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JUSSIAEA REPENS L. IS A NONTOXIC ANTIGONADAL HERB – A DOSE DEPENDENT STUDY ON MALE RATS.

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ABSTRACT

Oral administration of crude aqueous extract of *Jussiaea repens L* (family - Onagraceae) to adult male albino rats of Wistar strain at the doses of 100 mg (low dose ,Group-II), 200 mg (moderate dose, Group - III), and 400 mg (high dose, Group - IV) / kg body weight /day for 28 days, caused no significant change in body weight and organ weights like liver, kidney, spleen and heart but the weights of testis and cauda epididymis were significantly reduced in Group - III, where the weight of adrenal gland showed significant rise in Group - IV. Epididymal sperm concentration, motility and viability were significantly reduced but Sperm abnormality was markedly increased in Group - III and IV. SGOT,SGPT,ALP,ACP, Total protein, Urea and Creatinine level in serum were remained unchanged in treated groups. The fructose content of seminal vesicle and ventral prostate was reduced significantly in Group – III. All comparisons were made against the vehicle (water) treated control. So, the oral administration of aqueous extract of *Jussiaea repens L* may be considered as nontoxic antifertile agent in male rat in a dose dependent manner.

KEY WORDS: ***Jussiaea repens L ,*** **Reproduction, Sperm, Antifertility, Contraceptive.**

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INTRODUCTION

Over population is a worldwide burning problem and extra burden on the community. The census of 2011 showed that India, with 1.21 billion people is the second most populous country in the world [1]. The rapid population growth has caused a serious problem in economic growth and human development in developing countries like India. For this reason the World Health Organization (WHO) has constituted a population control programme, which includes studies having traditional medical practices [2]. So, family planning has been promoted through several methods of contraception, i.e., natural and synthetic. Though, most of the synthetic contraceptive agents are not to be used continuously because of their side effects , where herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects[3]. The plant *Ludwigia adscendens L.* (Synonym - *Jussiaea repens L*. family - onagraceae) [4] is one of such herbs, commonly known as creeping water prime rose. It grows in fresh water, ponds, canals of roadside and wetlands. Pharmacologists reported the clinical uses of this plant as hepatoprotective, anti inflammatory, antidiabetic [5] antibacterial [6] and fibrinolytic activity [7]. In different Districts of West Bengal, Jharkhand, Orissa [8] & Manipur [9] in India, some tribal people (Oraon & Chero) unknowingly consume this plant as vegetable and animal forage [10]. In Papua New Guinea, the leaves and stem of this plant are considered to have contraceptive (prevent pregnancy) properties[11]. Different Scientists [6,12-14] reported that aerial parts of this plant is composed of different metabolites like, rutin, kaempferol, quercetrin, quercetin, terpenes, triterpenes etc. Again, purified rutin, kaempferol, quercetin and triterpenes are potent antifertile compounds as reported by different investigators [15-18]. But no report has yet been available regarding the antifertile role of *Jussiaea repens L*. as a whole on male reproduction. So, our objective is to study the antigonadal effect of this plant in male rats in dose and a duration dependent manner.

MATERIALS AND METHODS

Plant Material

The plant *Jussiaea repens L.* was collected from wetlands of 24 Parganas (N), WB, India, during the month of March - April. The material was identified 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. Fresh plants were carefully washed under running tap water and finally with distilled water and air dried at 35-40°C for 4-5 days, then homogenized to a fine powder by mixer grinder and stored for extraction.

Extract Preparation

400 gm. dry powder of *J.repens L.* was taken for extraction in 4 liters of hot (50°C) distilled water for 30 minutes[19], then cooled and kept overnight at room temperature. The extract was filtered using clean muslin cloth, then by ordinary filter paper and finally by Whatman No. 1 filter paper . The residue was further extracted twice similarly. The resulting filtrate was then concentrated using a rotary evaporator, and further dried at 40°C. Finally yield 6.25 % solid crude extract which was stored in powdered form in an air tight container at 4˚C for further use in the experiment. The percentage extract yield was estimated according to Parekh and Chanda[20], as : Dry weigh t / Dry material weight × 100.

