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Effect on human sperm mitochondrial activity by *Piper betle* and *Calendula officinalis*

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ABSTRACT

About 90% of the word's contraceptive users are women. Contraceptive choices are available but with lot of side effects. Condom, vasectomy, withdrawal are very few contraceptive choices are available for males. However, as noted earlier, exploratory research has indicated that certain Piper betle and Calendula officinalis ingredients have contraceptive properties. This work gives a view mainly on the contraceptive properties of these plant extracts. Plant samples were collected from different states of India. Motility of sperm depends on mitochondrial activity present in mid-piece of sperm. In the present study, the mitochondrial activity of sperm was evaluated after treating semen with different concentrations of Piper betle and Calendula officinalis. The mitochondrial activity was also evaluated after subjecting the semen samples for different incubation time periods. Test was done on more then 75% motile normozoospermic semen sample. It was found that as the concentration of extracts increases the mitochondrial activity decreases significantly ($p < 0.001$), similar results were observed when constant concentration of extracts with increasing time intervals. The mitochondrial activity decreases significantly ($p < 0.001$) in 5 minutes to 20 minutes incubation time. Experiment indicates that Piper betle and Calendula officinalis have properties to decrease mitochondrial activity in human sperm.

Key words: Normozoospermic, sperm mitochondrial activity, contraceptive agent, spermicidal, *Piper betle, Calendula officinalis.*

INTRODUCTION

India is known as botanical garden of the world for its rich natural resources. Over 6,000 plants in India are used in traditional, folklore and herbal medicine [1]. The Indian system of medicine has identified various medicinal plants in which some are very commonly used [1]. Herbal

medicines are popular as remedies for diseases for a vast majority of the world's population. The demand for herbal medicines is increasing rapidly all over the country due to their lack of side effects and low cost [2]. Various plants have been used for the treatment of many diseases such as skin infection, ulcers, diabetes [3] and male reproduction [4] and many more.

Fertility control is an issue of global and national public health concern. Current methods of contraceptive result in an unacceptable rate of unintended pregnancies. Approximately 50% of all pregnancies are unintended at conception, 50% of those occurs in 94% of sexually active couples who report using some methods of contraception [5]. The only male specific contraceptive methods currently available are withdrawal, condoms, and vasectomy. As concern regarding side effect and convenience of these existing methods prevent their universal acceptance [6, 7]. The development of additional male methods of fertility control can provide tremendous social and public health benefits. Contraceptive vaccines, and inhibitors of spermatogenesis and sperm motility, provide a potential for non hormonal male contraceptives. However, new methods of male contraceptive become available, family planning and public health experts must cautiously evaluate the impact of this new technology before recommending them. Plant products as contraceptive will be more acceptable for economic reasons in term of self reliance and the possible practicability for a male as well as female contraceptive approach.

A very little work has been done on the biological activity and plausible contraceptive application of plant extracts or compounds isolated from plants. As the global scenario is now changing towards the use of nontoxic plant product having traditional medicinal use, development of modern contraceptive from plant origin should be emphasized for the control of population with male as well as female practicable use.

Two plant products which are very commonly used in daily life, *Calendula officinalis* (Compositae) and *Piper betle* also known to have antifertility effects in rodents [8, 9]. Contraceptive-like properties have also been reported in women by local tribes of Rajasthan and Bengal region of India as they use these for birth control also.

Calendula officinalis is commonly known as 'marigold'. It has traditionally been used for gastric ulcers and menstrual discomfort, skin disorders, as an antiseptic, and for anti-inflammatory diseases [10]. Antifertility potential of this plant has not been investigated in detail scientifically. *Calendula officinalis* is an important plant having several medicinal applications in India and all over world [2]. This plant is a native of southern Europe but occurrence of this plant is everywhere and on commercial scale, it is cultivated in North America, Eastern Europe and Germany [11]. Calendula is used in Ayurvedic and Homeopathic system of medicine for the treatment of fever and cancer since from long time [12]. It is recommended because of the broad area of biological activities like anti-inflammatory [13], anti mutagenic [14], wound healing [15, 16] diuretic [17], antispasmodic activities [18] and also used in gastrointestinal, gynecological, eye diseases, skin injuries and in some cases of burns [19].

