

IN VITRO STUDY OF THE CONTRACEPTIVE SPERMICIDAL ACTIVITY OF *ENSETE SUPERBUM* ON HUMAN SPERM

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ABSTRACT

Objective: The present study was conducted to determine the spermicidal and contraceptive efficacy of ethanolic extract of *Ensete superbum* seeds on human sperm *in vitro*.

Methods: The hypo-osmotic swelling (HOS) and the sperm viability test were used to detect the integrity of sperm membrane and vitality. The sperm revival test was also done to check the recovery of the sperm motility.

Results: *Ensete superbum* extract at 100 mg/ml concentration, induced complete immobilization of human spermatozoa and kill 100% spermatozoa within 20s *in vitro*. The sperm revival test did not show any spermatozoa that recovered their motilities. In the 100 mg/ml extract and N-9 treated groups, the rate of the normal HOS (swollen tails) and the viable sperms (unstained) was 0%, and the rate of the abnormal HOS (unswollen tails) and nonviable sperms (stained) was 100% indicating the plasma membrane degradation of the sperm.

Conclusion: The current study indicates that ethanolic seed extract of *Ensete superbum* possesses appreciable spermicidal potential, which may be explored as an effective vaginal contraceptive.

Keywords: Hypo-osmotic swelling, Sperm viability, Sperm membrane, Sperm motility, Vaginal contraceptive, *Ensete superbum*.

INTRODUCTION

Nature has been a source of natural medicines for thousands of years, since the beginning of civilization. Despite all the advances in modern and orthodox medicine, traditional medicine still plays a significant role in the lives of many people. Hence, the application of medicinal plants, especially in traditional medicine, is currently acknowledged and established as a viable profession [1].

At the present time, one of the social problems regarding world health is the stability of population growth. To control world population growth, male and female partners are equally responsible. Many methods have been devised for the female, whereas the male has not received enough attention in this respect [2]. So, the necessity to develop a safe, effective and affordable pre-coital spermicidal contraceptive to control pregnancy and population growth still exists.

Although many types of spermicidal contraceptives are available, they have side effects and are not easily accepted. The potent vaginal spermicidal nonoxynol-9 (N-9), an organic surfactant [3] causes the disruption of lipids within the sperm membrane and causes rapid loss of sperm mobility. However, N-9 causes inflammation and genital ulceration and thus increases the chance of HIV-1 infection on repeated use [4]. As a result, there is a growing interest in the search for contraceptives of natural origin. There are many medicinal plants that are known to possess antifertility properties either by suppressing spermatogenesis or by spermicidal action [1, 5]. A large number of plant species with antifertility properties were fortified by national and international agencies [6-8]. Several plant materials have been reported to possess spermicidal properties, of which saponin [9], NIM-76, an active fraction of neem oil [10] were reported to possess remarkable spermicidal actions. The spermatogenesis is inhibited by *Tripterygium wilfordii* and gossypol *in vivo* [11], which acts on different sites and stages of spermatogenesis in testis or sperm activity in epididymis.

Ensete superbum [12] is a member of the family musaceae, found in tropical and sub tropical regions of Asia and Africa indigenous to

India and Thailand. Traditionally, the powdered seeds are used for treating kidney stones and painful urination [13], given with milk for diabetes [14], and also for stomachache [15]. Antifertility activity of this plant has been reported. Previous studies indicate the anti-implantation activity of this plant. The anti-implantation activity of the fraction (VIDR-2GD), isolated from the seeds of *Ensete superbum* has been reported [16]. In another study the isolated compound derivatives 4- (4-hydroxy-3-methyl-hex-5-enyl)-chroman-2, 7-diol, also reported to possess significant anti-implantation activity [17].

At present there is no information about the spermicidal action of *Ensete superbum*. So the present investigation has been carried out to determine the spermicidal action and to evaluate different characteristics of sperm functions after *in vitro* exposure to extract of *Ensete superbum*.

