Available online on 25.04.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Sperm Immobilization Potential of Saponin Extract of *Ziziphus mauritiana*

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ABSTRACT

The contraceptive potential of Saponin extract of *Ziziphus mauritiana* is evaluated by human sperm immobilization assay. The leaves of *Ziziphus mauritiana* were subjected to successive solvent extraction. The dried methanolic extract was further solvent extracted with water saturated n-butanol and both the layers were separated. The organic layer was acidified with 1 N KOH to obtain the raw saponin extract. The extract was screened for spermicidal activity against human spermatozoa. The immobilization assay was performed on human ejaculate in 1:1 ratio according to modified waller method. Concentration showing motility inhibition was subjected to sperm viability assay using bakers medium. The sperm cell plasma membrane integrity study was done using hypo-osmotic swelling (HOS) test. The saponin extract at 0.1mg/ml & 0.5mg/ml concentration immobilize 80.68% to 100% and none of the spermatozoa recovered their motility in revival assay. The decrease in sperm viability was observed in range 35.6-56.68%. Significant morphological changes were observed under phase contrast microscope. The present study has pointed out that saponin extract shows good human spermatozoa immobilization capacity at concentration 0.5mg/ml. The damage to the sperm membrane architecture and impairment of functional integrity of the plasma membrane was evidenced by significant reduction in sperm viability and tail curling.

Article Info: Received 28 Feb 2019; Review Completed 30 March 2019; Accepted 19 April 2019; Available online 25 April 2019

Cite this article as:

Dubey R, Dubey K, Sperm Immobilization Potential of Saponin Extract of *Ziziphus mauritiana*, Journal of Drug Delivery and Therapeutics. 2019; 9(2-A):78-80

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INTRODUCTION

Population is a global problem which will grave implications related to food, water, healthcare, education, jobs etc. in developing countries. Numbers of medicinal plants been reported to possess contraceptive property some of which posses antifertility activity and they acted either by preventing implantation or by suppressing spermatogenesis. The commonly available synthetic agent is nonoxynol-9. [1-3]

Number of plant derived spermicides have been evaluated and were found to be triterpene saponins which probably causes instant immobilization of human spermatozoa within 20 seconds. [4-7].

Ziziphus mauritiana fast growing spiny shrub or tree consist of saponins as important major constitutent in almost all parts. [8-18] In the present work an attempt is made for the evaluating the sperm immobilization activity of the saponin extract of *Zizyphus mauritiana* Leaves.

MATERIALS AND METHODS

Test Materials

The Leaves of Ziziphus Mauritiana[10-12] were collected, authenticated, dried, powdered and stored in an air-tight container. Human ejaculates were obtained from Asian Institute of Infertility Management, Indore. The Borosil & Asgi glassware's and Chemicals from Sdfine, Loba Chem, HiMedia Lab were used.

Preparation of plant extract

The dried leaves were screened with 40 mesh sieve and soxhlet extracted with petroleum ether followed with 70% methanol and distilled water for 48hr under reflux condition. The methanolic extract is dried and solvent extracted by adding water-saturated n-butanol (1:1v/v).The aqueous phase and n-butanol phase was separated and organic phase was treated with 1M KOH solution. The raw precipitates of saponin was obtained, which was removed and evaporated to dryness. The extract was screened for phytochemical analysis. [10-15]

Immobilization assay

Three human ejaculates samples with routine semen analysis counting sperm >300 million/mL and viability >60 % with normal morphology, rapid and progressive motility was employed for the tests.

The saponin extract of concentration 0.1mg/ml and 0.5mg/ml was prepared in pysiological saline solution and as per modified Waller method were mixed with human ejaculate (>300 million/mL) thoroughly in 1:1 ratio. Drop of

the mixture was placed immediately on a pre-warmed slide and five fields were microscopically observed under high power (×400) for assessment of sperm motility at time interval of 20 Sec and 2 min. Physiological saline and sperm diluents (Formaldehyde) added to semen in 1:1 ratio served as control and standard respectively. The sample showing motility inhibitions were subjected to sperm revival test by incubating at 37°C for 30 min with Bakers Medium. [10, 14, 19-25]