Our second experimental plant is *Piper betle*, belongs to family Piperaceae. *Piper betel* is an edible plant with leaves that have been traditionally used in India, China and Thailand for prevention of oral malodor; it forms the most basic ingredient of a variety of widely used social and habitual masticators products. People in several parts of the world including the whole of the

north-eastern region, some western region of India, masticate it in raw form. In spite of its alienness, the plant is much more popular in India than in any other country of the world since the antiquity. This would be evident from the numerous citations laid down in the ancient literature, particularly the Indian scriptures. In these citations, significance of the leaves has been explained in relation to every sphere of human life including social, cultural, religious and even day-to-day life, which is very much relevant even these days. For example, a well-prepared betle quid is still regarded as an excellent mouth freshener and mild vitalizer routinely served on the social, cultural and religious occasions. . It is also used as a special item offered to the guests in order to show respect and for such traditional use of betle leaf in the Indian society, the leaf really stands alone without any parallel even today [20, 21]. In fact, this edible leaf has achieved an esteemed position in the human society right from the dawn of civilization, particularly in the countries like Bangladesh, Burma, China, India, Indonesia, Malaysia, Nepal, Pakistan, Philippines, South Africa, Sri Lanka, Thailand etc. [22, 23, 24], where leaves of this plant are traditionally used for chewing in their natural raw condition along with many other ingredients. It is a betel leaf, chewed as a palate cleanser, a breath freshener, and for digestive purposes. Anti fertility effect of *Piper betle* is mention by Sarkar *et al.*, [9].

Antifertility effect of these commonly used plants extracts are studied in details. Insufficient literature is available regarding effect of these plant extracts on sperm mitochondrial activity of human sperm. The present study was undertaken to find the effect of *Piper betel* and *Calendula officinalis* on sperm mitochondrial activity of human sperm.

MATERIALS AND METHODS

Plant materials

Fresh *Piper betle* leaves and flowers of *Calendula officinalis* were purchased from local market of different region of Uttar Pradesh, Haryana, Punjab and West Bengal, states of India.

Preparation of plant extract

Plant materials were shade dried, powdered and subject to soxhelet extraction with 50% ethanol [25]. The ethanol was evaporated under reduced pressure to obtain the crud extract in the form of a powder. 23% residue was obtained from *Calendula officinalis* flowers and 10% residue from leaves of *Piper betle*, which was dissolved in sterile Phosphate buffer saline (pH 7.2, 50mM) to use in desired concentrations.

Semen Sample

The semen samples were collected from human male volunteers and the study was carried out at laboratory of Oniosome Health Care Pvt. Ltd. (Punjab, India). Semen sample of normozoospermic men were obtained after five days of abstinence. After proper liquefaction, at room temperature, sperm motility was checked, 50 samples with more than 75% motile spermatozoa were used in test.

Sperm Mitochondrial Activity Index (SMAI) Test

For studying effect of *Piper betle* leaves and flowers of *Calendula officinalis* extracts on sperm mitochondrial activity of human sperm, 50 normozoospermic samples were used. Four different concentrations of these extracts were used in study with observations on three time intervals. In

control only sterile Phosphate buffer saline (pH 7.2, 50 m M) was used with sperm. Aliquots of 0.1 ml of sperm sample and extract solutions each were used. Sperm Mitochondrial Activity Index test was performed as mentioned by Gopalkrishna *et al.*, [26] with slight modification. Statistical analysis was done by Student's t test.

RESULTS

In experiment, constant concentration of *Piper betle* (figure 1) and *Calendula officinalis* (figure 2) extracts were used, with increasing time intervals significant ($p < 0.001$) decrease were recorded for SMAI.

