

**ANTI FERTILITY EFFECT OF *ZIZIPHUS JUJUBA MILL.*****Rekha.S *,¹ Chandrashekhara.S,²**

¹Dept. of Pharmaceutical chemistry, T. John College of Pharmacy, Bangalore, Karnataka, India.

²Dept. of Pharmaceutics, M.M College of Pharmacy, Belgaum, Karnataka, India.

Article Received on
01 January 2014,
Revised on 05 February
2014,
Accepted on 02 March 2014

Correspondence for Author*Rekha.S**

Dept. of Pharmaceutical
chemistry, T. John College of
Pharmacy, Bangalore,
Karnataka, India

ABSTRACT

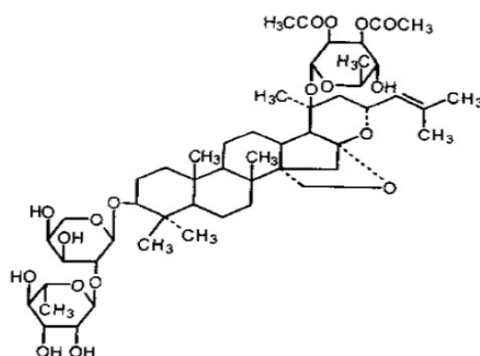
The Petroleum ether extract of *Zizyphus jujuba* leaves (EAZJ, Rhamnaceae) were evaluated for anti-fertility activity in the adult male mouse. This study was designed to assess the effect of EAZJ on sperm morphology, progressive motility and sperm concentration irreversibly in Wistar rats. Extracts were found to produce significant inhibition of sperm motility and cause reduction in viability of sperm cell. Saponin extract is found to be more active to cause immobilization. From this study, it is evident that *Zizyphus jujuba* severely affects male animal fertility parameters. It is clear that its antifertility effects are reversible.

Keywords: *Zizyphus jujuba* Mill, saponin, Antifertility/contraception, Wistar rats.

INTRODUCTION

Population explosion is leading cause of poverty in developing countries. There are several medicinal plants associated with antifertility properties. Fertility regulations with plants or plant preparations have been reported in the ancient literature of indigenous systems of medicine. Great attention is being given to plants with anti-fertility properties. These may act through effects upon sperm motility and viability, implantation of the fertilized egg or a rejection effect within the uterus. The biochemistry of these different pathways is complex, and the study of plants having such effects is revealing new mechanisms all the time. (R.T Mahajan., M.Z Chopda., 2009). According to the World Health Organization, medicinal plants can be the best source to obtain a different variety of drugs. (B Shashank., D Suresh., 2013). Medicinal plants have curative properties due to presence of various complex chemical substances of different composition which contain secondary metabolites such as

alkaloids, flavonoids, terpenoids, saponin and phenolic compounds distributed in different parts of the plants. *Ziziphus jujuba* Mill, a member of the family Rhamnaceae, commonly known as Ber, is used traditionally as tonic and aphrodisiac and sometimes as Hypnotic-sedative and Anxiolytic, anticancer (Melanoma cells), Antifungal, Antibacterial, Antiulcer, Anti-inflammatory, Cognitive, Antispastic, Antifertility/contraception, Hypotensive and Antinephritic, Cardiotonic, Antioxidant, Immunostimulant, and Wound healing properties. (R.T Mahajan., M.Z Chopda., 2009). A bulk of contraceptives on the market is women-oriented today. The ziziphin, a steroidal saponin which is extracted by dried leaves of *Z. jujube* was found to exhibit antifertility/contraception. It has a structure, 3-O-a-L-rhamnopyranosyl (1-2)-a-Larabinopyranosyl- 20-O- (2, 3)-di-O-acetyl-a-L-rhamnopyranosyl jujubogenin. (S.Azam-Ali *et.al*, 2001). The aim of this study was to investigate the effect of a medicinal herb, *Ziziphus jujuba* Mill on various parameters of male fertility using a rat model. (Fanuel Lampiao., 2013).



3-O-a-L-rhamnopyranosyl (1-2)-a-Larabinopyranosyl- 20-O- (2, 3)-di-O-acetyl-a-L-rhamnopyranosyl jujubogenin.

MATERIALS AND METHOD

Plant material and extraction procedure

The leaves of *Ziziphus jujuba* were collected from the forests of Biligiri rangana hills and was identified by Dr. Devaraj (Department of Botany Agriculture University, Bangalore) with voucher specimen no. BOT: 398. The leaves were collected from shade dried, powdered and soxhlated with methanol (50% v/v) at 55-60 °C for 36 h. the solvent was distilled off under petroleum ether, benzene, chloroform and acetone to remove impurities left, if any during extraction. Thus the resulting mass was dried under vacuum and kept at 4 °C.