Animal

32 adult male albino rats (*Rattus norvegicus L.*) of Wistar strain weighing 130g ±10g were selected for the experiment. The animals were acclimatized to the laboratory environment for a period of one week before starting the experiment. The animals were maintained under standard laboratory conditions, 12 hours light: 12 hours dark, 25±2°C and relative humidity (40-60%) with free access to standard normal diet, prescribed by ICMR,NIN, Hydrabad, India [21] and water *ad libitum*. Animal experiments were

performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) guided by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Ref.no. PU 796/03/ac/CPSEA.

Acute Toxicity Study

Acute toxicity study was performed to select the effective dose of the extract. The dose was selected on the basis of primary investigations according to Ghosh 1984[22] and Shivaraj et.al. 2011[23]. Acute toxicity study was performed in rats dividing into different groups of 8 animals each. Rats were kept fasting for overnight providing only water, after which the extract was administered orally at doses of 100, 200 and 400 mg /kg body weight and observed for toxic symptoms i.e., change in general behavior, skin effects, defecation, loss of hair or other physiological activities and mortality up to 72 hours. The dose up to 400 mg/ kg body weight did not produce any sign of toxicity . The animals were physically active, which showed that the aqueous crude extract of *J. repens L.* was safe and non-toxic at the doses used.

Animal Treatment

Animals were equally divided and randomly selected into 4 groups having 8 animals in each group and were treated daily for 28days, as - Group I : Control group, were given sterile distilled water of 0.5 ml/ 100 g body weight /day. Group II: Treated with aqueous extract (100 mg /kg body weight /day) (low dose group). Group III : Treated with aqueous extract (200 mg /kg body weight /day) (moderate dose group). Group IV: Treated with aqueous extract (400 mg /kg body weight /day) (high dose group).The dose was prepared as suspension of the extract in 0.5 ml of sterile water and administered daily to each animal orally with the help of 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. Treatment schedule was selected to determine the effects of *J.repens L.* extract for two seminiferous cycles (~ 28 days) consecutively .

Body Weight , Blood sample and Organ collection

The final body weights of all animals were recorded before sacrifice (at 29th.day). Animals were anaesthesized by diethyl ether and blood samples were collected from hepatic vein. Serum was separated and stored at - 20°C for different biochemical assay. The different organs i.e., testes, epididymis, seminal vesicle, ventral prostate and other vital organs like liver, heart, kidney, adrenal, spleen of each animal were dissected out, freed from adherent tissues, blotted free of blood and wet weights were recorded then kept in frozen condition (- 20°C) and were used immediately for estimation. Relative weight of organs was expressed per 100 g body weight.

Sperm Motility and Total Sperm count

One caudal epididymis of each animal from the right side was rinsed and gently minced in 2 ml of phosphate buffere saline (P^H 7.4). The fragments were allowed to sediment for 5 minutes. Epididymal sperm motility and sperm concentration was determined by WHO Manual [24]. The percentage motility was determined by counting both motile and immotile spermatozoa compared to total cells by Neubauer hemocytometer in WBC chamber. Again 40 µl of epididymal sperm suspension was diluted with 360 µl of diluents (50 gm sodium bicarbonate, 10 ml of 35% formalin and 0.25 gm trypan blue were added and made up to a final volume of 1 L with distilled water) and sperms was counted by Neubauer hemocytometer with a light microscope at 40x magnification in a RBC counting 5 major squares and were expressed as million/ml of suspension.

Sperm Viability

Sperm Viability was studied by mixing 50 µl of sperm suspension with 50 µl of eosin-nigrosin stain and incubated for 30 seconds at room temperature (20°C) then a thin smear was prepared. Two such smears were prepared from each sample. The smears were air dried and examined directly under microscope. At least

200 sperms were studied at magnification of 100x under oil immersion with a bright field objective. Unstained sperms were considered as live and pink or red colored sperm as dead [25].

