

Research Article



***Jussiaea repens* (L) Acts as an Antifertility Agent – A Search for Herbal Male Contraceptive**

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ABSTRACT

Overpopulation is a burning problem for developing countries like India. Several contraceptive measures have been adopted to control fertility but nowadays scientists are looking for herbal contraceptives which are cheaper and less or nontoxic. *Jussiaea repens* (L), a well known Indian medicinal plant, reported as nontoxic anti gonadal herb on male rats when administered orally. The present study was undertaken to investigate the probable mode of action of this plant (except root) as anti fertile agent and to study the reversible effect in rats, so that it can be used as alternative safe male herbal contraceptive in future. The aqueous extract at a dose of 200 mg/kg b.wt./day orally for 28 days has potent anti fertility activity that may be due to decreased testicular ascorbic acid, glycogen, $\Delta 5-3\beta$ and 17β HSD, G-6PDH, ATPase, LDH activities and increased level of cholesterol, which are directly related to testicular functions. The testicular protein content was unaltered but Zn content was reduced insignificantly than control. Serum triglycerides, VLDL, LDH and G-6PDH were significantly reduced in treated group keeping HDL unaltered but Zn content was reduced insignificantly where the significant rise of cholesterol and LDL level were noticed. A significant decrease of protein content, Zn, ATPase and LDH activities in epididymis were also observed after treatment, which supported the anti gonadal activity. Altered biochemical parameters may affect the maturation process of spermatogenesis which was reflected through increased percentage of denatured chromatin in spermatozoa. Restoration of all the biochemical parameters in the recovery group also supported the reversal effect of the extract after withdrawal of treatment. So, from the above studies it can be assumed that crude aqueous extract of *J. repens* at a regulated dose and duration, may be used as male herbal contraceptive without causing any permanent damage of the reproductive system or side effects in general.

Keywords: Herbal male contraceptive, *J.repens*, Reversibility, Spermatogenesis, Steroidogenesis.

INTRODUCTION

Fertility control is an issue of global and national public health concern as overpopulation continues to be a significant contributor to environmental degradation and human suffering worldwide. For this purpose several contraceptive methods have been adopted but are mostly synthetic which are costly and have lots of side effects. So, the World Health Organization has suggested the use of locally available plants, instead of synthetic drugs, as cost effective management for birth control.¹ Moreover, it has been observed that about 90% of the World's contraceptive users are women.² This gender-based usage is due to lack of effective male contraceptive in pill form. *Jussiaea repens* L, belonging to onagraceae family,³ is a well known medicinal plant, found in wetlands of different parts of India as well as in China, Malaysia, New Guinea and other countries,^{4,5} reported to have anti-diabetic, anti-inflammatory, hepatoprotective, antibacterial and other such remedial activities.⁶⁻⁹ Besides this, the plant is also widely used as vegetable and animal forage without knowing the presence of some anti fertile agents like rutin, quercetin, triterpenes, kaempferol etc. as it's active compounds.¹⁰⁻¹⁶ In our preliminary studies we have already reported that the crude aqueous extract of the plant (except root) has anti gonadal activity in a dose and duration depended manner¹⁷ on male reproductive system in rats when administered orally and it has no side effect on the vital organs like heart, kidney, liver etc. as a

whole.¹⁸ The main objectives of the present studies are to investigate the mode of action of *J. repens* as anti fertility agent and also to evaluate the reversibility of its action after withdrawal of treatment, so that this plant extract can be introduced as a non-hormonal, eco - friendly male contraceptive in future.

MATERIALS AND METHODS

Plant Material

The plant, *Jussiaea repens* L, was collected from wetlands of West Bengal, as reported earlier¹⁸ 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.¹⁸ 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 \pm 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 \pm 2°C and relative humidity 40-60%) with free access to standard diet¹⁹ 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.

On the 29th 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 57th day) all the animals from recovery group were anaesthetized by diethyl ether. Blood samples were collected for Serum preparation by centrifugation and reproductive organs i.e. testis and epididymis of each animal were dissected out, freed from adherent tissues, which was stored at - 20°C for different biochemical assay.

