. Leblond PC, Clermont Y. Definition of the stages of the seminiferous epithelium in the rat. Ann N Y Acad Sci ,1952; 55: 548-73.
16. Abercrombie M. Estimation of nuclear population from microtome sections. Anat Rec, 1946; 94: 239-47.
17. Tietz NW.Clinical Guide to Labooratory Tests. 3rd ed., Philadelpphia; WA Saunders Co: 1995.
18. Kosasa TS. Measurement of Human Luteinizing Hormone. Journal of Reproductive

Medicine, 1981; 26: 201-6.

19. Saxema BB, Demura H.M., et al, J Clin Endocrinol Metab, 1968;28: 591.
20. Li Shu, Lu DanDan, Zhang Yaling, Zhang Yi . Long-term treatment of hydrogen-rich saline abates testicular oxidative stress induced by nicotine in mice. Journal of Assisted Reproduction & Genetics, 2014; 31 (1): 109 – 114.
21. Levinsky H R, Singer M. Barnet M. Sangive M, Allaloaf D. Sialic acid content of human spermatozoa and seminal plasma in relation of sperms counts. Arch. Androl. Toxicol., 1983;74: 270-75.
22. Gupta RS, Yadav R, Dixit VP & Dobhal MP. Antifertility studies of Colebro oppositifolia leaf extract in male rats, with special reference to cell population dynamics. Fitoterapia, 2001; 73: 236-245.
23. Gupta R S, Kachhawa J B S, Chaudhary R. Antifertility effects of methanolic pod extract of Albizzia lebbeck (L.) Benth in male rats. Asian J Androl, 2004 ; 6: 155-159.
24. Verma C L, Dev Keshar, Gupta M L, Purohit R K. Biochemical alterations in mouse testis after the combined treatment of radiation and cadmium chloride.International Journal of Advanced Research, 2014; 2(6): 340-344.
25. Joshi Suresh C, Gajraj Anita. Induction of Hypocholesterolemia Associated with Testicular Dysfunction by Aloe barbedensis in Albino Rats. Pharmacologyonline, 2006; 3: 1- 8.
26. Dixit VP, Gupta RS, Kumar santosh, joshi B C. Reversible chemical sterilization:effects of cyclohexanol Administration on the testes and epididymides of male rabbit.Ind.J. Physio. Pharmac, 1980; 24 (4):278-286.
27. Reed DJ, & Fariss M W. Glutathione depletion and susceptibility. Pharmacological Reviews, 1984; 36: 25S-33S.
28. Luberda Zofia. The role of glutathione in mammalian gametes Reproductive Biology 2005; 5(1): 5-17.
29. Kaneko Tomoko, Iuchi Yoshihito, Kobayashi Takashi, Fujii Tsuneko, Saito Hidekazu, Kurachi Hirohisa and Fujii Junichi.Theexpression of glutathione reductase in the male reproductive system of rats supports the enzymatic basis of glutathione function in spermatogenesis. Eur. J. Biochem, 2002; 269:1570-1578.
30. Calvin H.I, Sot Turner S.I. High levels of glutathione attained during postnatal development of rat testis. journal of Experimental Zoology, 1982; 219: 389-393.
31. Grosshans K. & Calvin H I. Estimation of glutathione in purified populations of mouse testis germ cells. Biology of Reproduction, 1985; 33: 1197-1205.

