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EFFECTS OF *JUSSIAEA REPENS* L ON SPERM DNA INTEGRITY IN MALE ALBINO RATS

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ABSTRACT: Fertility control is an issue of global challenge. Researchers are trying to develop male herbal contraceptives from natural sources. *Jussiaea repens* L (JR) is such an herb which is reported to have non toxic antigonadal activity in male rats. The present study has designed to evaluate whether crude queous extract of JR affects the DNA integrity of spermatozoa and also to study whether its actions are reversible or not. In this study, toluidine blue (TB), acridine orange (AO) and aniline blue (AB) staining were used to assess sperm chromatin / DNA integrity and comet assay for sperm DNA damage. Results show that the DNA integrity or denaturation by TB, AO and AB positive staining of spermatozoa of JR treated group were significantly increased when compared with control. But TB positive staining was much higher (34.51%) than AO (27.06 %) and AB (18.91%) positive staining. But no denaturation was observed in epididymal spermatozoa of rats after withdrawal of extract treatment. Results of Comet assay also support the reduced change in Head DNA % and increase in Tail DNA %, Tail length (TL), Comet length (CL), Tail movement(TM) and Olive tail movement (OTM) than control groups. However, in the recovery group no significant changes were observed. All parameters returned almost towards control in withdrawal group suggesting the reversible action of extract. So, it may be concluded that *Jussiaea repens* at a dose of 200 mg/kg body weight/day for 28 days, induce temporary DNA damage of epididymal spermatozoa and infertility in rats. So, at regulated dose and duration, *Jussiaea repens* extract can be used as non-toxic male herbal contraceptive in future.

INTRODUCTION: Overpopulation is a global problem. So, Fertility control is a challenging issue. The World Health Organization has suggested the use of locally available plants, instead of synthetic drugs, as cost effective management for birth control [1]. It has been observed that about 90% of the World's contraceptive users are women [2]. But male contraceptive is very limited. Many researchers globally are trying to develop male herbal contraceptive from natural sources. *Jussiaea repens* L (JR) is such an herb that widely distributed in the different countries.

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In our previous study we have reported the non toxic antigonadal activity of JR by oral administration of its crude aqueous extract except root in adult male albino rats at a dose of 200 mg/kg body weight/day for 28 days caused significant decrease in weight of reproductive organs i.e. testis and cauda epididymis, sperm motility, sperm density and sperm viability [3].

In addition, spermatogenesis in seminiferous tubules and number of spermatogenic cells in the testis at stage VII were diminished and decreased serum testosterone, LH and FSH levels in treated rats [4]. The acridine orange stained fluorescent microscopic study of testis in the treated rats showed altered seminiferous tubules and large number of tail less spermatozoa in the lumen and having red or orange fluorescence nuclei which indicate the partial damage of DNA. Mating

experiment of our earlier study also revealed that when *Jussiaea repens* aqueous extract was fed orally to male albino rats a significant decrease in the mating index and fertility rate was zero [5]. The fertility process depends not only on sperm count, motility, viability and morphological abnormalities of spermatozoa but also on chromatin quality or DNA integrity. Because sperm concentration, motility and morphology give an approximate evaluation of the functional competence of spermatozoa, but do not always reflect the quality of sperm DNA [6-8]. Sperm DNA integrity is highly correlated with normal fertility. Any form of sperm chromatin abnormalities or DNA damage may result in male infertility [9, 10].

So, in the present study, the main objective is to investigate the *in vivo* effect of *Jussiaea repens* L aqueous extract in male rats on sperm chromatin quality or DNA integrity and sperm DNA damage by differential staining methods and single cell gel electrophoresis (comet assay), which may support our earlier studies regarding antifertility activity of *Jussiaea repens.*

MATERIALS AND METHODS:

Plant material:

The plant, *Jussiaea repens* L, was collected from wetlands of West Bengal, as reported earlier [3] 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 [3]. 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. 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. After the rats were sacrificed, cauda epididymis of each animal was used to asses sperm chromatin and DNA study.

Sperm chromatin and DNA study:

In this study toluidine blue (TB), acridine orange (AO), and aniline blue (AB) staining are used to assess sperm chromatin / DNA integrity and also comet assay used to asses sperm DNA damage.

