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HUMAN SPERM IMMOBILIZATION ACTIVITY OF CRUDE EXTRACT OF *SPONGIONELLA* SP. (A MARINE SPONGE).

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Abstract

The goal of this study was to investigate the effects of crude extract of a Sri Lankan marine sponge, *Spongionella* sp. on the motility of human spermatozoa *in vitro*. The solvent system used for the extraction was dichloromethane-methanol (1 : 1) and the concentrations tested were 3, 6, 12.5, 25 and 125 mg/ml The period of study was 60 min. The results show that the extract inhibited motility of sperm both in a dose - and time - dependent manner. Furthermore at higher concentrations, the extract caused complete immobilization of sperm. Accompanied with the impairment of motility there was also a curling of sperm tails and an agglutination of sperm heads.

Key Words : *Spongionella* sp (marine sponge). human spermatozoa, sperm motility.

1. Introduction

Marine sponges, a group of invertebrates, belonging to the phylum Porifera, have an unusual ability to biosynthesise organic compounds, especially long chain fatty acids, with amazing ease (Carballeira *et al.*, 1990). Extracts prepared from various marine sponges have been shown to possess a formidable array of potent bioactivities such as antibacterial (Jimenez *et al.*, 1990), anti fungal (Gunasekera *et al.*, 1990), antimalarial (Anger-hofer *et al.*, 1992) anti-inflammatory (Chan *et al.*, 1992), cytotoxic (Gunasekera *et al.*, 1990) antitumor (Sakemi *et al.*, 1987) or inhibition of thrombin (Fuestani *et al.*, 1990).

Sponges are a natural source of steroidal glycosides (Hirota *et al.*, 1990). We have recently shown that a number of alcyonacean soft corals possesse potent spermatostatic activity (Liyanage *et al.*, 1986) and one of the compounds

responsible for this activity was a novel steroidal glycoside (Tillekeratne *et al.*, 1989). In view of high steroidal glycoside content in sponges it is possible that they too possess potent sperm immobilization activity and be a rich natural source for the development of potential vaginal contraceptives for the future.

With this main goal in mind we investigated the effects of few Sri Lankan marine sponges on the motility of human spermatozoa *in vitro*. This study reports, for the first time, sperm immobilization activity of an extract prepared from a Sri Lankan marine sponge, *Spongionella* sp.

2. Materials and methods

Specimens of *Spongionella* sp. were collected from Galle harbour (situated in the West Coast of Sri Lanka), at a depth of 3 - 10 metres by snorkel diving. Voucher specimens were preserved in methanol and was deposited in the museum of the Department of Zoology, University of Colombo (code No : 63) and the rest were chopped into small pieces (approximate size $3 \times 3 \times 3$ cm) and stored in polyethylene vessels containing dichloromethane - methano, (1 : 1) solvent system. Following 1-2 months storage period, the organic phase was decanted off and dehydrated by adding anhydrous sodim sulphate (2.0 g/l). This was filtered and concentrated under negative pressure at 28 ± 2°C to obtain the crude extract. The last traces of the solvents were removed by storing the crude extract *in vaccuo* overnight at room temperature (28 - 30°C). This solvent free extract prepared in this manner was stored at -10°C until use.

For testing the sperm antimotility effects the crude extract was dissolved in absolute ethanol and mixed with an equal volume of ethanolic solution of polyvinylprodione (PVP) (1 : 1.5, crude extract : PVP by weight). The resulting mixture was gradually evaporated under negative pressure at 28 ± 2°C to dryness. The co-precipitate formed was dissolved in isotonic saline solution (0.9% NaCl, W/V). The concentrations of the stock solutions made for the evaluation of sperm antimotility activity were 6, 12, 25, 50 and 250 mg/ml.

