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HISTOARCHITECTURE AND BIOCHEMICAL STUDIES OF EPIDIDYMIS IN MALE RATS INDUCED BY AQUEOUS EXTRACT OF *JUSSIAEA REPENS* L

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Key words:

Jussiaea repens L, epididymis, testosterone, sialic acid, SEM, infertility

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ABSTRACT: Different herbal Plants are used globally for safe and natural source of medicine from ancient ages. *Jussiaea repens* L, is a medicinal herb that has been reported to have non toxic antigonadal activity, which reduced the sperm count, motility, viability and morphological alteration of spermatozoa in rats. It also alters histoarchitechture vis-à-vis functions of testicular tissues by altering the biochemical and hormonal profile. The present study was designed to evaluate whether *J.repens* crude aqueous extract treatment affects the histoarchitecture of epididymis and its biochemical alterations which are correlated with sperm maturation, as the relationship between spermatozoa and microenviroimient of epididymis during the sperms remain in it, is important for male fertility. Adult male albino rats were fed crude aqueous extract of *Jussiaea repens* at the dose of 200mg/kg b.wt /day for 28 days consecutively, which caused significant reduction in epididymal sialic acid, glycogen, phospholipid, GSH, and testosterone level. But no change in total lipid and MDA level. Histological studies of epididymis showed the epithelial lining and basement membrane was thin and disrupted, the luminal diameter, epithelial height and nuclear diameter significantly reduced in treated group compare to control. So, from these studies it may be concluded that JR extract altering the hormone and bio-molecular level which was reflected on histological alteration of epididymal tissue including spermatozoa and microenvironment during their sperm remain in the epididymis leads to infertility and all the effects were reversible on withdrawal of treatment.

INTRODUCTION: Various herbal plants have been used globally for safe and natural source of medicines including for inducing infertility. Many hormonal preparations are available for this purpose as contraceptives but have lots of side effects. So, cheap, safe, effective, easily available, low cost and easily administrable oral herbal contraceptives are mostly preferable. Many researchers globally are trying to develop male herbal contraceptive from natural sources [1, 2].

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Jussiaea repens L is such an herb which is widely distributed in different countries. In India, it is known by different names i.e., Keshardam (Bengali), Kessara (Hindi), Ising Kundo (Manipuri), Pani khutora (Assamese), Nir-charambu (Malayalam), Neerudantu (Kannada), Jadelo (Nepali), Neeti theegalu (Telugu) [3].

In our previous study we reported that oral administration of this plant extract in rats has no toxicity and affects the sperm quality, sperm morphology which was reflected through significant reduction in fertility rate. Our studies also showed that antifertile activity of this herb on male reproductive system is due to alterations of biochemical parameters in serum and testicular level and thus it may act as herbal male antifertility agent [4]. Recently we reported that the crude

aqueous extract of *Jussiaea repens* on male reproductive system of rat is mediated through alterations of histoarchitecture of testes and the reproductive hormone profile which directly or indirectly inhibit the steroidogenic and spermatogenic pathways and the effects were reversible after withdrawal of treatment [5]. The reversible action of this plant extract is highly encouraging for using the extract as contraceptive drug which will be a valuable outcome for herbal male contraceptive drug development.

The mammalian epididymis has attracted to the investigators because incoming spermatozoa from seminiferous tubule is immature and immature spermatozoa undergo changes in their morphology, biochemical constituents, process of sperm maturation and storage occur in this organ before ejaculation. Epididymis could be the extragonadal site which controls the fertilizing activity of spermatozoa in its microenvironment. So, Epididymis is considered as an excellent target for the development of male contraceptives [6, 7, 8]. Therefore, the present study was designed to evaluate the possible effects of crude aqueous extract of *Jussiaea repens* on the histoarchitecture of epididydimis and also to elicit the adroitness and mechanism of action of the extract on histoarchitecture of caudal epididymis in male rats.

MATERIALS AND METHODS:

Plant material:

The plant, *Jussiaea repens* L, was collected from wetlands of West Bengal, as reported earlier [9] 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.