Toluidine blue staining:
The TB stain was performed according to the method of Talebi 2008 [12]. Briefly, Air-dried smears were fixed in freshly prepared 96% ethanol-acetone (1:1) at 4°C for 1 hour and air dried, then hydrolysed in 0.1 N HCl at 4°C for 5 minutes and stained with 0.05% TB (0.05 gm toluidine blue in 50% citrate phosphate buffer, pH 3.5) for 5 minutes at room temperature then rinsed in distilled water. 200 spermatozoa were counted in different areas of each slide using X400 magnification. Sperm cell heads with good chromatin integrity were light blue; those of diminished integrity were deep violet (purple). Deep violet sperms were considered to be abnormal and the percentage of sperms with a deep violet color was determined.

Aniline blue staining:
The AB stain was performed according to the method of Talebi 2008 [12]. Air dried smears were fixed in 3% glutaraldehyde in 0.1M PBS for 30 minutes and stained with 5% aqueous AB in 4% acetic acid (pH 3.5) for 7 minutes then washed with PBS and air-dried. 100 spermatozoa were counted in different areas of each slide using X400 magnification. Sperm cell heads with good chromatin integrity were nearly colourless or pale-blue, whereas dark blue stained as abnormal chromatin integrity of spermatozoa was determined.

Acridine orange staining:
Air dried cauda epididymal sperm smear was 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, pH 4.0) for 5min.Slides were washed by distilled water for 5 min and air dried again [13]. The stained smear was observed under fluorescence microscope at X400 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.

Study of sperm DNA damage by comet assay:
The comet assay was performed under alkaline conditions, according to the method of Singh [14]. Frosted microscopic slides were covered with a thin layer of 1 % (w/v) normal melting point agarose in PBS at about 60°C. Cover slips were added and the slides were allowed to solidify for 15 min at room temperature. Upon solidification of the agarose, the cover slip was gently removed, 25 µl of sperm cell suspension (1–2 × 106 sperms per ml of 0.1 M PBS, pH 7.4) was mixed with 250 µl of 0.5% (w/v) Low Melting Point (LMP) agarose (1:10) at 37°C and 75 µl of the this mixture were rapidly spread onto the first agarose layer. Cover slips were added and the slides were allowed to gel at 4°C, for 20 min.

The cover slip was replaced and the 1 % (w/v) normal melting point agarose was again allowed to solidify for 10 min at 4°C. After subsequent removing the cover slip, the slides were dipped in freshly prepared lysing solution containing (2.5 M NaCl, 100 mM disodium EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO, 1% sodium lauryl sarcosinate, pH 10) for 2 hours at 4°C. After two hours, the slides were washed in the neutralization buffer (0.4 M Tris, pH 7.4) for 5 min and then slides were placed horizontally in electrophoresis tank containing alkaline buffer (300 mM NaOH, 1 mM EDTA, pH 13.0) for 30 min at room temperature to allow unwinding of DNA and electrophoresis was carried out for 20 min at 16V and 200 mA. After electrophoresis, slides were gently washed three times for 5 min in fresh neutralized buffer and stained with 25µl of ethidium bromide solution (20µg/ml) for 2 min. and covered with cover slip. The slides were viewed by fluorescence microscopy at 10x magnifications. 200 cells per group were captured and comet image was analyzed using the CASP image analysis software. Head DNA (%), tail DNA (%), tail length (TL), tail moment (TM) and olive tail moment (OTM) were used to estimate the DNA damage. The Olive tail moment is expressed [(tail mean – head mean) × (% tail DNA/100)] to quantify DNA damage.

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:

In the present study, the results of DNA integrity by toluidine blue (TB), aniline blue (AB) and acridine orange (AO) positive staining of spermatozoa of JR treated group were both significantly increased ($P<0.01$) when compared with control but denaturation by TB positive staining is much higher (34.51%) than AB (18.91%) and AO (27.06 %) positive staining. But after withdrawal of extract treatment there was no denaturation observed. **(Table 1, Fig. 1,2,3,4)**

TABLE 1: DNA INTEGRITY (ANILINE BLUE, TOLUIDINE BLUE AND ACRIDINE ORANGE STAINING) IN RATS EPIDIDYMAL SPERM

	Positive for Toluidine blue stain (TB+) %	Positive for Aniline blue stain (AB+) %	Positive for Acridine orange stain (AO+) %
Group I: Control group	18.419	10.875	7.517
	±1.138	± 0.767	±0.557
Group II: treated with aqueous extract (200 mg/kg body weight)	34.519 **	18.916 **	27.066 **
	±1.899	±1.174	±1.639
Group III: Recovery group	20.025	11.838	7.608
	±1.927	±1.053	±0.480

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).