Sperm Morphology

Sperm morphology was studied from a thin smear prepared from stained sperm suspension (eosin - nigrosin mixture). 200 spermatozoa per animal were observed under high power objective (magnification 400x) and classified as normal and abnormal types. The defective shape and structure of either head and or tail were considered as abnormal and the data was presented as percentage incidence of total abnormalities [26].

Tissue Fructose estimation

The seminal vesicle and ventral prostate was homogenized in 5% perchloric acid (100 mg tissue / ml of 5% perchloric acid) and protein-free extracts were obtained by centrifugation at 3000 rpm for 10 min. and 2 ml of clear supernatant was taken for analysis . 1ml of 0.1% resorcinol and 3ml of 30% HCl were added in 2 ml supernatant, mixed and kept for 10 min. For the blank, 2 ml of 5% perchloric acid was added and for the standard 2 ml of standard fructose (100 mg %) solution was added instead of supernatant . All the tubes were placed at 80^{o}C for 1 hour. The solutions were cooled and the reading was taken photometrically at 515 nm against reagent blank. The value was expressed in mg/gm of tissue [27] .

Biochemical estimations

The separated serum was subjected to estimate biochemical parameters like Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvate transaminase (SGPT) by the method of Reitman and Frankel,1957 [28]. Alkaline phosphatase (ALP) by Kind and King 1954 [29], acid phosphatase (ACP) by King and Jadeeshan 1956[30], Total Protein by Kaplan and Lavemel 1983[31], Urea by Donald and Wybenga 1971[32] and Creatinine by Toro and Ackermann 1975 [33] All the estimation was performed by using Laboratory kits (Span Diagnostics Ltd., Surat, India).

Statistical analysis

All the recorded values were expressed in mean ± SEM. The treated groups were compared to control using One way ANOVA with post hoc Dunnet's Multiple comparison test were performed using GraphPad version 3.00 Software. The value of $P<0.05$ was considered to be statistically significant.

RESULTS

Administration of the crude aqueous extract of *Jussiaea repens L.* for 28 days to male rats caused no significant change in body growth rate (Table 1, Figure 1),where as relative weights of testes and cauda epididymis were significantly decreased ($P<0.05$) in moderate dose (Group III) when compared to control (Group I). The relative weights of the seminal vesicle and ventral prostate in all treated groups when compared to control showed no significant change, (Table 2, Figure 2). The relative weights of vital organs like liver, heart, kidney and spleen remained unchanged in compared to control (Table 3,Figure 2), where weight of adrenal showed a significant rise in Group -IV (Table 3, Figure 3). The crude extract also caused a significant reduction ($P < 0.01$) in sperm motility, sperm concentration and viability in cauda epididymis at moderate dose. But in high dose (Group IV), only sperm motility and viability were significantly reduced ($p<0.05$) in respect to control group (Table 4). The sperm morphological abnormalities in caudal region of epididymis was marked and was significantly increased than control by gavaging the extract orally in moderate dose (Group III) ($P < 0.01$) and in high dose (Group IV) ($P<0.05$) in male rats (Table 4, Figure 4). The fructose content in seminal vesicle and ventral prostate was reduced significantly ($p<0.05$) in treated group having moderate dose (Group III) in compare to control (Table 5, Figure 5). Biochemical assay of different marker enzymes from serum like SGOT, SGPT, ALP and ACP showed no significant change in treated groups when compared with control. Again, estimation of total protein, Urea and Creatinine content in serum of treated animals also showed no significant change (Table -6).

Table-1

Body weights (gm) of male albino rats treated with aqueous crude extracts of Jussiaea repens L. at different doses for 28 days.