Methods of biochemical estimations

Estimation of serum biochemical parameters

Serum lipid profile including total cholesterol, triglycerides, LDL, VLDL, HDL cholesterol, Zn and LDH were measured by colorimetric methods using commercial kits (Span Diagnostics Ltd. and Crest Biosystems, India).²⁰⁻²³

Estimation of testicular and serum glucose-6-phosphate dehydrogenase (G - 6 -PDH) activity²⁴

Testicular tissue (50mg/ml) was homogenized in Tris – HCl buffer solution (0.1 M w/v, P^H 8.2), centrifuged at 10,000 \times g for 15 min at 4°C and supernatant was separated. For enzyme assay, Tris - HCl, NADP(0.2 mM

w/v) and MgCl₂ (0.1 M w/v) each of 0.1ml, 0.5 ml water and 0.1 ml supernatant were taken in cuvette. 0.1 ml glucose-6-phosphate (6mM w/v) was added as substrate and the change in optical density was measured at 340 nm against reagent blank. The enzyme activity was expressed as units / mg protein. In case of serum G-6-PDH activity estimation, 0.1 ml of serum was taken instead of supernatant.

Estimation of testicular cholesterol²⁵

Testicular tissue (50mg/ml) was homogenized in ether-alcohol mixture (1:3), centrifuged at 3000 rpm for 10 min, 0.1 ml supernatant was mixed with 4.9 ml ferric chloride solution (50 mg FeCl₃ 6H₂O, dissolved in 100 ml Acetic acid) in a screw capped centrifuge tube and allowed to stand for 15 min. The mixer was centrifuged at 3000 rpm for 10 min, 1.5ml of conc. H₂SO₄ was added to 2.5ml of the clear supernatant and incubated for about 30 min at room temperature for colour development. The colour intensity of unknown and standard were read against blank at 560 nm in a spectrophotometer. The result was expressed in mg/gm tissue.

Estimation of testicular Ascorbic acid²⁶

The testicular tissue (50mg/ml) was homogenized in 6% TCA, centrifuged at 3500g for 20 min, supernatant was mixed with a drop of bromine solution and kept for 10 min for complete oxidation of ascorbic acid into dehydroascorbic acid. In other tube, 2ml oxidized solution and 2ml thiourea reagent (2g of 2, 4-dinitrophenyl hydrazine dissolved in 100ml of 10N sulphuric acid, then 4g of thiourea was dissolved in this solution and mixed well by shaking) was mixed and incubated for 4 hours at 37°C. The tubes were kept in ice bath, 2.5ml 85% H₂SO₄ was added drop wise and mixed thoroughly. The solution was kept at room temperature for about 80 minutes for colour development. Ascorbic acid standard (10 μ g/ml) and the blank (6%TCA) were prepared parallel. The reading was taken at 540 nm in spectrophotometer and the value was expressed in mg/gm of tissue.

Estimation of testicular Glycogen²⁷

In 1ml 5 % TCA, 50mg testicular tissue 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 % H₂SO₄ v/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. The value of glycogen content was expressed in mg/gm of tissue.

Estimation of testicular Δ^5 , 3β -hydroxysteroid dehydrogenase (HSD) activity²⁸

Testicular tissue (50mg/ml) was homogenized in 20% spectroscopic grade glycerol containing 5mM potassium phosphate and 1mM EDTA at 4°C, centrifuged at 10,000×g for 30 min and 1ml of supernatant was mixed with 1 ml of 100 μ M of sodium pyrophosphate buffer (p^H 8.9), 40 μ l of ethanol containing 30 μ g of DHEA and 960 μ l of 25 mg % BSA. Enzyme activity was measured after addition of 100 μ l of 0.5 μ M (NAD) into the tissue supernatant mixture at 340 nm against blank (without NAD) was recorded at 30 sec interval for 3 min. One unit of enzyme activity was considered causing a change in absorbance of 0.001/min.

Estimation of testicular 17β -hydroxysteroid dehydrogenase (HSD) activity²⁹

For 17β -HSD activity measurement, 1ml of the same supernatant for 3β HSD was added with 1ml of 440 μ M sodium pyrophosphate buffer (p^H 10.2), 40 μ l ethanol containing 0.3 μ M testosterone and 960 μ l of 25 mg% BSA. Enzyme activity was assessed after addition of 100 μ l of 0.5 μ M NAD in the mixture as above.

Total tissue (testis and epididymis) protein estimation³⁰

The tissues (50mg/ml) of testis and epididymis were homogenized in phosphate buffer (P^H 7.4) and centrifuged at 2000 rpm for 15 min. Total protein was estimated against BSA (1 mg/ml) as standard. The intensity of colour development was read at 720 nm. The result was expressed as mg / gm of tissue.