FIG. 1: PHOTOGRAPH OF *JUSSIAEA REPENS* L (*LUDWIGIA ADSCENDENS* L)

Preparation of extract:

The plant extract (except root) was prepared following standard protocol [10]. 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 in the experiment.

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 [11] 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), all animals from control and treated groups, and after next 28 days (at 57 th. day) all the animals from recovery group were anaesthetized by diethyl ether. Cauda epididymis of each animal were dissected out, freed from adherent tissues, One cauda epididymis was stored at - 20°C for different biochemical assay and other was used for histological study.

Estimation of Cauda epididymal sialic acid:

Cauda epididymal tissue was homogenized in PBS buffer (50 mg /ml) (0.1M, PH 7.4). Homogenates were hydrolised with 0.1 N H_2SO_4 and centrifuged at 3000 rpm for 10 min. Then tissue Homogenates were incubated at 80°c for one hour, 0.5ml of the hydrolysate supernatant was taken in 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. After that, the 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 produced, disappeared after shaking. Then 3ml of thiobarbituric 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 the tubes, 4.5ml of cyclohexanone was added and shaked thoroughly for 15 seconds till the colour was taken up by the cyclohexanone supernatant. 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 [12]. The sialic acid content of the sample was determined from the standard curve of sialic acid and has been expressed in mg/g tissue.

Estimation of Cauda epididymal Glycogen:

In 1ml 5 % TCA, 50mg Cauda epididymal was homogenized and centrifuged at 3000 rpm for 15 min. In a centrifuge tube, 1ml supernatant and 5 ml 95 % ethanol was mixed and allowed in water bath at 37–40°C for 3 hours. After completion of precipitation, tubes were centrifuged at 3000 rpm for 15 min. The supernatant was gently decanted from packed glycogen and the tubes were allowed to drain in an inverted position for 10 minutes. The packed glycogen was dissolved by 2ml distilled water which was considered as sample and the blank was prepared by 2 ml water.

The standard was prepared by 2ml solution containing 0.1 mg glucose. 10 ml of anthrone reagent (0.05 % anthrone, 1 % thiourea and 72 % H_2S0_4v/v) were added into each tube with vigorous but consistent blowing and good mixing. All the tubes were kept in cold water, then immersed in boiling water bath for 15 minutes. The tubes were removed again to cold water bath and cooled to room temperature. The reading of unknown and standard were taken against blank at 620 nm in spectrophotometer [13]. The value of glycogen content was expressed in mg/g tissue.

Estimation of Cauda epididymal total lipid and phospholipid:

Cauda epididymal tissues were extracted according to the procedure of Folch *et al.* [14] 250 mg of the tissue was extracted with 6 ml of 3:1 (V/V) alcohol-ether mixture into test tubes 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 in another test tube. 6 ml of 3:1 alcohol- ether mixture was added to the residue and repeated twice in same procedure. The total volume of decanted filtrate was used for the estimation of total lipids, phospholipids (PL).

Estimation of total lipids:

Three test tubes were labelled as test, blank and standard. 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 a 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, with constant stirring add 5.0ml of water and stored in a brown bottle at room temperature] were added to all tubes and incubated at 37°c for 15 minutes. Lipids react with vanillin in the presence of sulphuric acid and phosphoric acid to form a pink colored complex. The optical densities were measured at 540 nm in a spectrophotometer [15]. Results were expressed as mg /g tissue.

Estimation of phospholipids:

0.5 ml tissue extract was mixed with 0.2 ml of ammonium ferrothiocyanate solution (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 of chloroform. The contents of the tubes were vortexed vigorously on cyclo-mixer for 15 seconds 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 using a blank [16]. Results were expressed as mg/g tissue.

Estimation of Cauda epididymal GSH (reduced glutathione) and MDA:

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

Estimation of reduced glutathione (GSH):

1.0 ml tissue supernatant was precipitated with 1.0 ml 10% TCA. The mixture was allowed to stand for 30 min. and centrifuged at 2500 rpm for 15 min. 0.5 ml supernatant was 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. of the 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 [17] and results were expressed in mg GSH /g tissue.