Semen samples were obtained from healthy adult volunteers (age: 20-35 yrs.) by self masturbation following at least 3-5 days of sexual abstinence Non was on any medication and they denied drug addiction. These samples were allowed to liquify at room temperature (30-32°C) for 15-20 min. and then were subjected to basic semen analyses according to the protocol laid down by the World Health Organization (WHO, 1987). The samples were thoroughly mixed with a Pasteur pipette to assure uniform distribution of sperm before removing an aliquot for analysis. All ejaculates selected (n=6) had a volume$>$ 1.5 ml ; sperm concentration $> 40 \times 10^6$ spermatozoa/ml ; motility of$>$ 50% ; and normal morphologu$>$ 60%. Spermatozoa were, separated from the seminal plasma by three cycles of centrifugation (500g for 5 min) is 8 ml volumes of medium BWW (Biggers *et al.*, 1977). The sperm concentration selected for the evaluation of antimotility effects of the extract was 20×10^6 spermatozoa/ml.

500ul of washed spermatazoa in BWW was placed in Falcon tubes and equal volume of vehicle (a solution containing 375 mg/ml of PVP in isotonic saline) or crude extract was added (final concentration : 3, 6, 12.5, 25 and 125 mg/ml), mixed well and the time was recorded (0 min). These sperm suspensions were then incubated for 60 min at room temperature (30 — 32°C) and 10 ul aliquots were transferred (at 0, + 5, + 15, + 30 and + 60 min onto separate clean glass slides (at 31°C) and covered immediately with a clean cover slip (22×22 mm).

The percentage motile spermatozoa (WHO, 1987) was counted (at least 100 cells) with each concentration and time point under phase contrast optics (× 400) using a squared grid (10 × 10 cm ; Fiscons Scientific Equipment Loughborough, UK). The external appearance of the spermatozoa was also noted.

The data are represented as means ± SEM. Statistical comparisons were made using Student's t-test. Level of Significance was fixed at $p < 0.05$.

3. Results

Data obtained are depicted in Table 1. Over the study period, the motility of the vehicle treated samples varied between 68.6% and 86%. The crude extract significantly ($p<0.05$) impaired the motility of sperm at all concentrations and time points except with the lowest concentration at 0 min. At the highest concentration (125 mg/ml) motility was totally inhbited instantaneously (from 0 min). Intermediate concentrations (25, 12.5 and 6 mg/ml) completely abolished motility of sperm only after 15 min of incubation. On the other hand, with the lowest concentration (3 mg/ml), complete immobilization of sperm was evident following a 30 min incubation period. Further more, at 25 and 125 mg/ml concentrations, the inhibitory effects of the extract on sperm motility was associated with moderate to high degree of head to head agglutination and curling of tails. In general, the nonmotile sperms usually formed aggregates and the motile sperm did not aggregate. It is also of interest to note that even when the sperm motility was not completely inhibited the sperms had no forward progression and the motility was restricted to wagging of their tails only.

4. Discussion

The sponge specimens used in this study belonged to the genus *Spongionella*, which was one of the widely distributed varieties at the Galle harbour. The solvent system employed for the extraction was dichloromethane -methanol (1 : 1) which is one of the most frequently used solvents in the natural product chemistry of marine invertebrates (Faulkner, 1987). The semen samples used were washed ones : in order to minimize the influence of naturally occuring sperm motility inhibitors (DeLamirande *et al.*, 1984

and other proteolytic enzymes (Huszar *et al.*, 1990) present in the seminal plasma which are acknowledged to be detrimental to sperm motility. Infact, several investigators have used washed spermatoza in investigating effects of various agents on motility of spermatozoa (Ratnasooriya *et al.*, 1991, Porat-Soldin *et al.*, 1992, Hong *et al.*, 1983). The vehicle control used was PVP in isotonic saline. Since the highest concentration of PVP (187.5 mg/ml) had no significant effect on sperm motility we did not examine the effects of lower concentrations of PVP on sperm motility.