Estimation of testicular and epididymal total ATPase activity^{31, 32}

50 mg tissue from testis and epididymis were homogenized in 1ml Tris – HCl buffer (0.1 M w/v, P^H 8.2), centrifuged at 10,000×g for 15 min at 4°C. For assay, 0.3 ml substrate buffer (3 mM ATP disodium salt in Tris – HCl buffer), 0.1 ml $MgCl_2$ (3 mM), 0.1 ml NaCl (150 mM) and 0.1 ml KCl (30 mM) was mixed with 0.2 ml supernatant and 0.2 ml Tris - Sucrose buffer. The blank was prepared by 0.3 ml of distilled water. Tubes were incubated at 37°C for 30 min. For termination of reaction, 0.5 ml 10% TCA was added and kept for precipitation at 4°C for 15 min. The solution then centrifuged at 3000 rpm for 15 min. The supernatant was used for determination of inorganic phosphate at 660 nm. The enzyme activity was expressed as μ mol of inorganic phosphate released/mg protein/30 min.

Estimation of testicular and epididymal lactate dehydrogenase (LDH) activity²³

Testicular and epididymal tissues were homogenized in phosphate buffer solution (0.1 M w/v, P^H -7.4) at a ratio of

1gm tissue: 10ml buffer and centrifuged at 10,000×g for 15 min at 4°C. The supernatant was taken for estimation of testicular LDH using standard Spectrophotometric kit²³ at 340 nm. The result was expressed as U / gm of tissue.

Estimation of testicular and epididymal zinc content³³

Testicular(0.5g/5ml) and epididymal (0.2g/5ml) tissues were digested with concentrated nitric acid at 100 °C for 2 hrs in boiling water bath and after 24 hrs, the clear digest was diluted to 10 ml with deionized water. The zinc level was estimated by atomic absorption (Model no. A Analyst-200, PerkinElmer Make) and was expressed in μ g/gm of wet tissue.

Evaluation of Sperm chromatin quality³⁴

A drop of cauda epididymal suspension was transferred to a glass slide and thin smear was prepared. The smear was air dried and fixed in methanol/acetic acid (3:1) for 2 hrs at 4 °C and stained with acridine orange (AO) solution (0.19% AO in phosphate citrate buffer, p^H 4.0) for 5 min. Slides were washed by distilled water for 5 min and air dried again. The stained smear was then observed under fluorescence microscope at 400X magnification and 100 cells were counted for each slide. Red to yellow stained heads of spermatozoa cells indicated on a denatured chromatin was considered abnormal while the green one on an intact chromatin was considered as normal spermatozoa. The denature chromatin % was calculated by abnormal spermatozoa in red fluorescence / total spermatozoa (normal and abnormal) in red and green fluorescence.

Statistical analysis of data

All the recorded values were expressed in mean \pm 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

Analysis of biochemical parameters from serum samples of different groups showed that lipid levels i.e. TG ($P < 0.05$) and VLDL ($P < 0.01$) were reduced significantly where the cholesterol and LDL were significantly ($P < 0.01$) increased but the HDL level showed no change in extract treated group when compared to control (Table 1, Figure 1A). The serum LDH and G6PDH activities were also decreased ($P < 0.01$) in treated group (Table 2, Figure 1B, 1C) but the Zn content of serum was decreased non significantly (Table 2, Figure 1B). Results from the testicular biochemical parameters in treated group showed the significant ($P < 0.01$) decrease in ascorbic acid, glycogen content (Table 3, Figure 1D), Δ^5 and 17β HSD, G-6PDH (Table 4, Figure 1E), ATPase ($P < 0.01$) & LDH ($P < 0.05$) (Table 5, Figure 1F) activities. But the significant ($P < 0.01$) increase of testicular cholesterol level (Table 3, Figure 1D) and non significant change of protein and Zn content (Table 5, Figure 1F) were observed in extract treated group when compared to untreated control. In

epididymis of extract treated animals, significant decrease ($P < 0.01$) in protein content, ATPase & LDH activities and Zn content were found (Table 5, Figure 1F). The observation of denatured chromatin % by Acridine Orange staining of spermatozoa in extract induced rats also showed significant ($P < 0.01$) increase (Table 6, Figure

2A, B, C). In the recovery group, all these parameters returned almost to control value.