Estimation of malondialdehyde (MDA):

1.0 ml tissue supernatant was added to 2ml of TCA-TBA HCL (1:1:1) reagent (thiobarbituric 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 was read at 532nm against a blank in a spectrophotometer [18]. MDA was calculated using the standard MDA (1,1,3,3 tetrahydroxypropane) and expressed in mg/ g tissue.

Estimation of Cauda epididymal testosterone level:

About 100 mg of Cauda epididymal tissue was homogenized in 1 ml of PBS buffer (PH – 7.4). Homogenates were centrifuged at 10000 rpm for 10min.[19] and the resultant supernatant then kept in -20° C and were used immediately for determination of testosterone by ELISA Method. Assay procedures were followed as specified by manufacturers instruction. The ELISA (AcccuBind™ ELISA) kits were used from Monobind Inc. Lake forest, CA 92630, USA. Cauda epididymal testosterone content was calculated as ng/ml/mg of Cauda epididymis.

Cauda epididymal histology:

For the histological studies, cauda epididymis were removed and immediately fixed in Bouins solution for 18 hours. After Bouin's fixation picric acid is removed through three changes in 70% ethanol (one hour each), then the tissues were dehydrated using graded concentrations of ethanol (70 %, 95

%, 100 % alcohol twice after 1 hour each). They were cleared in xylene by transferring them into equal volumes of absolute alcohol and xylene (1:1 v/v) for twice after 1 hour each. The tissues were then filtered in molten paraffin wax in an oven maintained at 58°C. Three changes of molten paraffin wax at one-hour intervals, finally embedded and blocked using L shaped metal molder. Serial sections were cut from the mid portion of cauda epididymis using the rotary microtome at 5 µ thickness, mounted on slides and were stained using H-E method [5]. Each section, finally mounted in DPX with cover slip and examined under microscope.

Scanning electron microscopy study:

Cauda epididymal tissue were fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.4) for 4 h. After the samples were washed and dehydrated in ascending grades of ethyl alcohol (50%- 70%- 80%- 90%- 100%), tissues were then further dehydrated in amyl acetate for 30 minutes and dried at critical point using liquid carbon dioxide in Hitachi critical point dryer. Cauda epididymal tissue samples were mounted on an aluminium stub and then coated with gold. The prepared samples of cauda epididymis were examined under the scanning electron microscope and photographs were taken under S-530 Hitachi SEM [5, 10, 20].

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 significant decrease in cauda epididymal sialic acid, glycogen ($P < 0.01$) and phospholipid ($P < 0.05$) content and also in GSH ($P < 0.01$) activity but no change was observed in total lipid and MDA level when compared with control **(Table 1).** Results presented in table 2, showed a significant reduction in cauda epididymal testosterone ($P<0.05$) level of *J. repens* treated animals than control which was restored towards normal in recovery group.

TABLE 1: CAUDA EPIDIDYMAL BIOCHEMICAL PARAMETERS IN DIFFERENT GROUPS.

	Cauda epididymal Sialic acid (mg/ gm tissue)	Cauda epididymal glycogen (mg/ gm tissue)	Cauda epididymal total lipid (mg/dl)	Cauda epididymal phospho Lipid (mg/gm tissue)	Cauda epididymal GSH (µmol/ gm tissue)	Cauda epididymal MDA (nmol/ gm tissue)
Group I: Control group	4.1238 ± 0.3016	2.2838 ±0.1203	14.2665 ±1.0778	55.2921 ±5.3314	1.9797 ±0.1277	16.3588 ±1.4794
Group II: treated with aqueous extract (200 mg/kg body weight)	2.8050 ** ±0.3341	1.6638** ±0.1328	11.2016 ±1.1660	40.0201 * ±3.1831	1.4649 ** ±0.1009	17.7600 ±1.3310
Group III: Recovery group	4.2350 ±0.2778	2.1300 ±0.1495	13.5929 ±1.8422	52.1909 ±3.8567	1.7450 ±0.1222	17.3875 ±1.3678