Sperm HOS & Viability Analysis

The assessment of plasma membrane functional integrity was done by hypo-osmotic swelling (HOS) tests according to WHO. The human ejaculated sperm were mixed separately with extract at a ratio of 1:1 and incubated for 30 min at 37°c. Similarly sperm samples mixed with saline solution was served as the controls. About 0.1 mL of above sample was mixed thoroughly with 1mL of HOS medium (1.47 % fructose and 2.7 % sodium citrate at 1:1 ratio) and incubated for 30 minutes at 37°c and the inflamed curling tails were examined under phase contrast microscope using ×100 magnification. For sperm viability test a drop of well mixed sperm sample was remixed thoroughly with eosin Y dye and was dropped onto a glass slide and observed under ×400 magnification. [10, 14, 26-28]

RESULTS

Sperm immobilization

The saponin extract at 0.1mg/ml and 0.5mg/ml concentration was able to immobilize 80.68% to 100% of the human spermatozoa instantly at 1:1 ratio. None of the spermatozoa, once immobilized, recovered their motility following removal of plant extracts and 30 minutes incubation with physiological saline, showed in Table 1.

Sperm HOS & Viability test

The significant decrease in sperm viability was observed i.e. reduction of 35.6-56.58% for saponin extract which indicated the spermicidal property of Extracts. (Table 1). Human spermatozoa showed typical morphological changes when subjected to hypo-osmotic shock. These changes were clearly visible by phase contrast microscopy. The controls showed the maximum amount of tail curling, while in extract treated spermatozoa, tail curling was significantly reduced (P<0.001), indicating the impairment of functional integrity of the plasma membrane. (Table 1).

Table 1: Sperm Immobilization and Viability test of Saponin extract of Ziziphus Mauritiana

Concentration	0.1mg/ml		0.5mg/ml		Solvent	Standard
	20 Sec	2Min	20 Sec	2Min	0-2min	0-2min
%M	19.318±0.31	0.207±0.20	7.537±0.28	0	74.73±1.80	0
% Im	80.68±0.31	99.79±0.20	92.46±0.28	100	25.26±1.80	100%
% IIm	55.42±1.74	74.52±1.628	67.19±1.629	74.73±1.80	-	-
% SV	47.25±0.433	37.25±0.28	35.16±0.30	26.33±0.441	82.91±0.464	-
%RSV	35.66±0.682	47.75±0.381	45.66±0.506	56.58±0.870	-	-
% HOS	34.58±0.220 (1%)		26.50±0.50 (2%)		84.75±0.520	
%RHOS	50.16±0.363 (1%)		59.08±0.961(2%)			

% M: Percentage Motility, % Im: Percentage Inhibition in Motility, %IIm: Increase in Percentage Inhibition in Motility, % SV: Sperm Viability, %RSV: Reduction in Percentage Sperm Viability, % HOS: Percentage Hypersomotic Swelling, %RHOS: Reduced Percentage Hypersomotic Swelling, %Mean of three replicates±SEM

CONCLUSION

The present study pointed out that saponin extract of *Ziziphus Mauritiana* is very potent spermicidial and have showed good human spermatozoa immobilize capacity at a concentration of 0.5 g/mL. It was concluded from the result that the damage to the membrane architecture was evidenced by the significant reduction in sperm viability and tail curling. The result also indicated the lost of plasma membrane integrity of sperm cells which will surely reduce the ability of the sperm cells to induce acrosome reaction and fertilization.