In all the result tables below (table 1-6), values are expressed as mean \pm 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 1: Serum lipid profile of different groups

| | Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | VLDL (mg/dl) | LDL (mg/dl) |
|---|-------------------------|-------------------------|----------------------|-------------------------|-------------------------|
| Group I: Control group | 152.3025 \pm 3.6037 | 118.6000 \pm 4.0401 | 33.3763 \pm 1.3310 | 23.7175 \pm 0.8081 | 95.2088 \pm 3.6729 |
| Group II: Treated with aqueous extract (200 mg/kg body wt) | 210.0000** \pm 9.2582 | 102.0700 * \pm 4.0108 | 34.7237 \pm 1.6717 | 20.4100 ** \pm 0.8018 | 154.8662** \pm 9.1049 |
| Group III: Recovery group | 160.0000 \pm 7.5593 | 116.7725 \pm 4.4644 | 33.0650 \pm 1.1731 | 22.7700 \pm 0.7291 | 104.2225 \pm 6.4036 |

Table 2: Serum zinc and enzyme activities

| | Serum LDH (U/dl) | Serum G-PDH (U/mg protein) | Serum Zinc (μ g/dl) |
|---|-------------------------|----------------------------|--------------------------|
| Group I: Control group | 190.3525 \pm 4.7312 | 0.371 \pm 0.045 | 154.676 \pm 9.882 |
| Group II: Treated with aqueous extract (200 mg/kg body wt/day) | 94.1763 ** \pm 4.7296 | 0.142 ** \pm 0.018 | 131.236 \pm 12.308 |
| Group III: Recovery group | 188.3525 \pm 5.0237 | 0.322 \pm 0.039 | 149.988 \pm 9.198 |

Table 3: Testicular biochemical parameters in different groups

| | Testicular Cholesterol (mg/gm tissue) | Testicular Ascorbic acid (mg/gm tissue) | Testicular Glycogen (mg/gm tissue) |
|---|---------------------------------------|---|------------------------------------|
| Group I: Control group | 10.5362 \pm 0.1729 | 0.8775 \pm 0.0846 | 2.5938 \pm 0.2125 |
| Group II: Treated with aqueous extract (200 mg/kg body wt/day) | 13.5038 ** \pm 0.8798 | 0.5238 ** \pm 0.0108 | 1.6462 ** \pm 0.1557 |
| Group III: Recovery group | 10.8875 \pm 0.2480 | 0.8225 \pm 0.0823 | 2.4925 \pm 0.1959 |

Table 4: Testicular enzymes activities in different groups

| | Testicular Δ 5-3 β HSD activity (U/mg protein) | Testicular 17 β -HSD activity (U/mg protein) | Testicular G-6PDH (U/mg protein) |
|---|---|--|----------------------------------|
| Group I: Control group | 11.7461 \pm 0.8405 | 13.2663 \pm 0.5885 | 10.437 \pm 0.571 |
| Group II: Treated with aqueous extract (200 mg/kg body wt/day) | 7.6238 ** \pm 0.6819 | 7.9275** \pm 0.8267 | 7.362 ** \pm 0.556 |
| Group III: Recovery group | 10.9637 \pm 0.6205 | 12.4350 \pm 0.6915 | 9.487 \pm 0.494 |

Table 5: Comparative studies of testicular and epididymal biochemical parameters

| | Protein (mg/gm tissue) | | ATPase (μ mole Pi/mg protein /30 min.) | | LDH (U/gm tissue) | | Zinc (μ g /gm tissue) | |
|---|------------------------|----------------------|---|---------------------|--------------------|---------------------|----------------------------|----------------------|
| | Testicular | Epididymal | Testicular | Epididymal | Testicular | Epididymal | Testicular | Epididymal |
| Group I: Control group | 59.955 \pm 1.944 | 83.960 \pm 5.020 | 2.568 \pm 0.148 | 2.648 \pm 0.032 | 53.442 \pm 1.664 | 8.395 \pm 0.592 | 44.125 \pm 6.523 | 56.522 \pm 1.545 |
| Group II: Treated with aqueous extract (200 mg/kg body wt) | 62.720 \pm 2.895 | 54.897** \pm 3.126 | 1.787** \pm 0.165 | 1.985** \pm 0.206 | 42.86* \pm 4.297 | 4.233** \pm 0.407 | 38.250 \pm 4.308 | 47.462** \pm 1.674 |
| Group III: Recovery group | 61.905 \pm 1.831 | 73.412 \pm 4.413 | 2.325 \pm 0.132 | 2.490 \pm 0.036 | 49.840 \pm 0.633 | 7.216 \pm 0.630 | 41.250 \pm 6.405 | 53.816 \pm 2.134 |