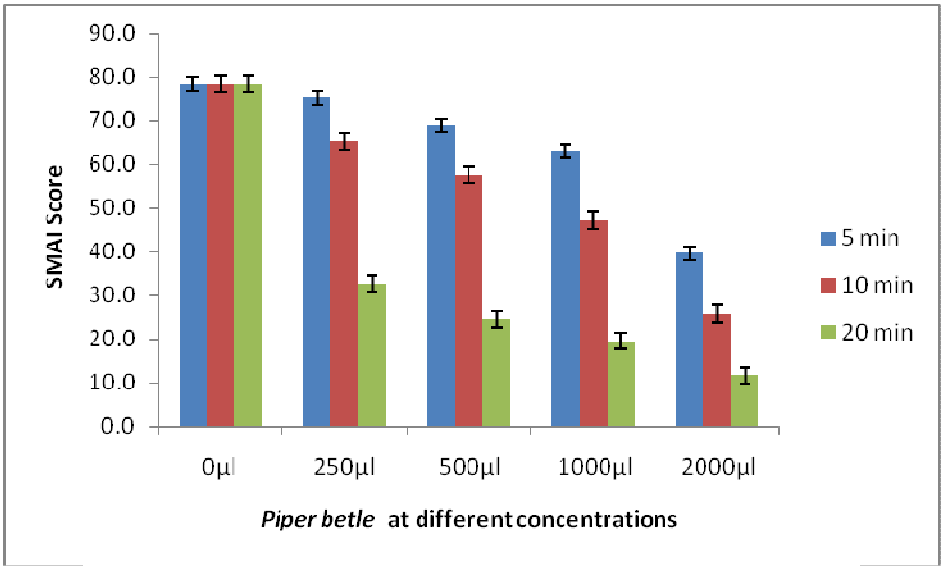

Figure 1: Effect of *Piper betle* on human sperm mitochondrial activity

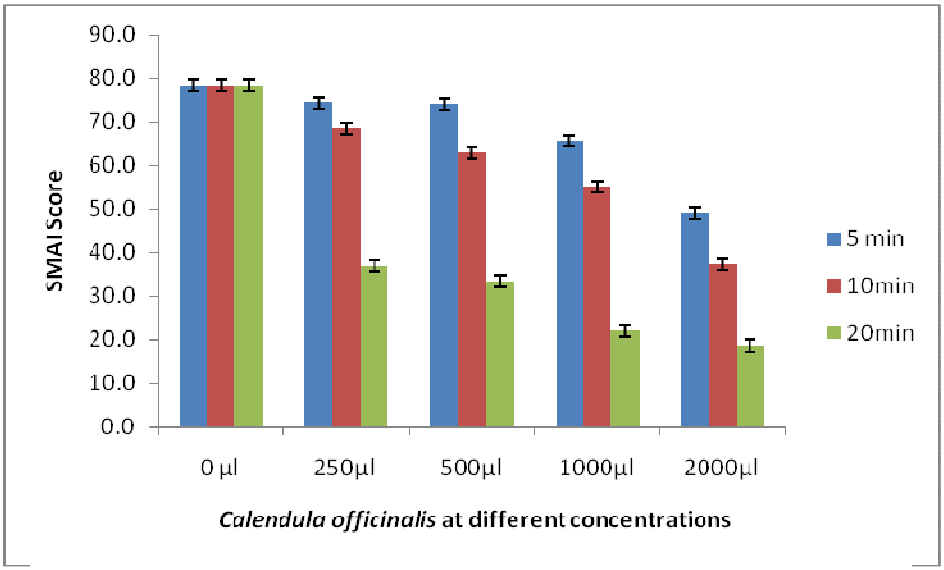

Figure 2: Effect of *Calendula officinalis* on human sperm mitochondrial activity

In increasing concentration of *Piper betle* and *Calendula officinalis* extracts at constant time and in increasing time intervals same significantly ($p < 0.001$) decreasing SMAI score were found as compare to control (figure 1 and 2). In observations, high concentration of extract shows decrease in SMAI score in less time interval as compare to low concentrations. Increase in time interval also effect SMAI score at every concentrations used and decreasing scores were recorded. In our observations we find SMAI score of *Piper betle* extract observation (figure 1) is much lower in comparison with *Calendula officinalis* extract (figure 2).

DISCUSSION

Piper betle and *Calendula officinalis* extracts suppress sperm mitochondrial activity and ability to work as contraceptive, even though further research is needed to identify mechanism of action which plays role in decreasing Sperm mitochondrial activity. These findings could also provide additional insight into the existing data available on plant extracts as antifertility agents. Keeping in view the importance of *Piper betle* and *Calendula officinalis* in national, regional and international perspective there is urgent need to locate collect and study its diversity and develop effective measure for future use. At the same time it is also essential to undertake ethno botanical studies to link its various therapeutic uses as well as contraceptive use. There is a dire need to documented folklore traditional knowledge, which is vanishing rapidly due to lack of awareness in these people. Not very much work has been done on the biological activity and possible medicinal application of these plant extract or compounds and hence extensive investigation is needed to exploit their therapeutic utility in fertility and diseases. A drug–development program should be undertaken to develop modern drugs with the compounds isolated from *Piper betle, Calendula officinalis* and many more medicinal plants.

The above study is helpful in showing that *Piper betle* and *Calendula officinalis* extracts are potent agents having adverse action on mitochondria and thus reducing sperm motility. Abundant availability of *Piper betle* and *Calendula officinalis* can make it a favorable agent of contraceptive. In our observations we find SMAI score of human sperm with *Piper betle* extract is much lower in comparison with *Calendula officinalis* extract. Although further studies are required to develop these extracts as non hormonal, pre and post coital contraceptive.

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