Animal model (Sachin Jain, Ankit Jain., 2013), (Yinusa Raji, Olumide S.,2005)

The experiments were approved by CPCSEA and the institutional ethics committee proposal. No. IAEC/TJCP/44/10. Sexually mature male Wistar rats (284–430 g body weight) were housed in standard rat cages and maintained under standard conditions (12 *hr* light/dark cycle; $25\pm 3^{\circ}\text{C}$ temperature; 35% – 60% relative humidity), provided with a standard laboratory chow and water ad libitum. The drug and/or vehicle were administered to all animals by oral intubation.

Treatment protocol

Male Wistar rats ($n = 40$) were randomly divided into three groups. One group received *Z. jujuba* while the other acted as controls. Ten animals from each group were sacrificed after 16 weeks. The corresponding recovery groups were also treated for 4 and 13 weeks and allowed to recover for 4 and 13 weeks, respectively. Blood was collected for hormonal analysis. The testis was removed for histological examination, while epididymal spermatozoa were retrieved for motility and morphological analysis.

A suspension of the extract was prepared in sterile distilled water (100 mg/mL) before administration. The required drug was administered orally with a glass syringe fitted with a feeding needle.

Sacrification schedule

At the end of the treatment and recovery periods, Twenty-four hours after their last dose, the rats were weighed and sacrificed under light ether anesthesia.

Sperm density and motility determination

The cauda epididymis was separated and minced using a pair of small scissors, to release the sperm into 10 ml of warmed physiological saline. The sperm suspension was placed in an incubator at 37°C for 10 min prior to total motility and progressive motility assessment. The aliquot of the sperm suspension was further diluted 5 times with warm physiological saline and then placed on Makler counting chamber and motile sperm were counted under a light microscope. Nine microscopic fields were observed per sample and averaged. Progressive and total sperm motility was expressed as a percent of motile sperm of the total sperm count. For sperm count, five counts per sample were made and averaged. Sperm count was expressed as *sperm/ml* of suspension solution.

Sperm morphology

Drops of rat sperm were placed on slides and smears were prepared. The smears were left to air dry before stained by Rapid Diff staining (Australian Biostain, Australia). Briefly, smears were submerged for 6 one-second dips in Rapid Diff fixative. The smears were then dipped six times for one second each in Rapid Diff Stain 1 followed by six dips in Rapid Diff Stain 2. Finally, the slides were rinsed in phosphate buffer (pH = 6.8) and air dried. Morphology was assessed under light microscopy with at least 200 cells assessed per slide.

Testicular histology

The right testes from both control and experimental groups were dissected out and fixed in formal saline. The tissues were processed for histological examination and paraffin sections were stained with hematoxylin and eosin and qualitative microscopic examination was made.

Fertility test

Male rats were introduced to female, 200-300 g (male: female ratio, 1:2) at 17:00 h after 55 days of treatment. The successful mating was confirmed in the forthcoming morning from 56 to 61 day by vaginal plug and spermatozoa in the vaginal smear. The inseminated female were separated and allowed to deliver at term, and the number of pups delivered and their characteristics were noted.

Body and organ weights

The initial and final body weights of the animals were recorded. The testes, epididymides, seminal vesicle and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed to the nearest milligram.

Statistical analysis

Data are expressed as mean \pm S.D. and analyzed for statistical significance by using one way analysis of variance (ANOVA). Results were considered significant at the $P \leq 0.05$ level .