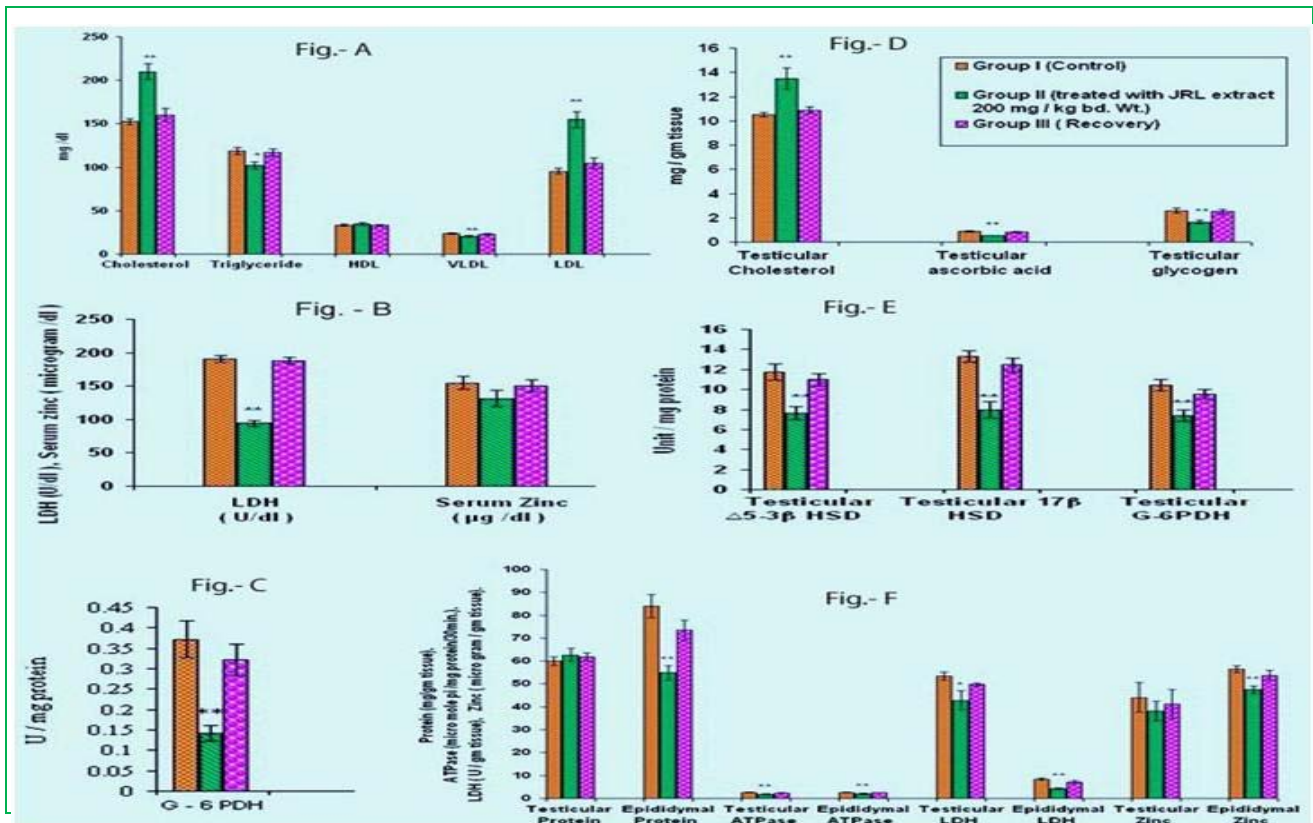


Figure 1: (A) Serum lipid profile, (B) Serum LDH activity and concentration of Zinc, (C) Serum G-6PDH activity, (D) Concentration of testicular cholesterol, ascorbic acid and glycogen, (E) Testicular Δ5-3β HSD, 17 β HSD and G-6PDH activity, (F) Testicular and epididymal Protein, ATPase, LDH activity and Zinc content - 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).

