



Original article

Spermicidal effects of lemon juice and juices from other natural products

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ABSTRACT

The study of spermicidal effects of lemon juice and juices from other natural products consisting of pineapple juice, apple juice and aloe vera juice, was carried out to develop methods of contraception using natural products. Semen was donated by 20 men from infertile couples who came to an infertility clinic at the Department of Obstetrics and Gynecology, Ramathibodi Hospital, Bangkok, Thailand from 1 November 2007 to 31 March 2010. Spermicidal effects were measured by observing changes in sperm viability, morphology and motility after the semen was mixed with lemon juice and the juices from the other natural products (pineapple juice, apple juice and aloe vera juice). Changes in sperm characteristics were compared with pure semen left at room temperature. After the semen was mixed with lemon juice, sperm were instantaneously immobilized and irreversibly deformed. A reduced spermicidal effect was observed when the semen was mixed with the other juices. A second, profound spermicidal effect was observed when semen was mixed with pineapple juice. The least effects were observed when the semen was mixed with aloe vera juice. This information can be used for the further development of natural barrier methods of contraception.

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Introduction

The use of available household or commercial products as spermicidal agents for contraception has long been investigated. [Umpierre et al. \(1985\)](#) found that Coca-Cola had a spermicidal effect. However, the study of [Hong et al. \(1987\)](#) provided quantitative evidence that Coca-Cola and Pepsi-Cola have little, if any, spermicidal effect and thus their application as a postcoital douching is a practice lacking scientific foundation. Lemon juice solutions have been shown to immobilize sperm in the laboratory ([Short et al., 2004](#)), as has Krest Bitter Lemon drink ([Nwoha, 1992](#)). While the author of the Krest Bitter Lemon study suggested its use as a postcoital douche, this is unlikely to be effective, as sperm begin leaving the ejaculate (out of the reach of any douche) within 1.5 min of deposition. No published studies appear to have been done on the effectiveness of lemon juice preparations in preventing pregnancy, though they are advocated by some as 'natural' spermicide.

[Short et al. \(2004\)](#) from the University of Melbourne reported that lemon juice is not only an effective form of contraception, but

also had effect on the HIV virus. The study of [Sagay et al. \(2009\)](#) on genital tract abnormalities among female sex workers who had douched with lemon/lime juice in Nigeria found that the practice of douching with citrus juice may be a risk factor for cervical dysplasia. They suggested that further studies to explore the association between douching with lime juice and cervical dysplasia are warranted in communities where this practice is common. Besides using lemon juice as a spermicidal agent, other natural products from plants have been studied to evaluate their effects on sperm ([Farnsworth and Wall, 1982](#)).

The current study investigated the spermicidal effects of lemon juice (*Citrus limon*) and juices of other natural products available—juice from pineapple (*Ananas comosus*), apple (*Malus estica*), and aloe vera (*Aloe vera*). Changes in sperm characteristics were compared with changes in control semen which was left at room temperature without mixing with any juices. The effects of the juices could lead to applicable information for the development of natural techniques of contraception.

Materials and methods

This research was approved by the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects,

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Faculty of Medicine, Ramathibodi Hospital, Mahidol University (MURA 2007/194) issued on 14 June 2007.

Semen samples were obtained from 20 volunteers who came to the Infertility Clinic, Ramathibodi Hospital, Bangkok, Thailand from 1 November 2007 to 31 March 2010. The semen samples were collected in the Reproductive Biology Laboratory of Ramathibodi Hospital and then were allowed to liquefy for at least 20 min at 37 °C in an incubator and were analyzed within 60 min. One milliliter of semen was mixed with 1 mL of each natural product (1:1 by volume) consisting of pure and diluted lemon juice, pineapple juice, apple juice and aloe vera juice. Changes in the characteristics of sperm were observed and compared with control semen which was left at room temperature without mixing with any of the solutions. Sperm concentration, viability, morphology and motility were evaluated using standard techniques (Schrader et al., 1992; World Health Organization, 1999). Sperm morphology was scored using the Tygerberg Kruger strict criteria (Kruger et al., 1987). Volunteers were aged 25–55 yr with no history of medical diseases such as diabetes mellitus, hypertension, hyper or hypothyroid or any autoimmune diseases and no history of chemotherapy or radiation. They had not been vasectomized and were not heavy smokers or heavy alcohol drinkers.