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 2: HORMONE PROFILE IN TREATED GROUPS

	Cauda epididymal Testosterone (ng/100 mg tissue)
Group I: Control group	0.575 ±0.044
Group II: treated with aqueous extract (200 mg/kg body weight)	0.400* ±0.036
Group III: Recovery group	0.547 ±0.055

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

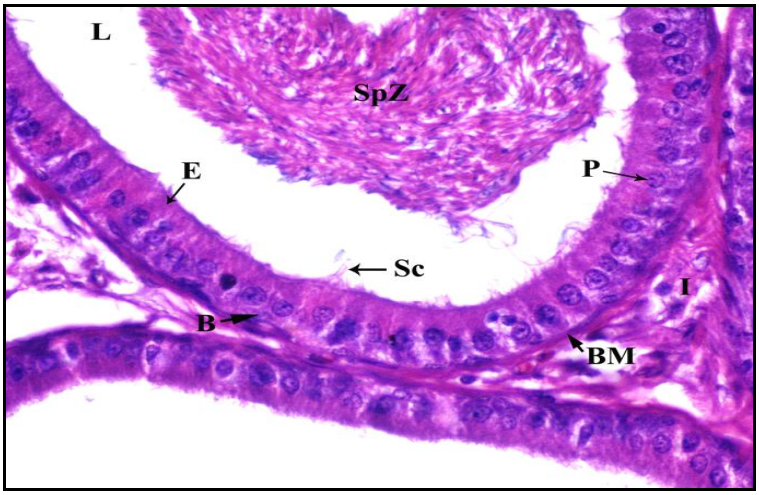

FIG. 2: PHOTOMICROGRAPH OF CAUDA EPIDIDYMIS OF CONTROL RAT SHOWING NORMAL ARCHITECTURE OF TUBULES AND INTERTUBULAR ELEMENTS, LUMEN CONTAINED DENSELY PACKED SPERMATOZOA, EPITHELIUM IS PSEUDOSTRATIFIED COLUMNAR WITH STEREOCILIA. THE INTERSTITIUM CONTAINED NUMEROUS INTERSTITIAL CELLS WITH ROUND NUCLEI AND CONNECTIVE TISSUE. L- LUMEN, E- EPITHELIUM, SC- STEREOCILIA, P- PRINCIPAL CELLS WITH ROUNDED NUCLEI,B- BASAL CELL, BM- BASAL MEMBRANE,I- INTERSTITIUM WITH CONNECTIVE TISSUE. (H&E STAINING, X400).

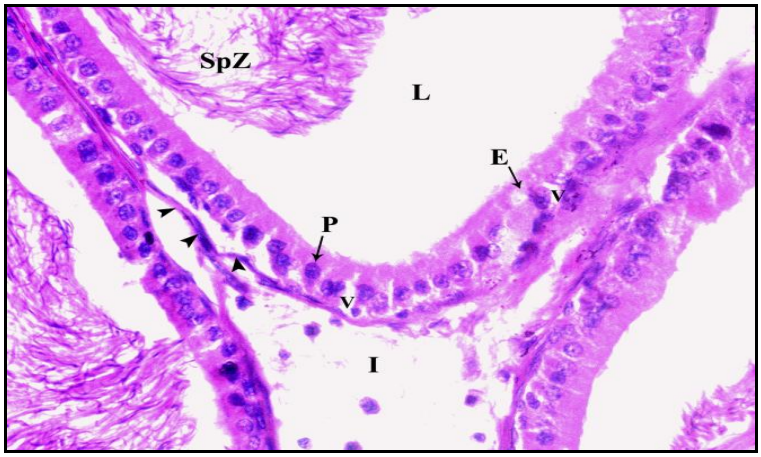

FIG. 3: PHOTOMICROGRAPH OF CAUDA EPIDIDYMIS OF JR EXTRACT TREATED RAT SHOWING DEGENERATION INTHE BASAL MEMBRAN (ARROW HEAD) WITH EPITHELIUM AND ALMOST ABSENCE OF STEREOCILIA.THE PRINCIPAL CELLS LOOSELY ARRANGED AND CYTOPLASMIC VACUOLATION (V) SEEN IN THE LATERAL SITE AND ALSO THE INTERSTITIUM CELL MASS REDUCED AND WIDE SPACES. (H&E STAINING, X 400).