The results of this study clearly show that the crude extract of *Spongionella* sp. inhibited the motility of human spermatzoa *in vitro*. This is a novel finding indicating the potential of marine sponges as a possible source for the development of vaginal contraceptive for thc 21st century. The results obtained also exqand the bioactivities of sponges heretofore publishcd (Jimenez *et al.*, 1980; Gunasekere *es al.*, 1990; Angeshofer *et al.*, 1992; Chan *et al.*, 1993; Sakemi *et al.*, 1987; and Fuestani *et al.*, 1990).

The onset of the antimotility effect of the crude extract of *Spongionella* sp. was almost instantaneous. This observation is indicative of a direct action of the extract on the sperm plasma membrane. However, it is unlikely to be a consequence of a non-specific perturbation of the plasmalemma, a phenomenon known to inhibit human sperm motility *in vitro* (Aitken *et al.*, 1985), but more likely to be a receptor-mediated action. The reasons being ; (a) the antimotility effect was achieved at fairly low concentrations and was dose-dependant ; and (b) two other marine sponge extracts were devoid of sperm immobilization activity (Ratnasooriya *et al.*, 1993). Alternatively, the immediate disruption of sperm motility could have resulted from an impairment of activity of a membrane bound enzyme/s (Veno *et. al.*, 1988) either directly or indirectly via a receptor. In complete contrast, an effect of the extract on ion channels could inhibit the sperm motility instantaneously. At the present time, we have no evidence against or in favour of such a mechanism of action of the extract.

The highest concentration of the extract caused total immobilization of sperm immediately. However, with prolonged incubation, intermediate and lower concentrations of the extract were also able to block sperm motility completely as the highest concentration did for a short contact period. This observation is suggestive of a build up of a second intracellular messenger, involved in the sperm motility blocking activity, in a time-dependent manner. Although, we do not know exactly whether the extract inhibited sperm motility, via a second intracellular messenger, the curling of tails, evident in the immobilized sperm suggests the involvement of calcium ions (Lindemann *et al.*, 1987 ; Serres *et al.*, 1991) in triggering the antimotility effect. It is well recognized that calcium ions indeed function as a second intracellular messenger. The

antimotility activity of the extract was accompanied with head to head agglutination of sperm although this was not quantified. Free fatty acids are shown to agglutinate human spermatozoa and to inhibit their motility (Siegal *et al.*, 1986). It is possible that fatty acids present in the extract may have precipitated this effect. This sperm agglutination effect of the extract (although how this happens is not clear), may also have contributed to its antimotility effects. In conclusion, the results of this study demonstrate that the crude extract of Sri Lankan marine sponge, *Spongionella* sp., possess potent sperm immobilization activity. In this study we have not attempted to isolate and characterize the active component/s precipitating the sperm antimotility effect. However, such studies are warrent as marine sponges appear to be a rich and a natural source that can be effectively harnased in the search and development of new vaginal contraceptives. Sponges contain the greatest variety of sterols of any animal group so far reported (Jemenez *et al.*, 1990).

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Table 1: Effects of the crude extraet of *Spongionella* sp. on the motility of human sperm.

Final Concentrations (mg. ml)	n	Percentage motility (means ± SEM) incubation time				
		0 min	5 min	15 min		60 min
Control (187.5mg/ml PVP)	6	86.00±7.34	88.92±10.51	75.82±13.48	81.55±13.48	68.65±13.92
Crude extract (125mg/ml	6	0**	0**	0**	0**	0**
Crude extraet (25mg/ml)	6	16.00±4.40**	10.32±5.46*	0**	0**	0**
Crude extract (12.5mg/ml)	6	47.00±10.63*	15.91±8.30*	0**	0**	0**
Crude extract (6mg/ml)	6	53.30±9.41*	27.00±11.31*	0**	0**	0**
Crude extract (3mg/ml)	6	63.31±10.83	31.79±9.21*	11.76±6.35**	0**	0**

PVP = Polyvinylphrodidone *P < 0.05, **p < 0.001

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