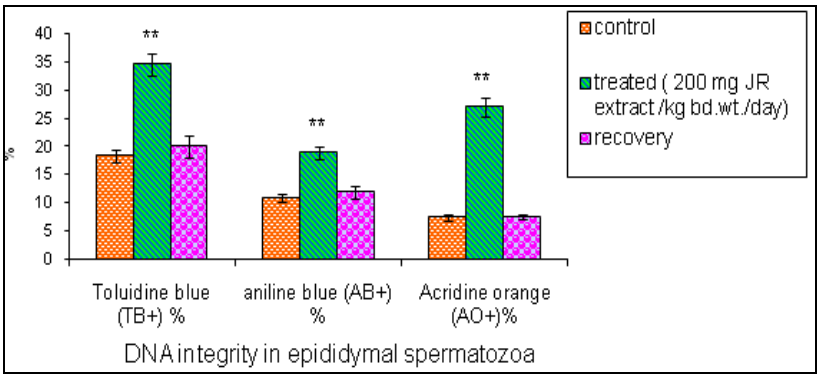

FIG. 1: EFFECT OF *JUSSIAEA REPENS* L EXTRACT IN CHROMATIN INTEGRITY OF EPIDIDYMAL SPERMATOZOA OF RAT STAINED WITH TOLUIDINE BLUE (TB), ANILINE BLUE(AB) AND ACRIDINE ORANGE (AO). VALUES WERE EXPRESSED AS MEAN ± SEM, N=8, **SIGNIFICANT (P<0.01).GROUP II (TREATED) WAS COMPARED WITH GROUP I (CONTROL) AND GROUP III (RECOVERY).

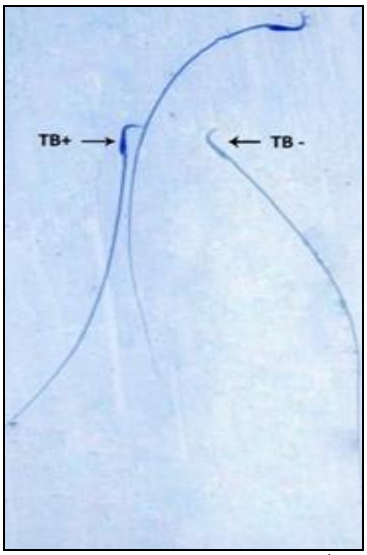

FIG.2: TOLUIDINE BLUE STAINING OF RAT'S SPERMATOZOA (TB^+ STAINING SHOWING SPERM CELLS WITH ABNORMAL CHROMATIN, TB^- STAINING SHOWING SPERM CELLS WITH NORMAL CHROMATIN)

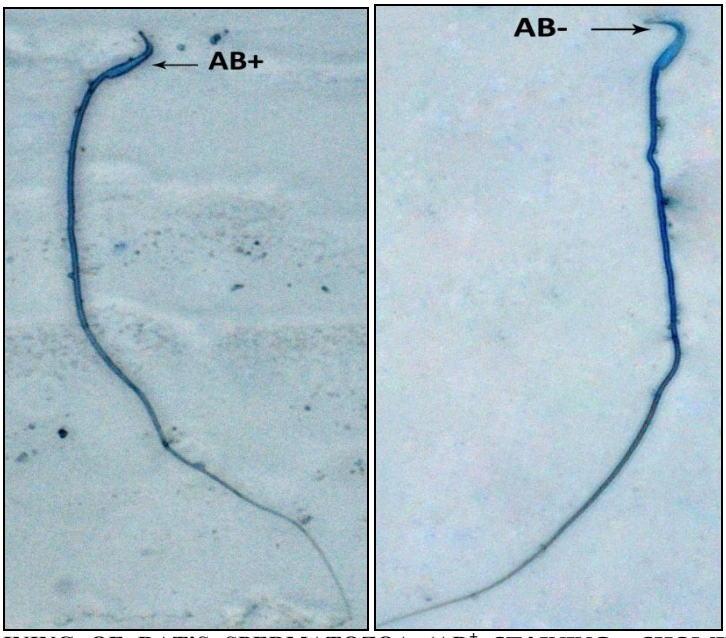

FIG.3: ANILINE BLUE STAINING OF RAT'S SPERMATOZOA (AB^+ STAINING SHOWING SPERM CELLS WITH ABNORMAL CHROMATIN, AB^- STAINING SHOWING SPERM CELLS WITH NORMAL CHROMATIN)

