

Evaluation of Spermicidal Potential of Aqueous Ethanolic Extract of *Madhuca indica* Leaves: Rising Approach towards Herbal Contraception

Zeba Khan¹, Syed Ibrahim Rizvi², Neetu Mishra^{3*}

^{1,3}Department of Home Science, University of Allahabad, Prayagraj, Uttar Pradesh, India.

²Department of Biochemistry, University of Allahabad, Prayagraj, Uttar Pradesh, India.

¹zeba.khan09@gmail.com

Abstract:

The aim of the current study was to determine whether an aqueous ethanolic extract of *Madhuca indica* leaves had spermicidal properties. Using the in vitro immobilisation assay, different concentrations of the plant extract were used to assess the impact on rat sperm motility. The outcome revealed that when the extract was applied, all of the spermatozoa instantly became immobile at a concentration of 160 mg/ml. At a concentration of 80 mg/ml, the sperm motility was “ 9.81 ± 0.13 , 9.70 ± 0.50 , 9.52 ± 0.10 , 9.38 ± 0.05 , 8.89 ± 0.04 and 8.17 ± 0.01 at 0, 15, 30, 60, 120 and 180 seconds respectively”. Similar to this, reduced sperm motility was seen at concentrations of 40 mg/ml, 20 mg/ml, and 10 mg/ml. This study concludes by explaining the existence of spermicidal activity in the crude extract of *Madhuca indica* leaves.

Keywords: contraceptive, herbal medicine, *Madhuca indica*, spermicidal.

INTRODUCTION

One of the important historical occurrences to consider in the modern era is the extraordinary increase in the global population. At the moment, there are about 8 billion people on the planet, with India having the largest population at 1.39 billion. The geometric increase in human population is one of the major issues facing developing nations like India. We now realise that our population growth is placing a strain on the planet's ability to provide food, energy, and raw materials. The mortality rate has significantly declined, and life expectancy has increased as a result of improvements in public health and medicine. Our socioeconomic infrastructure would be out of balance as a result of this population explosion, which will also negatively affect our economic policies. Since human fertility can be limited, controlling it is the most crucial and pressing biosocial and medical issue facing humanity today [1].

Traditional medicine has used medicinal plants for a very long time as a natural cure. Information on medicinal plants and how they are used by indigenous societies is useful for the creation of new drugs, the preservation of traditional cultures, and the preservation of biodiversity. The World Health Organization (WHO) is currently emphasising poor nations in order to encourage them to embrace herbal medicine, which they have historically used for generations [2].

Traditional medicine, which dates back thousands of years, is still used to treat and prevent both physical and mental illnesses. In the past, different societies have created a variety of practical healing techniques to treat a range of serious and life-threatening illnesses. Today, many nations still place a high value on traditional medicine, which is also referred to as complementary and alternative medicine or ethnic medicine. The majority of the medications used in conventional medicine come from natural sources. "Clinical trials" have been used in conventional medicine since the dawn of time [3].

The World Health Organization (WHO) has launched a programme to manage the population that includes research on conventional medical procedures. Numerous hormonal contraceptives have been created and used to date, but due to their chemical foundation, high cost, sophistication, and side effects, they did not satisfy the demand of developing nations. Herbal contraceptives have also been developed, but again, their effectiveness for humans is limited. Due to these issues, people are now looking into using herbal medicines to treat a variety of illnesses and regulate fertility. Nearly 80% of the world's population relies on conventional medicines for their primary healthcare, so research is being done to create herbal antifertility agents that are more effective than chemical compounds, socially and culturally acceptable, and compatible with humans. They also have fewer side effects [4].

Sapotaceae family member *Madhuca* is a significant medicinal plant. Large evergreen tree distributed in India; it is highly regarded as a universal panacea in tribal people's medicine. Many different diseases can be treated with the medicinal properties of mahua trees. Almost all of this plant's parts have been employed therapeutically in the indigenous medical system to treat a variety of human maladies.

Traditional remedies for diabetes, rheumatism, ulcers, haemorrhage, and tonsillitis include *Madhuca indica* bark. *Madhuca indica* has tremendous therapeutic potential in its flowers, seeds, and seed oil. The seed oil massage is particularly helpful in reducing discomfort on the outside. The Mahua explained numerous pharmacological potencies, some of which are still in use today, including anti-inflammatory, analgesic, antipyretic, anti-hyperglycemic, anti-ulcer, antioxidant, hepatoprotective, antibacterial, antiepileptic, anticancer, and toxicological properties [5].

So, utilising aqueous ethanolic extract of leaves at various doses in rats, the current investigation was carried out to assess the in vitro spermicidal activity of *Madhuca indica*.

MATERIAL AND METHODS

preparation of plant material

Madhuca indica leaves were procured from Prayagraj, Uttar Pradesh, India, and the plant specimen was verified at the Department of Botany, University of Allahabad, Prayagraj. The department herbarium received the voucher specimen for storage.