Treatment	Bodyweight (gm)		Weight gain %
	Initial	Final	
Group I: Control group	133.13 ± 2.48	162.75 ± 4.75	22.32 ± 3.19
Group II: Treated with aqueous extract (100 mg/kg body weight)	131.88 ± 2.66	168.50 ± 5.22	27.92 NS± 3.86
Group III: Treated with aqueous extract (200 mg/kg body weight)	130.00 ± 2.98	168.75 ± 5.48	29.68 NS± 2.24
Group IV: Treated with aqueous extract (400 mg/kg body weight)	129.38 ± 2.90	166.25 ± 6.25	28.52 NS± 4.02

NS= non significant Values are expressed as means ± S.E.M.; N=8.

Table -2

Relative weight of reproductive organs of male albino rats control Vs. treated with aqueous crude extracts of Jussiaea repens L.

Treatment	Relative weights of reproductive Organ (gm /100 gm. body weight)			
	Testes	Cauda Epididymis	Seminal vesicle	Ventral Prostate
Group I: Control group	1.330 ± 0.024	0.209 ± 0.006	0.270 ± 0.021	0.147 ± 0.013
Group II: Treated with aqueous extract (100 mg/kg body weight)	1.220 ± 0.070	0.202 ± 0.010	0.264 ± 0.017	0.142 ± 0.009
Group III: Treated with aqueous extract (200 mg/kg body weight)	1.050 * ± 0.060	0.177 * ± 0.006	0.255 ± 0.026	0.139 ± 0.007
Group IV: Treated with aqueous extract (400 mg/kg body weight)	1.200 ± 0.075	0.196 ± 0.007	0.274 ± 0.020	0.152 ± 0.011

Values are expressed as means ± S.E.M.; N=8. *Significant (P < 0.05) Group II , Group III and Group IV were compared to Group I (Control).

Table -3

Effect of aqueous crude extracts of Jussiaea repens L. on weight of vital organs of male albino rats .

Treatment	Weights of Vital Organ (gm /100 gm. body weight)				
	Liver	Heart	Kidney	Adrenal	Spleen
Group I: Control group	3.098 ± 0.074	0.3118 ± 0.009	0.6668 ± 0.016	0.0207 ± 0.001	0.3052 ± 0.029
Group II: Treated with aqueous extract (100 mg/kg body weight)	3.101 ± 0.097	0.3101 ± 0.012	0.6236 ± 0.027	0.0203 ± 0.001	0.2997 ± 0.019
Group III: Treated with aqueous extract (200 mg/kg body weight)	3.103 ± 0.094	0.3038 ± 0.020	0.6192 ± 0.040	0.0234 ± 0.001	0.3044 ± 0.025
Group IV: Treated with aqueous extract (400 mg/kg body weight)	3.111 ± 0.131	0.2943 ± 0.011	0.6344 ± 0.015	0.0252* ± 0.001	0.2964 ± 0.027

Values are expressed as means ± S.E.M.; N=8. *Significant (P < 0.05) Group II , Group III and Group IV were compared to Group I (Control).

Table – 4
Effect of aqueous crude extracts of Jussiaea repens L. on cauda epididymal sperm parameters of male albino rats .

Treatment	Sperm motility (%)	Sperm count (millions/ml)	Sperm Viability (%)	Sperm Morphology (%)	
				Normal (%)	Abnormal (%)
Group I: Control group	78.03 ± 1.94	30.61 ± 1.12	86.24 ± 2.86	89.75 ± 0.90	10.25 ± 0.90
Group II: Treated with aqueous extract (100 mg/kg body weight)	67.57 ± 3.50	26.69 ± 1.24	71.18 ± 3.80	81.50 ± 6.19	18.50 ± 6.19
Group III: Treated with aqueous extract (200 mg/kg body weight)	23.53 ** ± 6.51	14.29** ± 1.79	24.41** ± 4.60	42.25 ** ± 3.76	57.75** ± 3.76
Group IV: Treated with aqueous extract (400 mg/kg body weight)	59.33* ± 3.37	25.84 ± 1.38	64.91* ± 7.48	72.87* ± 2.81	27.13* ± 2.81

Values are expressed as means ± S.E.M.; N=8.* *Significant (P < 0.01), *Significant (P < 0.05) Group II , Group III and Group IV were compared to Group I (Control).