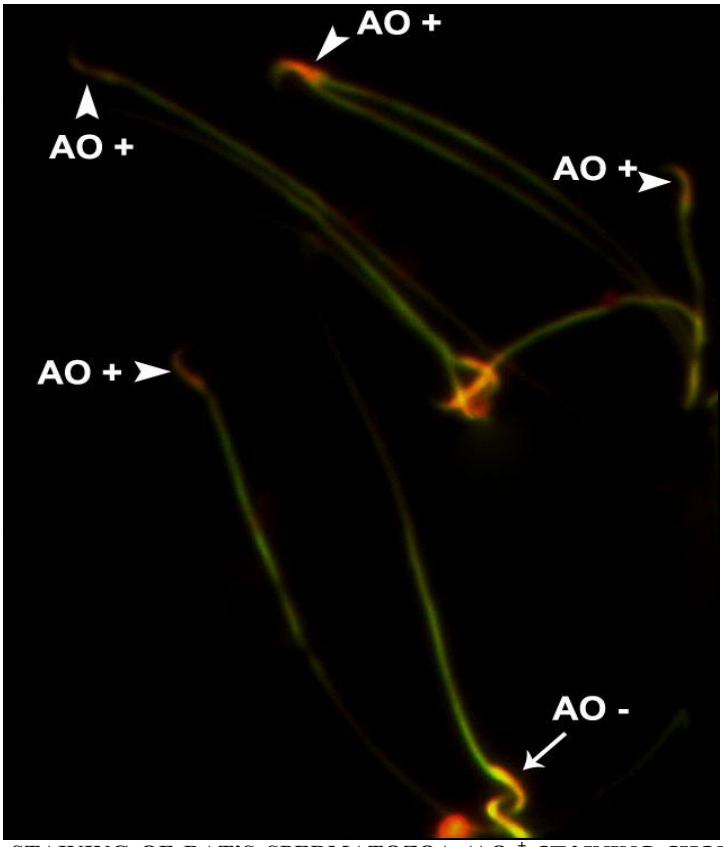

FIG.4 : ACRIDINE ORANGE STAINING OF RAT'S SPERMATOZOA (AO^+ STAINING SHOWING SPERM CELLS WITH ABNORMAL CHROMATIN, AO^- STAINING SHOWING SPERM CELLS WITH NORMAL CHROMATIN)

The results of sperm DNA damaged by comet assay indicates an increase in the percentage of sperm with fragmented DNA in the treated group where the lowest percent of DNA in comet head but increase the tail DNA %, tail length, comet length, tail moment and olive tail moment were observed. But in the withdrawal group there was no significant increase in the number of spermatozoa with damaged DNA **(Table -2, Fig. 5, 6, 7)**. All these comet parameters returned almost near to control value in withdrawal group.

TABLE 2: ANALYSIS OF SPERM DNA DAMAGE BY COMET ASSAY IN RATS

	Head DNA% (Px)	Tail DNA % (Px)	Tail length (Px)	Comet length (Px)	Tail movement (Px)	Olive tail movement (Px)
Group I: Control group	95.18 ±0.92	4.82 ± 0.92	3.03 ±0.02	14.03 ±0.19	0.15 ±0.02	0.27 ±0.05
Group II: treated with aqueous extract (200 mg/kg body weight)	62.04** ±2.42	37.96** ±2.42	5.94** ±0.40	18.76** ±0.60	2.39 ** ±0.28	1.93 ** ±0.18
Group III: Recovery group	95.07 ±1.13	4.93 ±1.13	3.12 ±0.05	14.47 ±0.30	0.17 ±0.04	0.28 ±0.06

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).

