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Sperm immobilization activity of aqueous, methanolic and saponins extract of bark of *Ziziphus Mauritiana*

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ABSTRACT

The aqueous, methanolic and saponin extracts of Zizyphus mauritiana bark were screened for spermicidal activities against human spermatozoa. Extracts were found to produce significant inhibition of sperm motility and cause reduction in viability of sperm cell. Reduction in Hypo-osmotic Swelling (HOS test) indicates the possibility of plasma membrane disintegration. Saponin extract is found to be more active to cause immobilization then aqueous and methanolic extract.

Key Words: Ziziphus Species, Sperm Immobilization, Human Spermatozoa, Saponin.

INTRODUCTION

Numbers of Indian medicinal plants were reported to possess antifertility property. They acted either by preventing implantation or by suppressing spermatogenesis. [1,2] The most potent spermicidal agent presently available in the market was a formulation of nonoxynol-9, however the product had been observed to cause inflammation and genital ulceration and thereby increased the risk of HIV-1 infection on repeated use. [3,4]

Majority of plant-derived spermicides were triterpene saponins of several structural types, flavonoids and phenol compounds. [5] The saponins of *Cyclomen persicum, Primula vulgaris* and *Gypsophyla paniculata* have been reported to cause almost instant immobilization of human spermatozoa within 20 seconds. [6] *Carica papaya* seed extract has also been shown to possess *in vitro* sperm immobilizing effect in human spermatozoa.[7] The purified fraction from the aqueous

crude extract of *Echeveria gibbiflora* had sperm immobilizing activity as well as strong agglutinating property in guinea-pig spermatozoa. [8]

Ziziphus belongs to the kingdom; plantae, order; roasles, division; magnoliophyta, class; magnoliopsida, family; rhamnaceae, genus; ziziphus. *Z. mauritiana* is a fast growing small to medium-sized, single or multi-stemmed, spiny shrub or tree, which is almost evergreen, but is deciduous during the dry season. It can reach up to 12 m tall and 30 cm diameter at breast height, but is highly variable in size and general appearance. The bark is dark grey, dull black or reddish with long vertical fissures, reddish and fibrous inside. The branches are spreading and droop at the ends. Stipules are mostly spines, in pairs with one hooked and one straight, or both hooked. [9-15]

The present paper here reported for the first time the sperm immobilization activity of the saponin, methanolic and aqueous extract of *Zizyphus nummularia* (Z. Rotundifolia) Barks.

MATERIALS AND METHODS

Test Materials

Bark of Ziziphus nummularia were collected from Malwa region of Madhya Pradesh in the month of Feb-March, 2007 and were identified by the Botany Department, Janata PG College, A.P.S. University, Rewa (M.P.). The bark were later air-dried, powdered and stored in an air-tight container for further use.

Human ejaculates were obtained from Asian Institute of Infertility Management and Test-tube Baby Centre, Indore, India. Chemicals were purchased from HiMedia Lab.

Preparation of sperm suspension

Human ejaculates (n=3) samples, from normal subjects after 05day of sexual abstinence were subjected to routine semen analysis following liquefaction at 37^{o}C.Sperm count above 300 million/mL and viability above 60 % with normal morphology, rapid and progressive motility was employed for the tests.

Preparation of plant extract

Bark were shattered and screened with 40 mesh. It was soxhlet extracted three times with petroleum ether for 4hr at 60^{o}C. After drying and levigation, one part of the residues were inverse flow extracted 10 times with 70% methanol for 4hr at 85^{o}C, then were filtrated and the other part was extracted with distilled water for 48hr under reflux condition. The alcohol solution (Filtrate) was evaporated to dryness and dissolved with water. After filteration and discarding the extraneous components, the solution was extracted by adding water-saturated n-butanol (1:1v/v), the n-butanol phase was then treated by 1N HCl, aqueous phase was removed. The n-butanol phase evaporated to dryness under pressure and the raw saponin was obtained. All extracts were screened for phytochemical analysis.

Immobilization assay

All Extracts of concentration 0.1mg/ml and 0.5mg/ml were prepared in pysiological saline solution and were mixed with human ejaculate (>300 million/mL) thoroughly in 1:1 ratio

according to a modified method of Waller.[16-21]A drop of the mixture was placed immediately on a pre-warmed slide and at least five fields were microscopically observed under high power (×400) for assessment of sperm motility at time interval of 20 Sec and 2 min. The drug extract showing 100% inhibition of motility within 20 Sec was considered as effective spermicidal. Physiological saline and sperm diluents (Formaldehyde) added to semen in 1:1 ratio served as control and standard respectively.

Sperm revival test

The samples showing motility inhibition by the test drug extract were subjected to this test. The sperms with inhibited motility were washed twice in physiological saline and incubated at 37^{o}C for 30 min with Bakers Medium. If reversal of motility in any sperm in test sample is observed the extract was considered to be failed for having spermicidal activity.