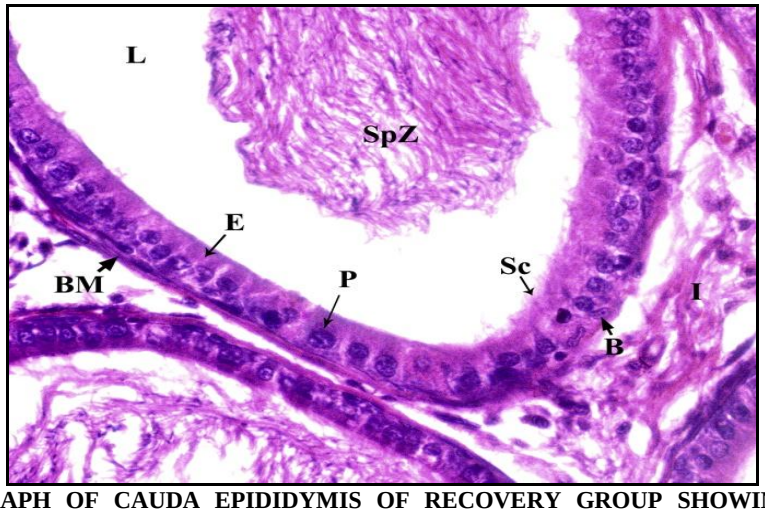

FIG. 4: PHOTOMICROGRAPH OF CAUDA EPIDIDYMIS OF RECOVERY GROUP SHOWING NEAR THE NORMAL ARCHITECTURE OF TUBULES AND INTERTUBULAR ELEMENTS, LUMEN CONTAINED SPERMATOZOA, EPITHELIUM IS PSEUDOSTRATIFIED COLUMNAR WITH STEREOCILIA. THE INTERSTITIUM CONTAINED NUMEROUS INTERSTITIAL CELLS WITH CONNECTIVE TISSUE. (H&E STAINING, X400).

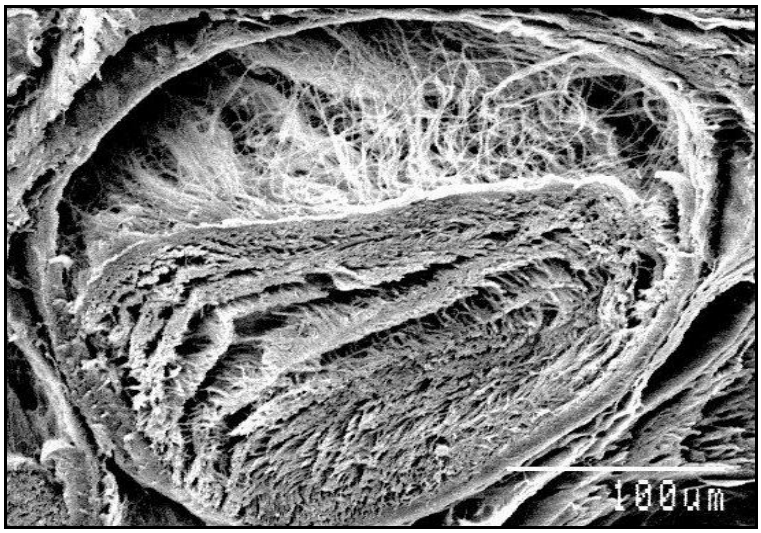

FIG. 5: SCANNING ELECTRON MICROGRAPH OF CAUDA EPIDIDYMIS SECTION OF CONTROL RAT SHOWING NORMAL ARCHITECTURE OF TUBULES WITH EPITHELIUM AND BASAL MEMBRANE ALSO LUMEN CONTAINED DENSELY PACKED SPERMATOZOA (X350 MAGNIFICATION)