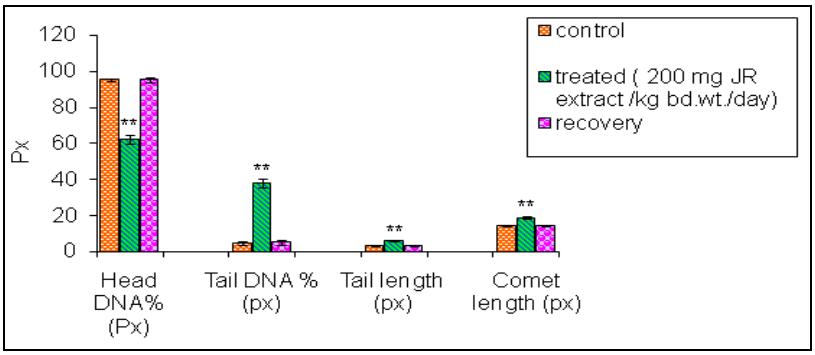

FIG. 5: EFFECT OF *JUSSIAEA REPENS* L EXTRACT ON PERCENTAGE OF SPERM DNA DAMAGE USING THE COMET ASSAY. VALUES WERE EXPRESSED AS MEAN ± SEM, N=8, **SIGNIFICANT (P<0.01). GROUP II (TREATED) WAS COMPARED WITH GROUP I (CONTROL) AND GROUP III (RECOVERY).

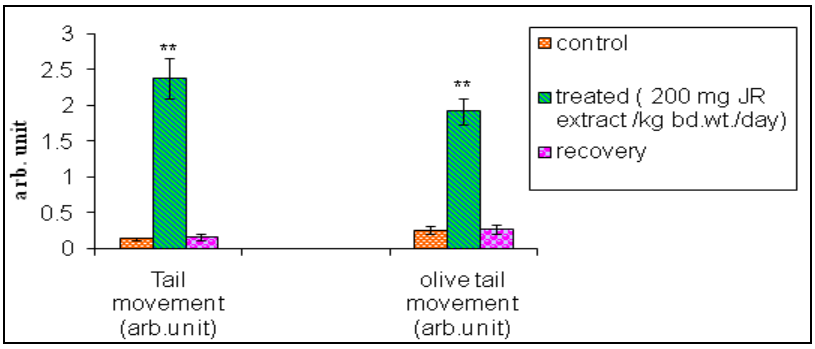

FIG. 6: EFFECT OF *JUSSIAEA REPENS* L EXTRACT ON PERCENTAGE OF SPERM DNA DAMAGE USING THE COMET ASSAY. VALUES WERE EXPRESSED AS MEAN ± SEM, N=8, **SIGNIFICANT (P<0.01). GROUP II (TREATED) WAS COMPARED WITH GROUP I (CONTROL) AND GROUP III (RECOVERY).