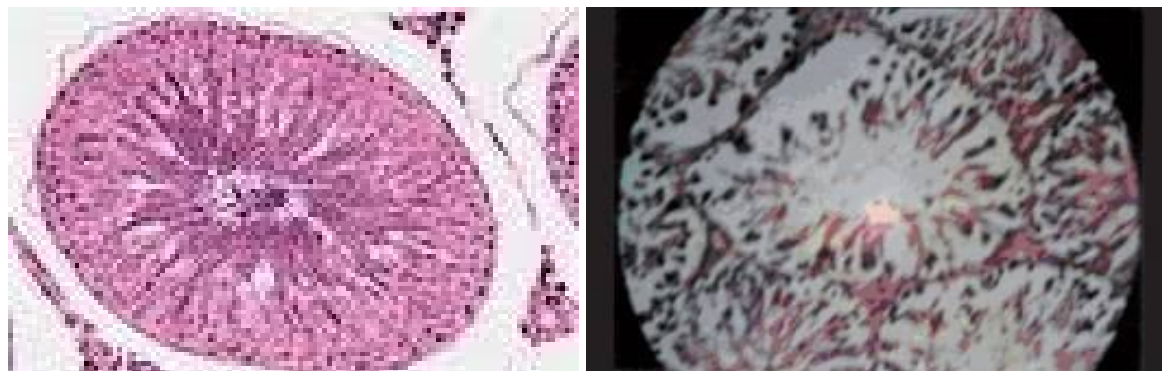


Fig 1 T.S of seminiferous tubules of control rat Fig 2 T.S of seminiferous tubules of *Z.jujuba* leaves treated rat showing less sperm cells

RESULTS

Sperm motility and count

Sperm motility, progressive motility and sperm concentration significantly decreased in treated animals compared to the controls ($p < 0.05$). Withdrawing the treatment restored these parameters ($p < 0.05$). Abnormal sperm morphology significantly increased in both the treated and treatment withdrawn groups when compared to the controls ($p < 0.05$). The significant differences were observed between the controls and the treated animals when treatment was withdrawn. Histological observations showed that *Ziziphus jujuba* treatment disrupted seminiferous tubule architecture and consequently the spermatogenesis process.

Fertility

The number of fertile males decreased in all treatment groups, leaving control and withdrawn group fertile in 60 days of treatment. The ratio between delivered and inseminated female (4/10 for treated, 6/10 for withdrawn versus 10/10 animals in control), and the number of pups (28, 49 versus 85 pups in control) dropped after 60 days of treatment. However, no significant difference was observed in the litter size of the female in any group. Spermatozoa with shortened and thinned flagella were present in the semen found in the vaginal smears of females, which were cohabited with the treated males. All delivered pups were normal healthy (Table 2).

Body and organ weights

The final body weight of all groups increased markedly when compared with their respective

initial body weights ($P \leq 0.01$). The final weights of treated and withdrawn group significantly increased when compared with the final body weight of control animals ($P \leq 0.01$). A great decline in the weights of testes, epididymides, seminal vesicle and ventral prostate (expressed in mg/100 g of body weight) were observed in all treatment groups when compared with control animals (Table 3).

Histopathology of testis

In the testicular histology study, seminiferous tubules of control animals showed clear organization of cells at various stages of spermatogenesis with clear spermatozoa maturation occurring near the lumen (Figure 1). In treated rats, the seminiferous tubules had very few spermatozoa. In some tubules the lumen was filled with debris. Most of the seminiferous tubules were azoospermic (Figure 2).

Table 1 The effect of *Z.jujuba* leaves on total sperm parameters in Wistar rats

	Control (Mean±SEM)	<i>Z.jujuba</i> treated (Mean±SEM)	<i>Z.jujuba</i> withdrawn (Mean±SEM)
Total motility (%)	61.6 ± 3.4% ^a	49 ± 3.96% ^b	50.5 ± 3.2% ^b
Progressive motility (%)	55.4 ± 3.65% ^a	43.1 ± 3.58% ^b	43.24 ± 2.65% ^b
Concentration (millions/ml)	40.8 ± 2.22 × 10 ⁶	32.23 ± 2.63 × 10 ⁶	29.89 ± 2 × 10 ⁶ ^b
Abnormal morphology (%)	29.3 ± 3% ^a	23.44 ± 5% ^b	19.43 ± 2% ^b

^{a,b}Values in rows are means±SEM. Means followed by the same letter in the row do not differ significantly. If the letter in the same row differs from that of the control, then $p < 0.05$

Table 2 Fertility of male rats after 60 days of treatment with 50% methanol extract of *Z.jujuba* leaf (Male: Female ratio, 1: 2.)

Sl. no	Treatment group	No of fertile males/no of treated males	No of female delivered/no of inseminated female	Total no of pops	Litter size±SD
1	Control	5/5	10/10	85	7.75±0.70
2	<i>Z.jujuba</i> treated	2/5	4/10	28	5.50±0.45
3	<i>Z.jujuba</i> withdrawn	3/5	6/10	49	6.25±0.35

n = 5 (male), n = 10 (female), compared with group I

Table 3 Body and organ weights after 60 days of treatment with 50% methanol extract of *Z.jujuba* leaf on male rats.