Assessment of plasma membrane integrity

Hypo-osmotic swelling (HOS) tests were done according to WHO [21], Eliasson & Treichl [22] and Jeyendran *et al*. [23] for assessing plasma membrane functional integrity. Human ejaculated sperm (>300 million/mL) were mixed separately with extracts at concentration 0.1mg/ml and 0.5mg/ml at a ratio of 1:1 and incubated for 30 min at 37^{o}c . Similarly, sperm samples in saline served as the controls. In HOS test 0.1 mL of aliquot was taken from each of the treated and control sample, mixed thoroughly with 1mL of HOS medium (1.47 % fructose and 2.7 % sodium citrate at 1:1 ratio), incubated for 30 minutes at 37^{o}c and the inflamed curling tails were examined under phase contrast microscope using ×100 magnification.

Analysis of sperm viability

Sperm viability test was also done according to WHO [21], Eliasson & Treichl [22] and Jeyendran *et al*. [23]. Human ejaculated sperm (>300 million/mL) were mixed separately with extracts at the concentration 0.1mg/ml and 0.5mg/ml at a ratio of 1:1 and incubated for 30 min at 37^{o}c. Similarly, sperm samples in saline served as the controls. For viability assessment one drop each of 0.5 % aqueous solution of eosin Y and. A drop of well mixed sperm sample was added to it and mixed thoroughly. The mixture was dropped onto a glass slide and observed under ×400 magnification.

Statistical analysis

Data were expressed in mean±SEM. Student's *t*-test was employed for statistical comparison.

RESULTS

Sperm immobilization

The saponin extracts at 0.1mg/ml and 0.5mg/ml concentration was able to immobilize 58.04% to 87.27% of the human spermatozoa instantly at 1:1 ratio. Methanolic and aqueous extract causes 42.39% to 72.95% inhibition in sperm motility at 0.1mg/ml and 0.5mg/ml concentration (Table 1).

Sperm revival test

None of the spermatozoa, once immobilized, recovered their motility following removal of plant extracts and 30 minutes incubation with physiological saline.

Sperm Viability

The significant decrease in sperm viability (reduction of 16-34.3% for saponin extract, 12.3-29.9% for alcoholic extract and 9.4-22.75% for aqueous extract) on treatment with the extract indicated the spermicidal property of Extracts. (Table 2).

Sperm membrane integrity

Human spermatozoa showed typical morphological changes when subjected to hypo-osmotic shock. These changes were clearly visible by phase contrast microscopy. In our experiment, 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 3).

Table 1 Sperm Immobilization by different extracts

Concentration		0.1mg/ml		0.5mg/ml		Solvent	Standard
		20 Sec	2Min	20 Sec	2Min	0-2min	0-2min
SE	%M	41.59±1.83	20.63±1.948	24.70±1.67	12.72±0.23	77.75±1.911	0
	% Im	58.04±1.83	79.36±1.948	75.29±1.67	87.27±0.23	22.24±1.911	100%
	% IIm	36.15±0.245	57.12±0.145	53.04±0.396	65.02±1.67	-	-
ME	M%	49.83±0.55	33.25±0.327	41.38±0.280	27.04±0.296	77.46±1.74	0
	Im%	50.16±0.55	66.74±0.327	58.61±0.280	72.95±0.29	22.53±1.74	100%
	IIM%	27.63±1.25	44.21±1.467	36.07±1.694	50.42±1.580	-	-
AE	M%	57.60±0.60	41.24±0.28	45.98±0.66	33.24±0.882	77.31±0.79	0
	Im%	42.39±0.60	58.75±0.28	54.01±0.66	66.75±0.882	22.68±0.79	100%
	IIM%	19.71±0.325	36.07±0.70	31.33±0.294	44.07±0.509	-	-

ME: Methanolic extract; AE: Aqueous Extract; SE: Saponin Extract
% M: Percentage Motility, % Im: Percentage Inhibition in Motility
%IIm: Increase in Percentage Inhibition in Motility
%Mean of three replicates±SEM

Table 2 Percentage Reduction in Sperm Plasma Membrane Integrity by viability test

Concentration			0.1mg/ml	0.5mg/ml	Control
SE	% SV	20 Sec	68.75±0.577	61.25±0.2887	84.75±0.9014
		2 min	58.75±0.520	50.41±0.5833	
	%RSV	20 Sec	16.0±1.181	23.5±0.629	
		2 min	26.0±1.040	34.33±0.333	
ME	% SV	20 Sec	73.08±0.5069	66.25±0.433	85.41±0.3632
		2 min	64.33±0.220	55.5±0.520	
	%RSV	20 Sec	12.3.3±0.16	19.16± 0.08	
		2 min	21.083±0.583	29.91±0.381	
AE	% SV	20 Sec	76.0±0.2887	69.50±0.2887	85.41±0.441
		2 min	67.58±0.3632	62.66±0.441	
	%RSV	20 Sec	9.41±0.726	15.91±0.33	
		2 min	17.83±0.6821	22.75±0.577	

ME: Methanolic extract; AE: Aqueous Extract; SE: Saponin Extract
% SV: Sperm Viibility, % Im: Percentage Inhibition in Motility
%RSV: Reduction in Percentage Sperm Viability
%Mean of three replicates±SEM