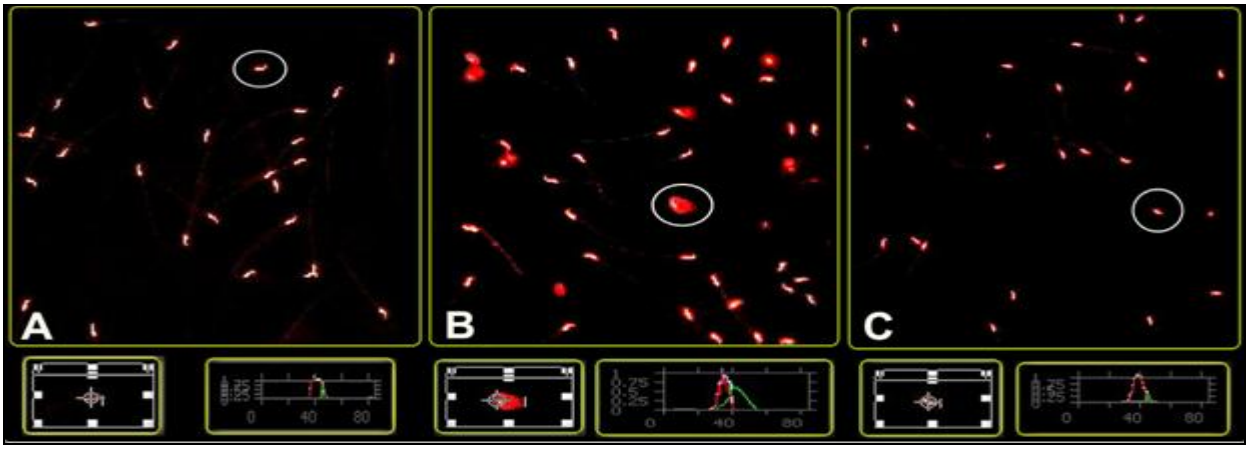

FIG.7: PHOTOMICROGRAPH OF DNA DAMAGE ON RAT EPIDIDYMAL SPERMATOZOA TREATED WITH JR EXTRACT IN THE COMET ASSAY. (A) INTACT SPERMATOZOA WITH UNDAMAGED DNA (WITH OUT COMET TAIL) IN CONTROL GROUP. (B) SPERMATOZOA WITH MEDIUM DNA DAMAGE (SHORT COMET TAIL) IN JR EXTRACT TREATED GROUP. (C) INTACT SPERMATOZOA WITH UNDAMAGED DNA (WITH OUT COMET TAIL) IN RECOVERY GROUP.

DISCUSSION: The fertilizing potential of sperm depends not only on the functional competence of spermatozoa but also on sperm DNA integrity, [6-8] Sperm chromatin condensation and stability which can detect the sperm maturation and possible disorders in spermatogenesis [15]. Some investigators reported that when >30% of sperm DNA is damaged, natural pregnancy is not possible, [16] which supported the results of our present study. We observed the DNA integrity or denaturation by TB, AB and AO positive staining of spermatozoa in *Jussiaea repens* (JR) treated group were significantly increased but denaturation by TB positive staining was much higher (34.51%) than AB (18.91%) and AO (27.06 %) positive staining **(Table 1, Fig. 1,2,3,4)**. But after withdrawal of extract treatment there was no denaturation observed. The DNA damage was measured by increased comet tail length using the comet assay [17, 18]. One of the principle of the comet assay is that nicked double stranded DNA tends to remain in the comet head, whereas short fragments of nicked double and single stranded DNA migrate into the tail area [19]. Thus, spermatozoa with high levels of DNA strand break would show increased comet tail fluorescent intensity [17] and comet tail length [18].

The results **(Table -2, Fig.- 5, 6,7)** of the present study indicated an increase in the percentage of sperm with fragmented DNA in the treated group where the lowest percent of DNA in comet head but higher the tail DNA %, tail length, comet length, tail moment and olive tail moment were observed. In the withdrawal group there was no significant increase in the number of spermatozoa with damaged DNA which indicated that after withdrawal of treatment, the damaged DNA was repaired. So, it is assumed that when JR aqueous extract was fed orally at a dose of 200 mg/kg body weight for 28 days, the DNA of epididymal spermatozoa was not permanently damaged. The exact mechanism by which chromatin abnormalities or DNA damage arise in the epididymal spermatozoa in the present study is not precisely understood.

Normally, Sperm nuclear chromatin abnormalities or DNA damage occur at the time of DNA packing in spermiogenesis [20, 21]. During this process, histones associated with round spermatid DNA are replaced by transition proteins and finally by protamines [22]. It is reported that, when compaction is poor and chromatin protamination is incomplete, sperm DNA is more vulnerable to oxidative stress [23]. Inadequate chromatin packaging during spermatogenesis may also render sperm DNA prone to damage [24]. Developing sperms are capable of repairing their DNA; therefore, damage occurring in the course of spermatogenesis is usually promptly repaired. Once the sperm is mature, however, there is no chance of further prefertilization DNA repair [24].

So, disturbance at any step could affect the process of spermatogenesis and the spermatozoa may become defective [4, 25]. Increased prevalence of genomic defects in spermatozoa may increase the risk of infertility [26]. In our previous studies we have reported that JR inhibited spermatogenesis process [4] and fertility rate was zero [5] which may be supported from the present study due to partial denaturation and loss of binding of DNA to basic protein and abnormal spermatid maturation.

The DNA denaturation also can be supported by our previous study, where epididymal Zn level was depleted by JR treatment [27]. Zn also play a role in the formation of S-Zn-S bridges, which additionally stabilize the chromatin structure [28, 29]. Deficiency of zinc associated with hypogonadism and failure of spermatogenesis [30, 31]. During spermatogenesis process defective spermatid protamination and appropriate disulfide bridge formation does not occur caused by inadequate oxidation of thiols during epididymal transit, resulting in diminished sperm chromatin packaging, makes sperm DNA more vulnerable to damage [32]. The glutathione (GSH) is the most abundant non-protein thiol in mammalian cells. It plays a key role in biological processes, including proteins and DNA synthesis and amino acid transport. However, its most important role is the protection of cells against oxidation, including control of male fertility [33]. High concentrations of GSH have been observed in rat testis during the onset of spermatogenesis [34].

