IN VITRO STUDY OF THE CONTRACEPTIVE SPERMICIDAL ACTIVITY OF Parmelia Perlata LICHEN ON HUMAN SPERM: RISING APPROACH TOWARDS HERBAL CONTRACEPTION

Dhara Patel*,1, Khushboo Patel1, Dhananjay Meshram1, Prachi Patel1

1Department of Quality Assurance, Pioneer Pharmacy Degree College, Nr.Ajwa Cross road, Sayajipura, Vadodara, Gujarat, India
2Department of Pharmaceutics, Pioneer Pharmacy Degree College, Nr.Ajwa Cross road, Sayajipura, Vadodara, Gujarat, India

ARTICLE INFO

Research Article

Received: 10th March, 2018
Accepted: 9th April, 2018

Corresponding Author:

Dhara Patel
E-mail: patel.dhara.j@gmail.com

Keywords: Hypo-osmotic swelling, Sperm viability, Sperm membrane, Sperm motility, vaginal contraceptive, Parmelia Perlata.

ABSTRACT

In recent times, 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. So, the necessity to develop a safe, effective and affordable precoital spermicidal contraceptive to control pregnancy and population growth still exists. The aim of this study was to highlight the work on extract of Parmelia Perlata lichen involved in male anti-fertility mechanism. 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. Parmelia perlata methanolic extract 20 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 20 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 (unsawnen tails) and nonviable sperms (stained) was 100% indicating the plasma membrane degradation of the sperm. The current study indicates that methanolic lichen extract of Parmelia perlata possesses appreciable spermicidal potential, which may be explored as an effective vaginal contraceptive.

© www.albertscience.com, All Right Reserved.

1. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years, since the beginning of humanity. For example, in India, many plants have medicinal value, and the application of medicinal plants, especially in traditional medicine, is currently acknowledged and established as a viable profession [1]. Exponential rise in human population in India has challenged all the development plans and has forced mankind to research on fertility regulation worldwide. The synthetic chemical agents currently being used as fertility regulating method possess the combination of hormonal and non hormonal compounds those have several side effects. The herbal drugs of Indian origin have revealed a significant fertility regulation potential of mammalian species which can be explored for developing an antifertility drug [2]. Herbal contraceptives are in popular demand because they are cost effective, readily available from local sources and have fewer side effects. However, herbal medicines may impair fertility in male and female animals or humans [3]. Whilst some medicinal plants tested for many plants used as contraceptives or sterility agents decrease spermatogenesis [4], impair implantation [5] or are spermicidal [6]. Some research findings have confirmed the spermicidal properties of Cestrum parqui [7], Carica papaya [8] and Hymenocardia acida [9]. Global search for antifertility agents as an alternative to resolve population explosion has continued to receive attention especially in developing countries. For decades, efforts have been made to develop safe and effective contraceptives from natural sources. Plants having folkloric reputation have been identified and evaluated for their contraceptive efficacy. In recent years, there is a renewed interest in the control of fertility by using plants as male contraceptives [10].

Parmelia perlata is a well known lichen of family Parmeliaceae. A lichen is an association of an alga and fungus, living together in a symbiotic relationship. Parmelia perlata is commonly called Stone flower or Chadilla. In India it is mainly found in Himachal Pradesh and West Bengal. It is used as food, fodder and medicine.
It is a good pain reliever and is used as a remedy for early healing of wounds. It cures many skin diseases and is considered to be an expectorant, astringent, resolvent, laxative, carminative and aphrodisiac. It is also used in treatment of fever, cough, dysentery and renal calculi. This lichen exhibits antimicrobial [11-12], antiviral [13], antitumor [14], antispasmodic [15], antioxidant [16] and antipyretic [17] activities.

At present there is no information about the in vitro spermicidal action of parmelia parlata. 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 parmelia parlata.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

The plant material Parmelia perlata (lichen) was collected from the hills of Himachal Pradesh (India) (Figure 1). Identification of the lichen was done with the help of Department of Botany, University of Rajasthan, Jaipur, Rajasthan-302004.

2.2 Extraction of lichen

The collected lichen materials were brought to the laboratory, air dried for three days, cleaned free of any other plant materials or mosses and then washed under running tap water. They were oven dried at 40°C for 42 h and ground into powder by using mixer. The powdered samples were stored in sterilized specimen bottles until when needed. Lichen constituents was extracted by cold extraction method (see below).

2.3 Chemicals

Eosin Y (1% solution in distilled water) and nigrosin (10% solution in distilled water) were of analytical grade and obtained from Loba Cheme Pvt. Ltd. (Mumbai, India).

2.4 Cold extraction:

10 g of lichen powder was added to 200 ml of acetone. The mixture was timed thoroughly by using shaker water bath for 5 h, then left at room temperature overnight and filtered using Whatman No. 1 filter paper. The filtrate was collected and solvent was removed using rotary evaporator; about 200 mg residues were recovered. The lichen powder that remained on the filter paper was dried and again extracted using 200 ml methanol. From this solvent, about 162 mg residue was recovered.

2.5 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 Isha Hospital, Vadodara were used for in vitro analysis studies. Pure N-9(500 μg/ml) was used as a reference standard for in vitro analysis (HOS and viability) [19]. Using an unstained sample of fresh semen, the number of motile sperm was counted until a total of 200 spermatozoa were assessed. In order to improve measurement accuracy, procedures have been carried out twice. The same donor's semen was divided into a number of equal portions for the following experiments.