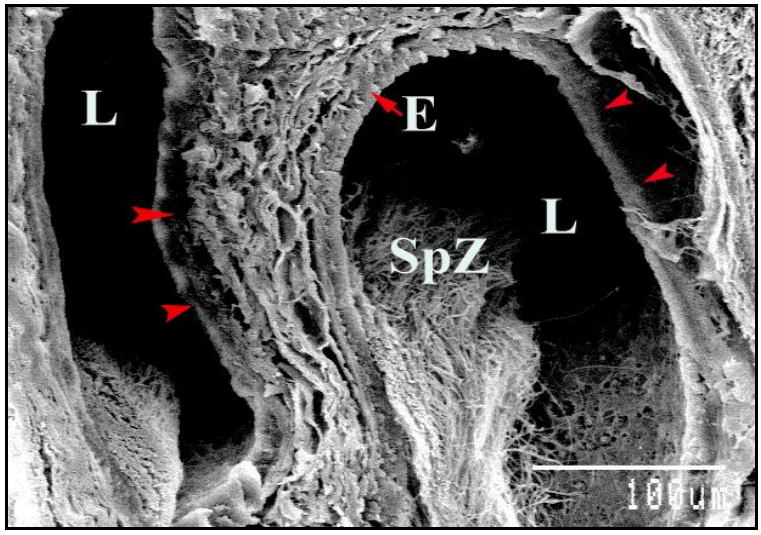

FIG. 6: SCANNING ELECTRON MICROGRAPH OF CAUDA EPIDIDYMIS SECTION OF JR EXTRACT TREATED RAT SHOWING DAMAGED EPITHELIUM AND BASAL MEMBRANE (ARROW HEAD) ALSO LUMEN CONTAINED LESS SPERMATOZOA (X350 MAGNIFICATION)

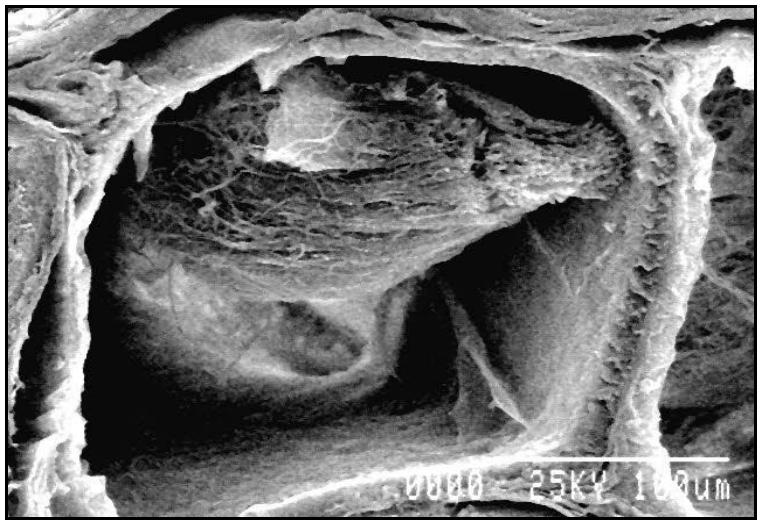

FIG. 7: SCANNING ELECTRON MICROGRAPH OF CAUDA EPIDIDYMIS SECTION OF RECOVERY GROUP SHOWING NORMAL ARCHITECTURE OF TUBULES WITH PLENTY OF SPERMATOZOA (X 350 MAGNIFICATION).

DISCUSSION: The epididymis is an important component of the male reproductive tract that is highly androgen**.** Dependent [21, 22] and plays a vital role in the male fertility. The present study is directed towards understanding the histological alteration of epididymis affects the post testicular sperm maturation in epididymis.

The epididymis provides a suitable environment for morphological and biochemical changes in spermatozoa [23]. It performs both secretory and absorptive functions [24]. The epididymal biochemistry of the present study of treated rats showed reduction of sialic acid, glycogen, phospholipid, and GSH level but no change was observed in total lipid and MDA level. The sialic acid is a normal constituent of glycoprotein, secreted by the epididymal epithelium which is an important constituent of cell membrane and basement membrane. Inside the epididymal lumen, sialic acid participates in maintaining osmotic balance in the cauda region and may facilitate sperm transport by lubricating action [25, 26, 27]. The synthesis of sialic acid in the epididymis is under androgenic control [28, 29].