In our previous studies, reduction of testicular GSH and insignificant rise of MDA in treated group when compared to control, Which indicate the

utilization of GSH for reduction of oxidative stress or it may be another possibility that oxidative stress can lead to sperm damage, deformity by adversely affecting the quality of sperm DNA and eventually, male infertility [35, 36]. Efficient energy metabolism is also an another crucial point for sperm survival, motility and capacity for fertilization. Oxidative stress resulting from normal metabolism in the mature sperm is a significant source of DNA damage. The high levels of oxidative phosphorylation needed to maintain sperm motility are unavoidably associated with increased amounts of reactive oxygen species that may damage DNA of the sperm [24]. In our previous findings that inhibition of ATPase activity in testis and epididymis after extract treatment the suppression of energy metabolism and inhibition of male gonadal functions supports the alteration in acrosomal membrane leading to infertility [27]. Again, the spermatogonia give rise to mature spermatozoa under the hormonal influences of gonadotropins and the testosterone is required for successful completion of the spermatogenesis process. [4, 37] which also supported our earlier studies, where testosterone reduced in JR treated rats leading to inhibition of spermatogenesis process and infertility.

CONCLUSION: In the present study, it may be concluded that *Jussiaea repens* L at a dose of 200 mg/kg body weight for 28 days, induce DNA damage of epididymal spermatozoa temporarily and infertility of rats. So, at regulated dose and duration, the crude aqueous extract of *Jussiaea repens* L can be used as non-toxic male herbal contraceptive in future.

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REFERENCES:

1. Umadevi M, Sampath Kumar PK, Bhowmik Debjit and Duraivel S: Medicinal plants with potential antifertility activity. Journal of Medicinal Plants Studies 2013; 1(1): 26-33.
2. Joshi Suresh C, Sharma Aksha and Chaturvedi Mridula: Antifertility potential of some medicinal plants in males: an overview. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(5): 204-217.
3. Pradhan NK, Ghosal S and Chakraborty I: *Jussiae repens* is a nontoxic antigonadal herb- A dose dependent study on male rats. International Journal of Pharma and Bio Sciences 2013; 4(2): 131-143.
4. Ghosal S, Chakraborty I and Pradhan NK: Reversible action of *Jussiaea repens* L induced alterations of histoarchitechture *vis-à-vis* functions in testicular tissues of rat. World Journal of Pharmaceutical Research 2015; 4 (5): 1667-1687.
5. Ghosal S, Chakraborty I and 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.
6. Sharma RK, Said T and Agarwal A: Sperm DNA damage and its clinical relevance in assessing reproductive outcome. Asian Journal of Andrology 2004; 6(2):139-148.
7. Sun JG, Jurisicova A and Casper RF: Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biology of Reproduction 1997; 56(3): 602-607.
8. Saleh RA, Agarwal A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, et al.: Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertility and Sterility 2003; 79 (Suppl 3): 1597-1605.
9. Modarresi Chahardehi A, Ibrahim D and Fariza Sulaiman S: Antioxidant, Antimicrobial Activity and Toxicity Test of *Pilea microphylla*. International Journal of Microbiology 2010; 2010: 826 - 830.
10. Cocuzza M, Sikka SC, Athayde KS, et al.: Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: an evidence based analysis. International Brazilian Journal of Urology 2007; 33(5): 603-21.
11. Mathur JN: National Centre for Laboratory Animal Sciences (NCLAS) – A profile. The Indian Council of Medical Research, Bulletin, April 2004; 34(4): 21-28.
12. Talebi AR, Moein MR, Tabibnejad N and Ghasemzadeh J: Effect of varicocele on chromatin condensation and DNA integrity of ejaculated spermatozoa using cytochemical tests. Andrologia 2008; 40: 245-251.
13. Tejada RI, Mitchell JC, Norman A et al.: A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertility and Sterility 1984; 42(1):87-91.
14. Singh NP, McCoy MT, Tice RR and Schneider EL: A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research 1988; 175: 184 -191.
15. Hingst O, Blottner S and Franz C: Chromatin condensation in cat spermatozoa during epididymal transit as studied by aniline blue and acridine orange staining. Andrologia 1995; 27: 275 - 279.
16. Evenson, DP, Larson KL and Jost LK: Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with the other techniques. Journal of Andrology 2002; 23: 25 - 43.
17. Hughes CM, Lewis SE, McKelvey-Martin VJ and Thompson W: A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay. Molecular Human Reproduction1996; 2: 613 - 619.