MATERIALS AND METHODS

Preparation of the plant extract

The seeds of *Ensete superbum* were purchased from a herbarium in Mangalore, India and authenticated by Dr MP Sharma, Department of Botany, Jamia Hamdard. The voucher specimen has been deposited in the Herbarium of the same Department, Faculty of Science, Jamia Hamdard, New Delhi, India. The fruits were ground into powder and exhaustively extracted thrice with absolute ethanol (10 times of the initial sample weight) for 36 hours in an orbital shaker. The extract was filtered and concentrated *in vacuo* to get black solid material. The ethanolic extract of *Ensete superbum* were collected and stored at -20°C till further use.

Semen preparation

Sperm count above 100 millions/ml and viability above 60% with normal morphology, rapid and progressive motility were used for *in vitro* analysis (WHO, 2009) [18]. Semen samples from healthy fertile men with the above properties collected from NIHF clinic were used for *in vitro* analysis studies. N-9 (500 µg/ml) was used as a reference standard for *in vitro* analysis (HOS and viability). The dose of N-9 was taken according to the reference of Xu et al. [19].

Assessment of plasma membrane integrity

Hypo-osmotic swelling test

Sperm viability and hypo-osmotic swelling (HOS) tests were performed according to WHO manual (2009) [18] for assessing plasma membrane functional integrity. Sperm suspension (100 million/ml) was mixed with the test samples separately for *in vitro* analysis. Similarly, sperm suspension in saline served as the control.

Sperm viability test

Eosin nigrosin staining was performed to assess sperm viability. The prepared slide was examined using a phase contrast microscope. Pink-stained dead sperm were differentiated from unstained live sperm, and their numbers were recorded.

Sperm immobilization assay

Sperm immobilization assay was carried out by treating the diluted semen sample with ethanolic extract of different concentrations ranging from 0 to 100 mg/ml. The sample was added to the diluted semen (1:1) and the time taken for immobilization was recorded using a phase contrast microscope. Sperm suspension in saline served as the control.

Sperm revival test

After the completion of the experiment, the spermatozoa were washed twice in physiological saline and incubated once again in an extract free Ham's F-10 media at 37°C for 30 minutes to observe a reversal of sperm motility, if any. Immobile sperms showing vibratory movement to progressive motility after incubation were considered revived.

RESULTS AND DISCUSSION

In the present study we have reported spermicidal activity of *Ensete superbum*. Some of the plant extract has been reported to possess spermicidal activity [10, 20]. Peptides from natural origin were also reported to possess sperm immobilization activity. Nisin, a naturally occurring antimicrobial cationic peptide from *Lactococcus lactis* [21], subtilosin, a cyclopeptide from *Bacillus subtilis* [22] and magainins, a class of peptides from the skin of African clawed frog, *Xenopus laevis* [23] caused complete immobilization of human spermatozoa.

The spermicidal activity of seeds of *Ensete superbum* was evaluated by a series of *in vitro* experiments. The results demonstrated that *Ensete superbum* seeds extract exerted dose-dependent sperm immobilization effect. 100 mg/ml of ethanolic extract is required to immobilize and kill 100% of 1 million sperm within 20 seconds (Fig. 1). N-9 at 500µg/ml concentration for 30 seconds also caused complete immobilization of human sperm. The sperm revival test showed that the effect of extract was spermicidal and there was no

revival of motility even after incubation for 30 min in the media. Similar results were observed in the case of N- 9 also.

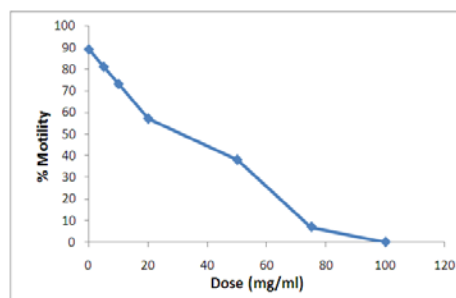


Fig. 1: Effect on percent motility of 1 million human sperm after exposure to different doses of *Ensete superbum* seed extract for 20s.

Sperm fertilizing ability is dependent not only on its motility but also on other functional characteristics. Therefore, besides motility, membrane integrity status is also important for a successful fertilization process.