All 20 semen samples were processed and analyzed by researchers at the laboratory. A routine semen analysis was performed which included the following parameters: semen volume, sperm concentration, sperm viability, sperm morphology and sperm motility.

Sperm concentration

Measurement of sperm concentration was carried out under a microscope (40×) after the semen had been dropped into a Neubauer Hemocytometer. Scanning the slide and estimating the numbers of spermatozoa (both viable and non-viable) per field or part of a field equivalent to 1 mL gave an approximate sperm concentration in millions per milliliter.

Sperm viability

The viability of sperm was determined using eosin staining and examination under a microscope (100×). Two hundred spermatozoa were counted with a light or phase contrast microscope before differentiating the live (unstained) spermatozoa from the dead (stained) cells.

Sperm morphology

Morphological measurements of the sperm were undertaken by examining the sperm under a microscope (100×) after staining with methyl alcohol, eosin, and methylene blue. The percentage of normal morphological sperm was recorded. As each slide was examined systematically from one field of the microscope to the next, all normal spermatozoa were assessed and scored and the defects of the abnormal spermatozoa were noted. For normal morphology characteristics of sperm, the sperm must be vigorously motile and the sperm head must be a symmetrical, oval shape of the appropriate size (World Health Organization, 1999).

Sperm motility

Sperm motility was observed and classified. At least five fields of the microscope were assessed in a systematic way to classify 200 spermatozoa. The motility of each spermatozoon was graded *a*, *b*, *c* or *d*, according to whether it showed the following characteristics: *a* indicated rapid progressive motility (at least 25 µm/s at 37 °C and

at least 20 µm/s at 20 °C); *b* indicated slow or sluggish progressive motility; *c* indicated non-progressive motility (less than 5 µm/s); and *d* indicated immotility.

Within a defined area of the fields indicated by lines A and B formed by a graticule in the focal plane of the microscope, all spermatozoa with grade *a* and *b* motility were counted first. Subsequently, the spermatozoa with non progressive motility (grade *c*) and immotile spermatozoa (grade *d*) were counted in the same areas. The numbers of spermatozoa in each category were tallied with the aid of a laboratory counter. The count of 200 spermatozoa was repeated on a separate 1 mL specimen from the same semen sample and the percentages in each motility grade from the two independent counts were compared and averaged. Sperm motility was also scored for statistical analysis. Levels *a*, *b*, *c* and *d* were allocated score of 3, 2, 1 and 0, respectively.

Preparation of juices of natural products

- 1) The lemons bought from a supermarket were cleaned and washed with soap and tap water. Then, each lemon was divided in half before squeezing to obtain the juice which was filtered through a clean cloth. About 2 mL of juice was obtained from one lemon. Diluted lemon juice was prepared by adding 1 mL of sterile water into 1 mL of fresh lemon juice (1:1 dilution). Only 1 mL of diluted lemon juice was mixed with semen.
- 2) The natural products consisting of apple, pineapple, and aloe vera were bought from a supermarket. All the fruits were washed and cleaned with soap and tap water. The skins of the fruits were peeled before dividing each fruit into small pieces of 2–3 mm³, which were then ground using a grinding machine and squeezed to obtain the juice. The juices were then filtered through a clean cloth to eliminate the sediments.

Each type of the juice was prepared fresh just before mixing with semen. Data analysis was done using the SPSS Statistics 18.0 Mahidol package (SPSS Inc; Chicago, IL, USA). Comparison of qualities of sperm within and between groups was undertaken using the General Linear Model (GLM) for repeated measurement.

Results

Sperm concentration

In the control semen, the mean number of sperm concentration was stable over time at 55–64 × 10⁶ per mm³. After the semen was mixed with pure lemon juice, the mean concentration of sperm decreased instantaneously to zero and remained so. When semen was mixed with the other kinds of juice, the mean concentration of sperm decreased gradually and significantly (*p* < 0.001) when compared with the control. Among the other juices, the pineapple juice had the lowest mean concentration of spermatozoa (Table 1). When the mean concentrations of sperms were compared between each mixture of sperm and juice from the natural products, there were statistically significant differences between each type of mixture except between semen mixed with diluted lemon juice and semen mixed with aloe vera juice (both not significant) as shown in Table 1.

Sperm viability

The viability of sperm in the control semen left at room temperature gradually decreased over time. Mean numbers of viable sperm were significantly different (*p* < 0.001) between the control semen and the semen mixed with each kind of juice. The viability of the sperm after mixing with lemon juice decreased immediately to

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