Table-5
Effect of aqueous crude extracts of Jussiaea repens L. on fructose content of Seminal Vesicle and Ventral prostate of male albino rats.

Treatment	Seminal vesicle Fructose(mg/gm)	Ventral Prostate Fructose(mg/gm)
Group I: Control group	0.501 ± 0.018	0.396 ± 0.025
Group II: Treated with aqueous extract (100 mg/kg body weight)	0.497 ±0.017	0.393 ± 0.011
Group III: Treated with aqueous extract (200 mg/kg body weight)	0.378* ± 0.030	0.326* ± 0.012
Group IV: Treated with aqueous extract (400 mg/kg body weight)	0.506 ± 0.030	0.380 ± 0.019

Values are expressed as means ± S.E.M.; N=8. *Significant (P < 0.05) Group II , Group III and Group IV were compared to Group I (Control).

Table – 6
Study of toxicity markers from serum of male albino rats treated with aqueous crude extracts of Jussiaea repens L.

Treatment	SGOT IU/100 ml	SGPT IU/100 ml	ALP KA units	ACP KA units	Total protein gm/dl	Urea mg/100 ml	Creatinine mg/dl
Group I: Control group	24.036 ± 0.614	16.093 ± 1.232	22.400 ± 1.680	5.306 ± 0.261	6.514 ± 0.281	27.448 ± 0.725	1.559 ± 0.180
Group II: Treated with aqueous extract (100 mg/kg body Weight)	21.571 ± 1.961	16.328 ± 1.339	17.650 ± 2.206	5.506 ± 0.358	6.191 ± 0.214	26.276 ± 0.587	1.496 ± 0.312
Group III: Treated with aqueous extract (200 mg/kg body weight)	19.658 ± 2.310	14.609 ± 1.075	22.913 ± 1.587	5.963 ± 0.439	6.251 ± 0.316	27.213 ± 0.772	1.441 ± 0.292
Group IV: Treated with aqueous extract (400 mg/kg body weight)	23.286 ± 1.121	15.313 ± 1.451	21.038 ± 2.785	6.275 ± 0.422	5.986 ± 0.166	28.729 ± 0.466	1.638 ± 0.318

Values are expressed as means ± S.E.M.; N=8.

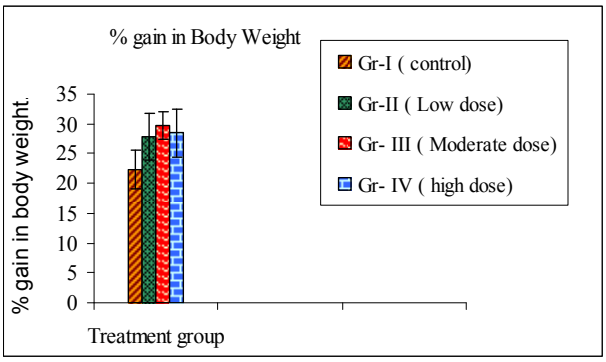

Figure 1

% gain in body weights of male albino rats treated with aqueous crude extracts of Jussiaea repens L.at different doses for 28 days.

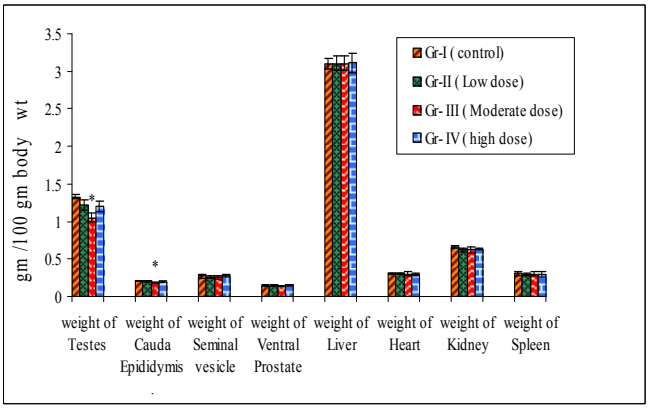

Figure 2

Relative weight of reproductive & vital organs(gm) of male albino rats treated with aqueous crude extracts of Jussiaea repens L. at different doses for 28 days. *Significant (P < 0.05).