Functional degradation of sperm membrane was evidenced by HOS response test. Following exposure to hypoosmotic solution, intact sperm membrane permits free passage of fluid into the cell to reach osmotic equilibrium. As a result sperm volume increases and plasma membrane bulges. Because, the plasma membrane around the sperm tail fiber is more loosely attached than that around other parts, sperm tail is particularly susceptible to hypoosmotic exposure and responds by coiling [24]. About 87% of the control sperm showed HOS response, whereas 100 mg/ml extract and N-9 treated sperms failed to retain plasma membrane integrity, as there was no tail curling in treated group when compared to the control (Fig. 2) (Table 1).

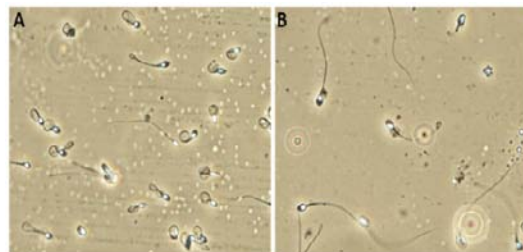


Fig. 2: Hypo-osmotic swelling test of human sperm treated with *Ensete superbum* seed extract. A. Control, B. Treated (100 mg/ml). Control group showed maximum tail curling when compared with treated group (400 x magnifications).

Table: Effect of *Ensete superbum* seed extract on human spermatozoa *in vitro*.

| Groups | Hypo-osmotic swelling (%) | | Viability (%) | |
|-----------------|---------------------------|------------|---------------------|-------------------|
| | Normal | Abnormal | Unstained (Eosin Y) | Stained (Eosin Y) |
| Control | 87.33±4.77 | 12.66±2.86 | 92.67±8.11 | 7.33±2.33 |
| ESE (100 mg/ml) | 0 | 100* | 0 | 100* |
| N9 (500 µg/ml) | 0 | 100* | 0 | 100* |

Data are mean ± S.E.M. of 10 replicates, * Significant difference at $p < 0.0001$ level, when compared with control group

Loss of membrane integrity causes excessive leakage of ATP and other metabolites which affect sperm motility and viability with time because of non-availability of metabolites for metabolism [25].

In our study we found a significant increase in the number of nonviable sperm in the extract treated and N-9 treated groups in comparison to the control group. Viability reduced significantly in the extract treated group (100 mg/ml). The results were similar to N-9 treated groups where both kill 100% of 1 million sperm within 20-30 seconds (Fig. 3) (Table 1).

A. Control, B. Treated (100 mg/ml). Dead spermatozoa with damaged plasma membrane stain pinkish violet and the live ones remain unstained (400 x magnifications).

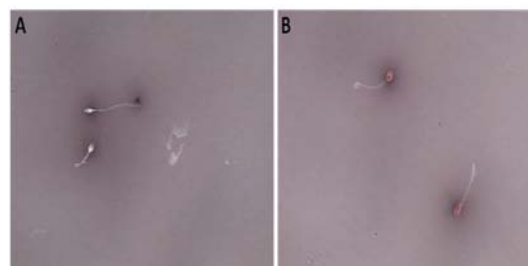


Fig. 3: Sperm viability test of human sperm treated with *Ensete superbum* seed extract.

So we observed that ethanolic extract of seeds of *Ensete superbum* severely inhibited motility and damaged the membrane of human spermatozoa. These observations suggested that cytoplasmic membrane was severely hampered by the exposure of *Ensete superbum* extract resulting in unbalanced metabolism and fertilizing ability of sperms. Hence, the spermicidal effects of *Ensete superbum* make it a highly desirable candidate in future to serve as a potent male contraceptive agent.

CONCLUSION

In conclusion, this study has shown that the ethanolic seed extract of *Ensete superbum* possesses spermicidal property. Further investigations, which include isolation of major compounds from the extract as well as *in vivo* study for the execution of spermicidal properties, will be carried out.

CONFLICT OF INTERESTS

Declared None

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