REFERENCES

1. Verma PK, Sharma A, Mathur A, Sharma P, Gupta RS, Joshi SC. Effect of *Sarcostemma acidum* stem extract on spermatogenesis in male albino rats. Asian Journal of Andrology, 2002; 4: 43-7.
2. Weir SS, Roddy RE, Zekeng L, Feldblum PJ. Nonoxynol-9 use, genital ulcers, and HIV infection in a cohort of sex workers. Genitourinary Medicine, 1995; 71: 78-81.
3. Fichorova RN, Tucker LD, Anderson DJ. The molecular basis of nonxynol-9-induced vaginal inflammation and its possible relevance to human immunodeficiency virus type 1 transmission. Journal of Infectious Disease, 2001; 184: 418-28.
4. Farnsworth NR, Waller DP. Current status of plant products reported to inhibit sperm. In: Zatuchni GI, editor. Research frontiers in fertility regulation, 1982; 2: 1-16.
5. Primorac M, Sekulovic D, Antonic S. *In vitro* determination of the spermicidal activity of plant saponins. Pharmazie, 1985; 40: 585.
6. Lohiya NK, Kothari LK, Manivannan B, Mishra PK, Pathak N. Human sperm immobilization effect of *Carica papaya* seed extracts: an *in vitro* study. Asian Journal of Andrology, 2000; 2: 103-9.
7. Delgado NM. Effects of a purified fraction from *Echeveria gibbiflora* aqueous crude extract on guinea-pig spermatozoa. Phytotheraphy Research, 1999; 13: 46-9.
8. Dubey R, Dubey K, Janapati YK, Shridhar C, Jayveera KN. Comparative anti microbial studies of aqueous, methanolic and saponins extract of seeds of Trigonella Foenum-Graecum on human vaginal pathogens causing UTI infection. Der Pharma Chemica, 2010; 2(5): 84-88
9. Dubey R, Dubey K, Gupta R, Gupta A. Antidiabetic potential of aqueous methanolic and saponin extract of Ziziphus nummlaria Linn. , Journal of Drug Delivery and Therapeutics, 2017; 7(7): 173-174.
10. Dubey R, Dubey K, Shridhar C, Jayveera KN. Sperm immobilization activity of aqueous, methanolic and saponins extract of bark of Ziziphus Mauritiana. Der pharma Sinica, 2011; 2(2): 11-16.
11. Dubey R, Dubey K, Shridhar C, Jayveera KN. Human Vaginal Pathogen Inhibition Studies on aqueous, methanolic and Saponins extracts of stem barks of Ziziphus Mauritiana. International Journal of Pharmaceutical Sciences Research, 2011; 2 (3): 659-663.
12. Dubey R, Dubey K, Janapati YK, Shridhar C, Jayveera KN. Anti-microbial studies of aqueous, methanolic and saponins extract of leaf of Ziziphus mauritiana on human vaginal pathogens

causing UTI infection. International Journal Pharmceutical and Life Sciences, 2010; 1(2):77-81
13. Dubey R, Dubey K, Dwivedi S, Janapati YK, Shridhar C, Jayveera KN. Standardization leaves of Ziziphus nummularia Linn. - An effective herb for UTI infections, International Journal Drug Discovery and Herbal Research, 2011; 1: 5-7
14. Dubey R, Dubey K, Shridhar C, Jayveera KN. Spermicidal Potential of aqueous, methanolic and saponins extract of bark of Ziziphus Nummlaria. Ethanopharmacology, 2012; 3(12): 612-614.
15. Liu MJ, Cheng CY. A taxonomic study of the genus Ziziphus. Acta Horticulturae, 1995; 390: 161-165.
16. Singh JP, Singh IS. Some promising varieties of ber. Indian Horticulture, 1973; 18(2): 3-28.
17. Srivastava SK, Srivastava SD. Structure of Zizogenin, a new sapogenin from Ziziphus mauritiana. Phytochemistry, 1979; 18(10): 1758-1759.
18. Huang RL, Wang W-Y, Kuob Y-H, Lina Y-L. Cytotoxic Triterpenes from the Fruit of Ziziphus jujube, The Chinese Pharmaceutical Journal, 2001; 53: 179-184.
19. Qian SZ, Wang ZG. Gossypol: a potential antifertility agent for males. Annual Review of Pharmacology and Toxicology, 1984; 24: 329-60.
20. Waller DP, Zaneveld LJD, Fong HHS. *In vitro* spermicidal activity of Gossypol. Contraception, 1980; 2: 183-7.
21. Ratnasooriya WD, Amarasekera AS, Perera NS, Premkumara GA. Sperm antimotility properties of a seed extract of Abrus precatorius. Journal of Ethnopharmacology, 1991; 33: 85-90.
22. Suttiyotin P, Thwaites CJ. Evaluation of ram semen motility by as swim up technique. Journal of Reproduction and Fertility, 1993; 97: 339-45.
23. Qian YX, Shen PJ. Spermicidal effect in vitro by the active principle of garlic. Contraception, 1986; 34: 295-302.
24. WHO Laboratory manual for the examination of human semen and sperm cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999.
25. Eliasson R, Treichl L. Supravital staining of human spermatozoa. Fertility and Sterility, 1971; 22: 134-137.
26. Jeyendran RS, Van der ven HW, Perez Pelaez M, Carbo BG, Zaneveld LJD. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. Journal of Reproduction and Fertility, 1984; 70:219-28.
27. Schill WB, Wolff HH. Ultrastructure of human spermatozoa in the presence of the spermicide nonoxynol-9. Andrologia, 1981; 13: 42-9.
28. Wilborn WH, Mahn DW, Mcguire JJ. Scanning electron microscopy of human soermatozoa after incubation with the spermicide nonoxynol-9. Fertility and Sterility, 1983; 39:717-9.