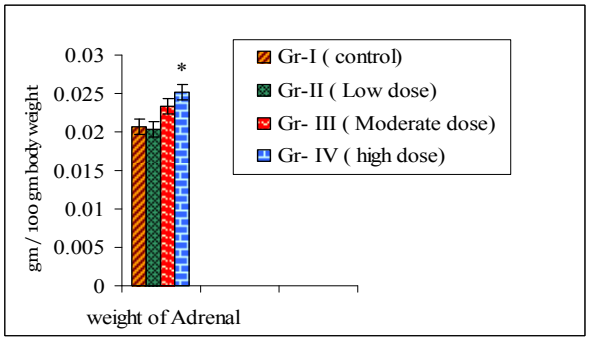

Figure 3

Relative weight of vital organ(gm) of male albino rats treated with aqueous crude extracts of Jussiaea repens L. at different doses for 28 days. *Significant (P < 0.05).

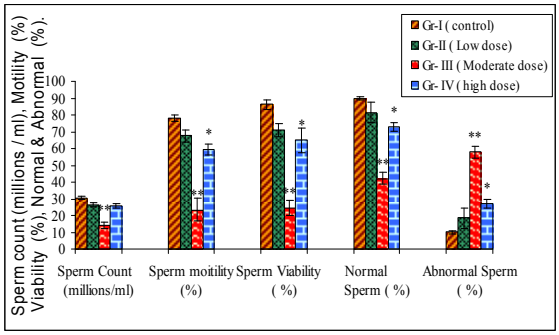

Figure 4

Cauda epididymal sperm characteristics of adult male albino rats treated with aqueous crude extracts of Jussiaea repens L. at different doses for 28 days. *Significant (P < 0.05),* *Significant (P < 0.01).

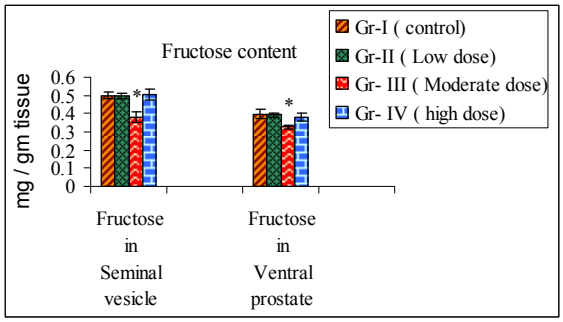

Figure 5

Fructose content in Seminal Vesicle and Ventral prostate of adult male albino rats treated with aqueous crude extracts of Jussiaea repens L at different doses for 28 days *Significant (P < 0.05).

DISCUSSION

In the present study the administration of the crude aqueous extract of *Jussiaea repens L*. at different doses, caused no significant alteration in somatic growth and relative weights of some vital organs in respect to control, which suggest the non toxicity of the plant extract up to 400 mg crude extract /kg body weight/ day for 28 days (table – 1, 3. figure- 1, 2) . Possibly the extract of *J. repens* has no inhibitory or stimulatory effect on different metabolic enzymes otherwise it would cause change in body weights in treated groups as claimed by different investigators in other studies [34-38]. This was also supported by Thanabhorn et.al.[39], as a reduction in body weight gain and weights of internal organs serve as the toxicity index on long term exposure to toxic substances. Again, other investigators [38,40] have reported the rise in weights of different internal organs is due to the accumulation of metabolic debris produced by cellular degeneration. The rise of adrenal weight in higher dose (400 mg / kg body weight) treated group (table- 3,figure - 3), is also may be due to hypertrophy caused by a defect in conversion of pregnenolone to progesterone [41]. The significant reduction in weights of testes and cauda epididymis (table-2, figure -2) by *J. repens* treatment at moderate dose (200mg / kg body weight) indicates the possible inhibition of androgen synthesis, as different investigators [42-45] reported the change in circulating androgens would affect the internal