2.6 Assessment of plasma membrane integrity:

2.6.1 Hypo-osmotic swelling test

A functional membrane is requisite for the fertilizing ability of spermatozoa, as it plays an integral role in sperm capacitation, acrosome reaction, and binding of the sperm to the egg surface. The hypo-osmotic swelling (HOS) test evaluates the functional integrity of the sperm's plasma membrane and also serves as a useful indicator of fertility potential of sperm. The HOS test is a semi-quantitative test based on the semi-permeability of the intact cell membrane, which causes spermatozoa to “swell” under hypo-osmotic conditions, when an influx of water results in an expansion of cell volume [20]. The test was introduced by Jeyendran et al. [21]. It is simple to perform and easy to score and gives additional information on the integrity of the cell membrane of the sperm tail. The HOS test may help in assessing the diagnosis and the management of male infertility. The HOS test was performed as recommended by WHO [22]. Preparation of the HOS solution was as follows: 0.735 g of sodium citrate dihydrate and 1.351 g of d-fructose were dissolved in 100 ml of purified water. A total of 0.1 ml of semen sample was mixed with 1.0 ml of a hypo-osmotic solution (150 mOsm). After incubation for 5 min at 37 °C, ≥200 spermatozoa were analyzed by phase-contrast microscopy at 400 magnification, evaluating the modifications of the sperm tail to score swollen sperm (normal HOS) and unswollen sperm (abnormal HOS), which were reported as a percentage of all sperm observed. Sperm samples in physiological saline (1:1, v:v) served as the controls.

2.6.2 Sperm viability test:

Sperm viability test was determined by the eosin nigrosin staining technique [22]. One drop of the mixture was mixed with two drops of 1% eosin Y. After 30 seconds, three drops of 10% nigrosin solution was added and mixed. A drop each of the extract-treated semen-eosin-nigrosin mixture and the PSS-treated semen-eosin-nigrosin mixture (control) was observed under a microscope. Unstained sperms were counted as live and stained sperms were counted as dead.

2.6.3 Sperm morphology

Sperm morphology was observed under a microscope by the eosin-nigrosin staining technique [22]. Any change in morphology was noted.

2.6.4 Sperm immobilization assay

Sperm immobilization assay was carried out by treating the diluted semen sample with methanolic extract of different concentrations ranging from 0 to 150 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. For immobilization assay from the stock solution of the extract, concentrations of 1, 3, 5, 10, and 20 mg/ml were prepared in normal saline and were mixed at the ratio of 1:1 with the sperm suspension. Physiological saline solution (PSS), pH 7.4, was mixed at a ratio of 1:1.

Table 1: Results of sperm HOS, EY tests in control, extract and N-9 group

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm Membrane Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal HOS</td>
</tr>
<tr>
<td>Control</td>
<td>78.2 ± 0.12</td>
</tr>
<tr>
<td>Extract</td>
<td>0</td>
</tr>
<tr>
<td>N-9</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1: Sperm membrane changes in the HOS test and EY staining, after treatment with extract or N-9

3.2 Sperm Immobilization Assay:

Sperm death was evaluated in the drug-treated samples using the eosin-nigrosin staining technique. Sperm viability was decreased dose-dependently. A total decrease in sperm viability in the group treated with extract at a concentration of 20 mg/mL was observed in 5 minutes (Table 2).

Table 2: Effect of different concentration of methanolic lichen extract of parmelia perlata on the motility of human sperm

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control ± SD</th>
<th>1 mg/ml ± SD</th>
<th>3 mg/ml ± SD</th>
<th>5 mg/ml ± SD</th>
<th>10 mg/ml ± SD</th>
<th>20 mg/mL SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85.12 ±0.33</td>
<td>83.12 ±0.03</td>
<td>81.12 ±0.48</td>
<td>81.02 ±0.33</td>
<td>80.13 ±0.34</td>
<td>81.19 ±0.21</td>
</tr>
<tr>
<td>1</td>
<td>85.02 ±0.68</td>
<td>72.45 ±0.11</td>
<td>70.21 ±0.29</td>
<td>68.25 ±0.14</td>
<td>50.12 ±0.54</td>
<td>20.35 ±0.14</td>
</tr>
<tr>
<td>2</td>
<td>83.88 ±0.11</td>
<td>70.14 ±0.71</td>
<td>65.55 ±0.41</td>
<td>55.28 ±0.11</td>
<td>35.21 ±0.19</td>
<td>10.25 ±0.91</td>
</tr>
<tr>
<td>3</td>
<td>80.72 ±0.58</td>
<td>68.24 ±0.51</td>
<td>60.72 ±0.19</td>
<td>46.23 ±0.28</td>
<td>30.11 ±0.58</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>73.42 ±0.41</td>
<td>60.12 ±0.11</td>
<td>50.85 ±0.11</td>
<td>41.23 ±0.84</td>
<td>14.23 ±0.33</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>72.12 ±0.61</td>
<td>50.31 ±0.31</td>
<td>38.12 ±0.67</td>
<td>35.12 ±0.15</td>
<td>6.05 ±0.14</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2: Immobilization of Sperm (20 mg/ml) dead cell- red colour
3.3 Sperm morphology:
There was no change in the morphology of sperm treated with the extract compared with untreated sperm.

3.4 Sperm revival test:
Sperms immobilized by the treatment of the extract were studied for revival, but none of the sperms regained motility when washed with PSS solution and incubation at 37°C for 30 minutes.

CONCLUSION
The spermicidal activity of lichen extract of Parmelia perlata was evaluated by a series of in vitro experiments. The results demonstrated that Parmelia perlata lichen extract exerted dose-dependent sperm immobilization effect. 20 mg/ml of methanolic 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. We conclude that the ethanol extract of Parmelia perlata lichen possesses an immobilizing factor that probably reduces motility by causing sperm non viability by disrupting the membrane architecture of the sperm cell. Therefore, the future potential of the lichen Parmelia perlata as a local spermicidal agent appears to be promising.

REFERENCES