32. Knapen M F, Zusterzeel P L, Peters W H. & Steegers E A. Glutathione and glutathione-related enzymes in reproduction. A review. Eur. J. Obstet. Gynecol. Reprod. Biol., 1999; 82:171-184.
33. Den Boer PJ, Poot M, Verkerk A, Jansen R, Mackenbach P, Grootegoed JA,Glutathione-dependent defence mechanisms in isolated round spermatids from the rat. Int J Androl., 1990; 13 (1): 26-38.
34. Bo zhang, Sen Lin. Effects of 3, 4-Dichloroaniline on Testicle Enzymes as Biological Markers in Rats. Biomedical and environmental sciences, 2009; 22(1):40-43.
35. Clermont Y, Morgentaler H. Quantitative study of spermatogenesis in hypophysectomized rats. Endocrinology, 1955; 57: 369–382.
36. Sharma Priyanka, Sharma Aksha, Agarwal Meera, Joshi Suresh C, Contraceptive Potential of Calendula officinalis Aqueous Extract in Male Rats. Int.J. Pharm.Sci. Rev.Res., 2013;22(1): 192-197.
37. Lee DJ, Barnes MM, The effect of a single dose of cadmium chloride on the phospholipid fatty acids of the rat testis. J Reprod Fertil, 1971;27(1): 25-9.
38. Connel GM, Eik-Nes KB. Testosterone productionby rabbit testis slices. Steroids,1968; 2(4): 507-16.
39. Russell LD, Alger LE, Nequin LG. Hormonal control of pubertal spermatogenesis. Endocrinology, 1987; 120: 1615-32.
40. Haneji T, Maekawa M, Nishimune Y. Vitamin A and FSH synergistically induce differentiation of type A spermatogonia in adult mouse cryptorchid testes in vitro. Endocrinology, 1984; 114: 801-5.
41. 41.Seetharam Y N, Sujeeth H, Jyothishwaran G, Barad Arvind, Sharanabasappa G, Umareddy B, Vijaykumar M B, Patil Saraswati B. Antifertility effect of ethanolic extract of Amalakyadi churna in male albino mice. Asian J Androl, 2003; 5: 247-250.
42. Elshennawy Waslat W, Abo Elwafa Hanaa R. Histological and Ultrastructural Changes in Mammalian Testis under the Effect of Hydrocortisone. Journal of American Science, 2011; 7(9): 38-48.
43. Kusemiju TO, Osinubi AA, Noronha CC, Okanlawon AO. Effect of aqueous extract of the bark of Carica papaya on the testicular histology in Sprague- Dawley rats. Nig.Qt.J. Hosp Med, 2010; 20(3):133-137.
44. Cameron DF, Murray FT, Drylie DD. Interstitial compartment pathology and spermatogenic disruption in testes from impotent diabetic men. Anat Rec., 1985; 213(1): 53-62.

45. Sharma Aksha, Sharma Priyanka, Chaturvedi Mridula and Joshi Suresh C. Contraceptive Efficacy of Citrullus colocynthis Methanolic Extract in Male Rats. American Journal of Phytomedicine and Clinical Therapeutics, 2014;2: 229-241.
46. Onyeka CA, Aligwekwe AU, Olawuyi TS, Nwakanma AA, Kalu EC, Oyeyemi AW. Antifertility Effects of Ethanolic Root Bark Extract of Chrysophyllum albidum in Male Albino Rats. International Journal of Applied Research in Natural Products,2012; 5(1):12-17.
47. Uguralp S , Bay K.A, Mizrak B, Kaymaz F, Kiziltay A, Hasirci N. The effect of sustained and local administration of epidermal growth factor on improving bilateral testicular tissue after torsion. Urol. Res., 2004; 32: 323-31.
48. Ballester J, Carmen Munoz M, Dominguez J, Rigau T, Guinovart JJ, Rodríguez-Gil JE. Insulin-Dependent Diabetes Affect Testicular Function by FSH- and LH-linked Mechanisms. J Androl., 2004; 25:706-19.
49. Udoh P, Kehinde A. Studies on antifertility effect of Pawpaw seeds (Carica papaya) on the gonads of male albino rats. Phytother res., 1999; 13: 226-228.
50. Tejada R I, Mitchell J C, Norman A, Marik J J, Friedman S. A test for the practical evaluation of male fertility by acridine orange (ao) fluorescence. Fertil. Steril., 1984; 42: 87–91.
51. Hingst O, Blottner S. & Franz C. Chromatin condensation in cat spermatozoa during epididymal transit as studied by aniline blue and acridine orange staining. Andrologia.1995; 27: 275-279.
52. Erenpreiss J, Spano M, Erenpreisa J, Bungum M, Giwercman A. Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J. Androl., 2006; 8: 11–29.
53. Fernandez J L, de la Calle J F V, Tamayo M, Cajigal D, Agarwal A, Gosalvez J. Sperm DNA integrity and male infertility: current perspectives. Arch. Med. Sci., 2009; 5: S55–S62.
54. Henkel R, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, Menkveld R, Gips H, Schill WB, Kruger TF. Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil. Steril. 2004; 81: 965–972.
55. Dehghani F, Talaei-Khozani T, Panjehshahin M. R, Panahi Z. toxic effects of hydroalcoholic extract of kiwi (actinidia Chinensis) on histological structure of the male Sprague-dawley rat reproductive tissues. The Islamic Republic of Iran, 2006; 19-25.
56. Shaikh MA, Naqvi SNH and Chaudhry MZ. Effect of neem oil on the structure and function of the mature male albino rat testes. Braz. J. Morphol. Sci., 2009; 26(1): 49-54.