microenvironment of epididymis and thereby lead the alteration in sperm motility and metabolism. Chitra et.al.,[46] reported the reduction in sperm motility is directly related to androgen deficiency . In the present study the reduction in progressive motility of epididymal sperms (table-4 ,figure- 4)may be responsible for decreased fertility , which may be due to the presence of some spermatogenic inhibitory agent (mostly flavonoids)[15-18] as supported by many investigators [47-49]. They also claimed the low level of androgen is not enough to maintain the weights of gonads and accessories , which are directly androgen dependent target organs. The reduction in epididymal sperm count, viability and rise of abnormal sperm morphology in *J. repens L* treated rats (table - 4, figure -4) may be due to the inhibitory activity of the extract towards spermatogenesis and steroidogenesis. ZemJanis, in 1977 [50] reported that animal having more than 10% abnormal sperms are considered as infertile. Chinoy et.al.,[51] reported the androgen depletion causes reduction in sperm count, sperm motility and maturation of sperm which is highly correlative with our findings. But in high dose treatment (400 mg /kg body weight /day) , the stimulatory effects in sperm motility, sperm viability and reduction of sperm abnormalities are not clear . It may be due to the presence of some active oxidative and antioxidative components in this crude extract which can overcome the extract induced stress in male gonads, as have been reported by different investigators[52,53]. Similar stimulatory effects in higher dose treatment also have been found in others studies [52,54].In the biochemical studies, the reduction of fructose content in ventral prostate and seminal vesicle (table – 5, figure - 5) also further supports the inhibition of androgen production by *J.repens*, because , fructose synthesis in gonads is directly androgen dependent [55,56]. Again , low level of fructose could inhibit the sperm motility by deficient generation of ATP [57]. The SGPT appears in higher concentrations in a number of tissues i.e., liver, kidney,heart and pancreas , where the SGOT is localized primarily in the cytosol of hepatocytes .Again SGOT is considered as more sensitive marker of hepatocellular degeneration than SGPT and within its limits can provide a quantitative assessment of the degree of liver damage [58]. The acid phosphatase enzyme is abundant in the prostate and seminal fluid, it also occurs in significant quantity in spleen, liver, kidney, red cells and bone [59]. The rise in serum levels of SGOT, SGPT, ALP and ACP has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damage [60]. So, in the present investigation, no change in the activities of some toxicological marker enzymes i.e., SGOT, SGPT, ALP and ACP in *J.repens* treated rats showed no toxicity (table-6).In 2007, Saidu *et al*., [61] reported the rise of plasma immunoglobulin concentration in infection and the plasma concentration of proteins may also decrease as a result of over hydration, impaired protein synthesis due to malnutrition, malabsorption, liver disease, hypogammaglobulinaemia or increased protein loss due to renal, gastrointestinal and skin disorders. But in the estimation of total protein in serum (table- 6) showed no significant change indicating no above disorders expressing nontoxicity of the extract in rats . Furthermore , estimation of serum urea and creatinine (table -6) showed no alteration in *J.repens* treated rats leading no nephrotoxicity. As urea is the principal end product of protein catabolism in liver [62]. Again the elevation of blood Urea and creatinine are also the good indicators for kidney disorders [63] . The appearance of creatinine in the serum is proportional to the body's muscle mass and is more readily excreted by the kidney than urea [64]. Whitby et.al., in 1989 [65], reported the inverse relation between serum creatinine level and glomerulus filtration rate . Similar findings also were reported by V,Saritha et.al., [66] in aloevera extract fed rats.

CONCLUSION

So, we may conclude that, the crude aqueous extract of *Jussiaea repens L.,* is antigonadal in a dose and duration dependent manner in male rats when administered orally. Therefore, the aqueous plant extract can be used as safe nontoxic antigonadal agent in the near future.

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