The fluctuation in the level of sialic acid in reproductive tissues indicates the altered levels of testosterone or FSH and LH, needed for the functioning of gonads. A significant decrease in the sialic acid concentration in our present study, which may be due to the antispermatogenic activity of JR extract. Again, reduced glycogen level may be due to interference in energy metabolic pathway. Since glycogen is an energy source for general metabolism and constant supply of energy substrate which is essential for proper functioning of epididymis [30].

The epididymis is an androgen targeted tissue and its metabolism and epithelial secretory functions depend on the presence of androgens [22]. In the present study the cauda epididymal testosterone level was decreased which further indicated the alteration of epithelial secretory functions and leads to decline the biochemical components which are directly or indirectly inhibited the fertilization process [4]. Again Glutathione is a natural reservoir of reducing power, which can be quickly used by the cells as a defense against oxidative stress. Decrease of glutathione level in treated group may affect oxido-reductase metabolism or reduced GSH, which is utilized to minimize oxidative stress [31].

The histoarchitecture of cauda epididymis of control rat showed that the tubules were normal in size and lined with pseudostratified columnar epithelial cells with prominent stereocilia. Inter tubular stroma contains connective tissues and blood vessels. The lumen is filled with mature spermatozoa. Whereas the histoarchitecture of cauda epididymal epithelium of JR treated rats was moderately change possibly due to deficiency of androgen which was reported in our previous study [5].Reduction in the cauda epididymal tubular diameter, reduced number of spermatozoa within the cauda epididymal lumen exhibit the androgen deficiency [32]. The disorganized epithelium of cauda epididymis and cells showed vacuolization with signs of degeneration due to the treatment of aqueous extract of *J.repens*, which supports the indirect evidence for the antiandrogenic action of the plant extract.

Male fertility depends also on sperm kinetics i.e, count, motility, morphology and testicular spermatogenesis and also the epididymal sperm maturation. In our previous study we reported that JR treatment altered all of these parameters which indicate the disturbances in spermatogenesis and sperm maturation by this plant extract, which was further recovered after withdrawal of treatment [4, 5]. Again, in JR treated rats, reductions in total proteins [4], sialic acid and androgen hormone in the epididymis could be responsible for morphological abnormalities observed in cauda epididymal spermatozoa [5]. It is probably due to inhibition of spermatogenesis in the testis, as a result, scanty of spermatozoa in epididymal lumen and degeneration of epithelium with basement membrane and also reduced stereocilia were observed.

Besides this, reduction in epididymal total lipid and phospholipid of JR treated rats supported the immaturation of spermatozoa inside the epididymis. As, Brooks et al. 1974 [33] reported the energy requirement in sperm maturation and storage activity, which is also related with oxidation of lipid. Lipids also maintain the

structural and functional integrity of the epididymis [34, 35]. Phospholipids are principal constituent of plasma membrane and control the movement of molecules that are crucial for events taking place in the epididymis [36]. Anoptimal level of phospholipid in plasma membrane is essential for the optimal secretory activity of epithelial cells [37]. Thus, maturity of spermatozoa and successful fertilization depends on both the integrity of the spermatozoal membrane and its adequate phospholipid composition in caudal region [38].

In the present study, the histoarchetecture of epididymis is critically correlated with the biochemical alterations of its microenvironment, indicated that JR has inhibitory role in epididymal functions in rats leading to male infertility [4].

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 epididymal epithelium which is affects the normal resorptive activities of the cauda epithelium and altered the biochemical microenvironment which influence the disturb sperm maturation and fertility. These effects are reversible on withdrawal of treatment. Therefore *Jussiaea repens* (except root), at a dose of 200mg/kg b.wt/day for 28 days consecutively use for the development of herbal male contraceptive in future.

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