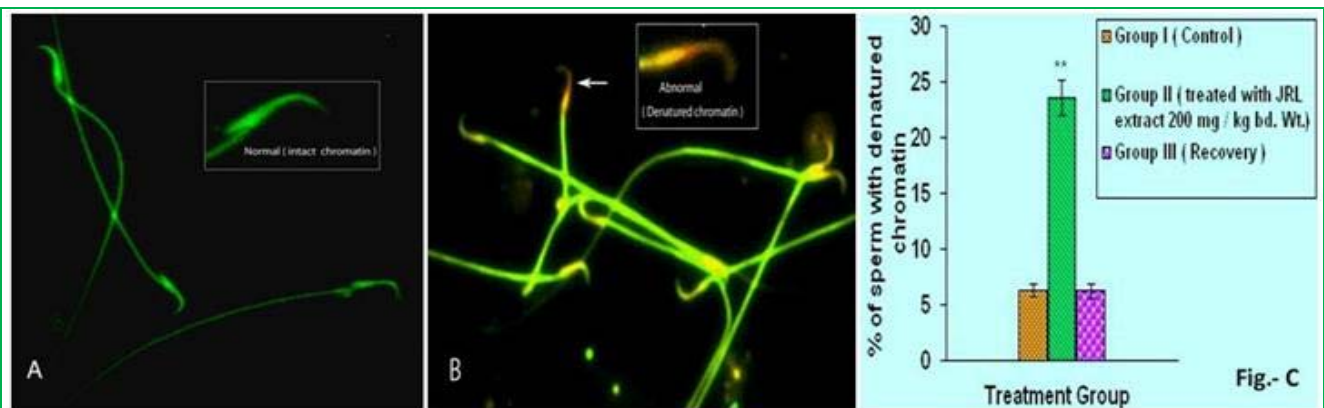


Figure 2: Effect of *J. repens* (*L*) extract in chromatin integrity of epididymal spermatozoa of rat stained with acridine orange (AO). A = sperm with intact chromatin (green) or normal. B = sperm with abnormal chromatin or denatured chromatin (arrow) (400 X magnification). C = graph representing percentage of denatured chromatin in epididymal spermatozoa of Control, treated and recovery groups. Values were expressed as mean ± S.E.M.; N=8. **Significant (P < 0.01), Group II was compared with Group I (Control) and Group III (Recovery).

Table 6: Effect of *J repens L* extract in rat epididymal sperm chromatin denaturation.

| Group | % of sperm with abnormal chromatin integrity |
|--|--|
| Group I: Control group | 6.2675 ± 0.5893 |
| Group II: treated with aqueous extract (200 mg/kg body weight) | 23.5925** ± 1.5536 |
| Group III: Recovery group | 6.2338 ± 0.6933 |

DISCUSSION

For a developing country, the lack of basic education and access to health care services, limits male involvement in family planning. Scientists are trying to develop male contraceptives from plants which will be more acceptable for economic and safety reasons. Our previous studies showed that *J. repens* has anti fertility effect on male reproductive system of rat¹⁸ and caused morphological alterations of spermatozoa which was reflected through

significant reduction in fertility rate.¹⁷ Similar studies of medicinal plants with detrimental effects on male fertility also has been reported by different authors.³⁵⁻³⁸