Sl.no	Treatment group	Body weight (gm)		Testes	Epididymis	Ventral prostate	Seminal vesicle
		Initial	Final				
1	Control	265.0± 8.7	331.0± 16.0 ^b	1790.0± 15	465.0± 9.7	325.0± 7.7	435.0± 86.7
2	<i>Z.jujuba</i> treated	250.9± 10.7	365.0± 48.7 ^{b,c}	1065.0± 148.7 ^c	365.0± 11.7 ^c	165.0± 5.7 ^c	346.0± 198.7 ^c
3	<i>Z.jujuba</i> withdrawn	257.0± 34.7	325.0± 33.3 ^b	1265.0± 108.7 ^c	415.0± 6.7 ^c	136.0± 8.2 ^c	405.0± 77.7 ^c

Data are expressed as mean±S.D.,^a mg/100 g of body weight, ^bP < 0.01 compared with corresponding initial body weight, ^c P < 0.01 compared with corresponding group I.

CONCLUSION

From this study, it is evident that *Z.jujuba* severely affects male animal fertility parameters. It is, however, clear that its antifertility effects are reversible.

REFERENCES

1. RT Mahajan, MZ Chopda. (Phyto-Pharmacology of *Ziziphus jujuba* Mill- A plant review). *Pharmacognosy Review*, 2009; 3(6): 320-329.
2. B Shashank, D Suresh. (Quantification of secondary metabolites from *ziziphus mauritiana* lam. Bark). *Int J Bio-Tech and Res*, 2013; 3(2): 1-6.
3. S Azam-Ali, E Bonkougou, CB Godara, JT Williams. *Ber and other jujubes*. 1sted, University of Southampton; International Centre for Underutilised Crops: 2001, pp.93-113.
4. Fanuel Lampiao. (The Anti-fertility Effects of *Acacia nilotica* in Male Wistar Rats). *J Reprod Infertil*, 2013; 14(1): 39–42.
5. J Sachin, J Ankit, P Pritesh, SS Shailendra. (The Anti-fertility Effects of *Acacia nilotica* in female Wistar Rats). *As Pac J Trop Med*, 2012; 3(1): 547-551.
6. R Yinusa, S Olumide, Akinsomisoye, M Toyin, Salman.(Antispermato-genic activity of *Morinda lucida* extract in male rats). *As J Androl*, 2005; 7 (4): 405–410.

7. AMM San, S Thongpraditchote, P Sithisarn, W Gritsanapan. (Total phenolics and total flavonoids contents and hypnotic effect in mice of *Ziziphus mauritiana* Lam. seed extract). *Evid bas Comp and Alt Med*, 2013; 4(1): 835-844.
8. X Lin, L Yao, Y Li, RWu, X Pang. (Identification of genes associated with fruit ripening in *Ziziphus jujuba* using suppression subtractive hybridization approach). *Acta Phys Plant*, 2013; 35(6): 1997-2008
9. EJ Seo, SY Lee, SS Kang, YS Jung. (*Zizyphus jujuba* and its active component jujuboside B inhibit platelet aggregation). *Phytotherapy Res*. 2013; 27(6): 829-834
10. A Balakrishnan, B Pamaiah, B. (Hypoglycemic and hypolipidemic effects of *zizyphus jujuba* lam. In streptozotocin-induced diabetic rats). *Res J of Pharma, Bio and Chem Sci*. 2013; 4(2): 611-616.
11. SY Lee, JS Kim, JH Lee, YS Kim, SS Kang. (A new saponin from the seeds of *Zizyphus jujuba* var. *spinosa*). *Bul of the Korean Chem Soc*. 2013; 34(2): 657-660.
12. ZN Cruz, P Rodr guez, P, A Galindo, E Torrecillas, S Ondo o, CDMellisho, Torrecillas. (A Leaf mechanisms for drought resistance in *Zizyphus jujuba* trees Cruz). *Plant Sci*. 2012; 197: 77-83
13. S Das. (Antimicrobial and antioxidant activities of green and ripe fruits of *Averrhoa carambola* Linn. and *Zizyphus mauritiana* Lam). *Asi J Pharma and Clin Res*.2012; 5(3): 102-105.
14. S Ebrahimi, S Ashkani-Esfahani, A Poormahmudi. (Investigating the efficacy of *Zizyphus Jujuba* on neonatal jaundice). *Iran J of Pedia*. 2011; 21(3): 320-324.
15. A Shanmugavasan, KSR Vaitheeswaran, T Ramachandran. (Design and development of pyrolyser to extract medicinal oil from the stem of *Ziziphus jujube*). *J of Anal and App Pyrolysis*.2011; 92(1): 176-183.