Table 3 Hypo-Osmotic Swelling (HOS) tests reading under Phase contrast Microscope

Concentration		1%	2%	Con
SE	% HOS	56.08±0.441	44.08±0.794	83.16±0.939
	%RHOS	27.08±0.712	39.083±1.54	-
ME	% HOS	59.91±0.650	51.08±0.726	83.66±0.87
	%RHOS	23.75±0.7637	32.58±1.46	-
AE	% HOS	66.58±0.794	58.33±0.506	83.83±0.506
	%RHOS	17.25±1.299	25.16±0.166	-

% HOS: Percentage Hypersomotic Swelling,
%RHOS: Reduced Percentage Hypersomotic Swelling
%Mean of three replicates±SEM
Met: Methanolic extract; AE: Aqueous Extract; SE: Saponin Extract; Con: Control

DISCUSSION

Saponin extract was shown to be the most active. The present study pointed out that at a concentration of 0.5 g/mL extracts shows good human spermatozoa immobilize capicity.

Most of plant spermicidal compounds act on the sperm surface, disrupting the plasma membrane. The currently used active principle of vaginal spermicide, nonoxynol-9, acted in a similar manner. It produced disruption of lipids within the sperm membrane, particularly, on the acrosome and mid-piece causing rapid loss of sperm motility. [4-6], [24-25]

In the present study the damage to the membrane architecture was evidenced by the significant reduction in sperm viability and tail curling. A property of the cell membrane was its ability to permit the transport of molecules selectively. This is not only essential for the maintenance of sperm motility, but also for the induction of the acrosome reaction and possibly other key events related to fertilization. On incubation of motile sperm with extract this general property of the plasma membrane was lost.

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REFERENCES

[1] Chaudhury RR. Plants with possible antifertility activity. Published by Indian Council of Medical Research **1966**; Special Report Series No. 55.
[2] Verma PK, Sharma A, Mathur A, Sharma P, Gupta RS, Joshi SC, *et al*. *Asian J Androl* **2002;** 4: 43-7.
[3] Weir SS, Roddy RE, Zekeng L, Feldblum PJ. *Genitourin Med* **1995**; 71: 78-81.
[4] Fichorova RN, Tucker LD, Anderson DJ. *J Infect Dis* **2001**; 184: 418-28.
[5] Farnsworth NR, Waller DP. *Research frontiers in fertility regulation* **1982**; 2: 1-16.
[6] Primorac M, Sekulovic D, Antonic S. *Pharmazie* **1985**; 40: 585.
[7] Lohiya NK, Kothari LK, Manivannan B, Mishra PK, Pathak N. *Asian J Androl* **2000**; 2: 103-9.
[8] Delgado NM. *Phytother Res* **1999**; 13: 46-9.

[9] Liu, M. J.; Cheng, C. Y. ***Acta Hortic.*** **1995**, 390, 161-165.
[10] Bhansali, A. K. Monographic Study of the family Rhamnaceae of India. Ph.D. thesis, University of Jodhpur, India, **1975**
[11] Singh, J. P.; Singh, I.S. *Indian Hortic.* **1973**, 18(2), 3-28.
[12] Jawanda, J. S.; Bal, J. S.; Josan, J. S.; Mann, S. S. *Punjab Hortic. Journal* **1981**, 21, 17-22.
[13] Srivastava, S. K.; Srivastava, S. D. *Phytochemistry* **1979**, 18(10), 1758-1759.
[14] Huang, R.L.; Wang, W-Y.; Kuob, Y-H.; Lina, Y-L. *The Chinese Pharmaceutical Journal* **2001**, 53, 179-184.
[15] Jiang, J-G.; Huang, X-J.; Chen, J.; Lin, Q-S. *Natural Product Research* **2007**, 21(4), 310-320.
[16] Qian SZ, Wang ZG. *Annual Rev Pharmacol Toxicol* **1984;** 24: 329-60.
[17] Waller DP, Zaneveld LJD, Fong HHS. *Contraception* **1980;** 2: 183-7.
[18] Ratnasooriya WD, Amarasekera AS, Perera NS, Premkumara GA. *J Ethnopharmacol* **1991;** 33: 85-90.
[19] Suttiyotin P, Thwaites CJ. *J Reprod Fertil* **1993;** 97: 339-45.
[20] Qian YX, Shen PJ. *Contraception* **1986;** 34: 295-302.
[21] WHO Laboratory manual for the examination of human semen and sperm cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; **1999.**
[22] Eliasson R, Treichl L. *Fertil Steril* **1971**; 22: 134-7.
[23] Jeyendran RS, Van der ven HW, Perez Pelaez M, Carbo BG, Zaneveld LJD. *J Reprod Fert* **1984**; 70:219-28.
[24] Schill WB, Wolff HH. *Andrologia* **1981**; 13: 42-9.
[25] Wilborn WH, Mahn DW, Mcguire JJ. *Fertil Steril* **1983;** 39: 717-9.