The present study was designed to explain the probable mode of action of crude aqueous extract (except root) from *Jussiaea repens* L (JR) as anti gonadal factor. Results of biochemical parameters from serum and gonadal tissues both show that extract treatment caused alterations in most of the essential components which are directly or indirectly related with steroidogenesis or maturation of spermatozoa. It is well known that cholesterol is an essential precursor for the production of testosterone³⁹ which is actually responsible for maintaining the normal activities of male reproductive system.⁴⁰ In our present study, JR extract treatment shows alterations of lipid profile in male rats. The enhanced level of serum and testicular cholesterol (Table 1, 3, Figure 1A, D) indicates that cholesterol was not utilized for testosterone production^{41,42} which can be supported by the reduced activities of two testicular steroidogenic key enzymes $\Delta 5-3\beta$ and 17β HSD in the extract treated group (Table 4, Figure 1E), supports our previous studies leading to male infertility.^{43,17} So, the decreased activity of these two enzymes caused decreased serum testosterone level (data unpublished) and hamper the normal testicular and epididymal functions.^{38,44} On the other hand, ascorbic acid, a cofactor of steroidogenic enzymes, plays key role in the synthesis of testosterone,^{45,46} which in turn is essential for the maintenance of the structural and functional integrity of androgen targeted reproductive organs.⁴⁷ Few early studies demonstrate that the deficiency of ascorbic acid cause degeneration of the testicular germinal epithelium,⁴⁸ reduced sperm concentration and motility⁴⁹ which are associated with poor breeding performance.⁵⁰ The decline in testicular ascorbic acid concentration in the JR treated group of our study (Table 3, Figure 1D), can be correlated with a decrease in steroidogenesis⁵¹ as HSD enzymes are NADPH dependent and NADPH production depends also on DNPH oxidation by ascorbic acid.⁵² NADPH is also required for the conversion of cholesterol to testosterone.⁵² But due to the decreased activity of glucose 6-phosphate dehydrogenase in serum and testis (Table 2,4, Figure 1C, E) after extract treatment, the supply of this coenzyme (NADPH) may be reduced which may be the another cause of reduced production of androgen⁵³ as well as hamper the spermatogenesis process (data unpublished). Again, glycogen is the main source of energy of sperm cells⁵⁴ which is directly proportional to the steroid hormone level.⁵⁵ Here, decreased level of testicular glycogen (Table 3, Figure 1D) in extract treated group, indicates the deficiency of nutrients for the development of sperms. Reduced glycogen and fructose content in testis and seminal vesicle¹⁸ cause the decreased number of matured and motile sperms as reported by others.⁵⁶ In sperm, the ATP required for motility due to flagellar beat⁵⁷ and is hydrolyzed by ATPase, an important testicular metabolic marker enzyme.⁵⁸ So, in this study, inhibition of ATPase

activity in testis and epididymis after extract treatment (Table 5, Figure 1F), indicates the suppression of energy metabolism and inhibition of male gonadal functions.^{59,60} The inhibition of ATPase activity in this study also supports the alteration in acrosomal membrane⁶¹ in JR treated rats, as we reported earlier¹⁷ leading to infertility. Lactate dehydrogenase (LDH) is a marker of carbohydrate metabolism, which is required for germ-cell production and differentiation.⁶² The decreased activity of LDH in serum, testis and epididymis in treated group (Table 2, 5, Figure 1B, F) suggests a shift in tissue respiration from anaerobic to aerobic type which is detrimental for the metabolism of spermatozoa.⁶³ Similar finding was also reported by El-Kashoury et.al., where in-vivo reduction of LDH activity indicates the inhibition of androgen.⁶⁴ The decreased level of protein content in testis and epididymis in JR treated rats (Table 5, Figure 1F) revealing androgen depreivation leading to defect in sperm maturation.^{65,66} In the present study (Table 2, 5; Figure 1B, F), decreased zinc level in serum, testis and epididymis by JR treatment may have the negative effect on serum testosterone concentration, as Zn controls the testosterone metabolism and semen volume,⁶⁷ sperm maturation and maintenance of germinal epithelium,⁶⁸ testicular regression, low sperm count,⁶⁹ atrophy of the seminiferous tubules, failure of spermatogenesis⁷⁰ and abnormal sperm production.⁷¹ This reduction of Zn content in our study also supports the denaturation of sperm chromatin integrity (Table 6, Figure 2A, B, C) causing male infertility.⁷² Zn may play a role in the formation of S-Zn-S bridges, which additionally stabilize the chromatin structure.^{73,74}

Results of the above studies show that the anti gonadal activity of JR crude aqueous extract on male reproductive system of rat is mediated through alterations of several biochemical parameters at serum and testicular level, which directly or indirectly inhibit the steroidogenic and spermatogenic pathways and thereby reduce the fertility rate and the effects were reversible after withdrawal of treatment.¹⁷

The reversible action of this plant extract is highly encouraging for using this extract as contraceptive drug. Our present study shows that after four spermatogenic cycles, mostly, all extract induced altered biochemical parameters affected the normal fertilization processes, returned back towards normal control level. Our fertility and mating experiments with recovery group also supported the reversible action of this extract.¹⁷

CONCLUSION

From the above studies it is concluded that crude aqueous extract of *Jussiaea repens* herb (except root) in a regulated dose and duration manner can be introduced as a safe herbal contraceptive for male in a pill form in future.

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