18. Singh NP and Stephens RE: X-ray induced DNA double-strand breaks in human sperm. Mutagenesis 1998; 13:75-79.
19. Klaude M, Eriksson S, Nygren J and Ahnstrom G: The comet assay: mechanism and technical considerations. Mutation Research 1996; 363: 89-96.
20. Sailer B, Jost L and Evenson D: Mammalian sperm DNA susceptibility to in situ denaturation associated with the presence of DNA strand breaks as measured by the terminal deoxynucleotidyl transferase assay. Journal of Andrology 1995; 16: 80 -7.
21. Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi PG and Bianchi U: Origin of DNA damage in ejaculated human spermatozoa. Reviews of Reproduction 1999; 4: 31-7
22. Grimes SR, Meistrich ML, Platz RD and Hnilica LS: Nuclear protein transitions in rat testis spermatids. Experimental Cell Research 1977; 110:31-39.
23. Iommiello Valeria Maria, Albani Elena, Di Rosa Alessandra, Marras Alessandra, Menduni Francesca , Morreale Giovanna , Levi Shanti Lia , Pisano Benedetta and Levi-Setti Paolo Emanuele : Ejaculate Oxidative Stress Is Related with Sperm DNA Fragmentation and Round Cells**.** International Journal of Endocrinology 2015; (2015): 1-6.
24. Vazharova Radoslava and Kremensky Ivo: Individual capacity for DNA repair and maintenance of genomic integrity: a fertile ground for studies in the field of assisted reproduction. Biotechnology & Biotechnological Equipment 2016; 30 (3): 419-433.
25. Ghosal S, Chakraborty I and Pradhan NK: Histoarchitecture and Biochemical Studies of Epididymis in Male Rats Induced by Aqueous Extract of Jussiaea Repens L.International Journal of Pharmaceutical Sciences and Research 2016; 7(5): 2209-18
26. Belloc S, Hazout A, Zini A, Merviel P, Cabry R, Chahine H, Copin H and Benkhalifa M : How to overcome male infertility after 40: influence of paternal age on fertility. Maturitas 2014; 78(1): 22-29.
27. Chakraborty I, Ghosal S and Pradhan NK: *Jussiaea repens* L Acts as an Antifertility Agent – A Search for Herbal Male Contraceptive. International Journal of Pharmaceutical Sciences Review and Research 2014; 24(2): 288-296.
28. Kvist V and Eliasson R: Sperm nuclear chromatin decondensation ability. Acta Physiologica Scandinavica 1980; 109 (Supplement) 486: 1-24.
29. Mardani Mohammad, Vaez Ahmad and Razavi Shahnaz : Effect of saffron on rat sperm chromatin integrity. Iranian Journal of Reproductive Medicine 2014; 12(5): 343–350
30. Prasad AS: Discovery of humen zinc deficiency and studies in an experimental human model. American Journal of Clinical Nutrition 1991; 53: 403-412.
31. Endre L,Beck F and Prasad : The role of zinc in human health. The Journal of Trace Elements in Experimental Medicine 1990; 3: 333- 375.
32. Gardner David K, Weissman Ariel, Howles Colin M and Shoham Zeev: Textbook of Assisted Reproductive Technologies, Laboratory and Clinical Perspectives. Informa healthcare, UK, Third Edition 2009.
33. Luberda Z: The role of glutathione in mammalian gametes. Reproductive Biology 2005; 5(1): 5–17.
34. Donnelly ET, Mcclur N and Lewis SE: Glutathione and hypotaurine in vitro: effects on human sperm motility, DNA integrity and production of reactive oxygen species. Mutagenesis 2000; 15(1): 61–68.
35. Makker K, Agarwal A and Sharma R: Oxidative stress & male infertility, Review Article. Indian Journal of Medical Research 2009 129: 357-367.
36. Agarwal Ashok, Virk Gurpriya, Ong Chloe and Sdu Plessis Stefan: Effect of Oxidative Stress on Male Reproduction. The World Journal of Mens Health 2014; 32(1): 1–17.
37. 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

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