Extraction of plant-based material

In 500 litres of 70% ethanol, 100 grammes of a powdered leaf were soaked for 48 hours at room temperature while being stirred occasionally. Using Whatman (No. 1) filter paper, the substance was purified. The resulting filtrates were then dried at room temperature. When not in use, the extract was kept at 40 C in sealed containers.

Animals

Adult white male albino rats were housed in polypropylene cages at room temperature, with a 12-hour cycle of light and darkness. They were given free access to water for a week, and commercial meal was permitted. The experiment's protocols adhered to the standards for the treatment and welfare of research animals.

Experimental Design

Diethyl ether was used to anaesthetize the rats. To externalise the testis and epididymis, a scrotal slash was performed. The testes and epididymis were carefully divided, and any blood was wiped from the area. In order to collect epididymal sperms for the creation of sperm suspension, the cauda epididymis was chopped and put in a watch glass with 2 ml of physiological saline (kept at 37 °C). As was previously mentioned, [8] each rat's sperm solution was gathered and used for the in vitro immobilisation activity. Ten microliters of the extract were diluted in physiological saline solution at a range of concentrations (1%, 2%, 4%, 8%, and 16%) before being coupled with an epididymal sperm suspension (1:1 v/v) in order to study the effects of the plant leaf extract on sperm motility. On an uncontaminated, dry glass slide with a cover slip put on a heated stage, a drop of the uniformly mixed sample was applied right away. A binocular microscope (Olympus, Japan) was then used to magnify this slide by x10 and x40. 100 spermatozoa were counted after a quick inspection of five fields minimum. 10 l of physiological saline was used as the control in place of plant extract. At various time intervals, the spermatozoa's motility was noted (15, 30 60, 90, 120 and 180 seconds).

Statistical Analysis

Utilizing Graph Pad Prism Version 3.0 for Windows, the data was analysed and presented as mean S.E.M.

RESULTS

Table 1 displays the effect of the *Madhuca indica* leaf aqueous ethanolic extract on sperm motility at various times (duration in seconds). In a concentration-dependent manner, the plant extract significantly (P 0.05) reduced the motility of spermatozoa. When spermatozoa were exposed to a 16% concentration, an immediate immobilisation was seen.

Table 1: Effect of *Madhuca indica* leaves extract on spermatozoa motility (expressed as a percentage). Data are expressed as mean \pm S.E.M.

Concentration	0 seconds	15 Seconds	30 seconds	60 seconds	120 seconds	180 seconds
Normal saline	76.86 \pm 1.00	75.53 \pm 0.85	72.66 \pm 1.50	71.6 \pm 1.34	65.95 \pm 0.65	61.18 \pm 0.68
10mg/ml	27.03 \pm 0.05	26.87 \pm 0.32	25.83 \pm 0.11	25.06 \pm 0.25	24.45 \pm 0.57	23.88 \pm 0.25
20mg/ml	21.02 \pm 0.30	20.57 \pm 0.13	20.26 \pm 0.20	18.88 \pm 0.11	17.76 \pm 0.22	16.17 \pm 0.06
40mg/ml	13.47 \pm 0.24	13.11 \pm 0.01	12.44 \pm 0.11	12.16 \pm 0.20	11.88 \pm 0.02	11.12 \pm 0.01
80mg/ml	9.81 \pm 0.13	9.70 \pm 0.05	9.52 \pm 0.10	9.38 \pm 0.05	8.86 \pm 0.04	8.17 \pm 0.01
160mg/ml	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

DISCUSSION

The results of the current study demonstrated the spermicidal effects of an aqueous ethanolic extract of *Madhuca indica* leaves and demonstrated a concentration- and time-dependent reduction in sperm cell motility. Immediately immobilised rat epididymal spermatozoa were observed in a similar study on the stem bark extract of *Hymenocardia acida* at 10% concentration [6][14]. But when applied, the *Madhuca indica* extract at a 16% concentration immediately immobilised sperm cells. The findings of the present study are in line with those of an earlier investigation that discovered partially purified compounds of ethyl acetate sub-fractions of *Carica papaya* seeds decreased spermatozoa motility when given at a 2% concentration [7][13].

Achyranthes aspera and *Stephania hernandifolia* were reported to have immobilised sperm by impairing motility [8]. The hydro ethanolic extract of *Madhuca* leaves has a concentration- and time-dependent immobilisation effect on rat sperm in vitro, similar to what was seen with *Cestrum parqui* [9].

It has been noted that a variety of phytochemicals found in plants that exhibit spermicidal or sperm immobilisation properties comprise secondary metabolites like saponins, flavonoids, and

phenol compounds [11]. Human sperm are said to virtually instantly become immobile in 20 seconds when exposed to the saponins of *Cyclomen persicum*, *Primula vulgaris*, and *Gypsophila paniculata*. Furthermore, it has been established that the majority of plant-derived spermicides that immobilised sperm in both animals and humans contained saponins [12].

Saponins and other phytoconstituents were detected in the *Madhuca indica* leaf extract. The findings of this study demonstrated the spermicidal properties of the aqueous ethanolic extract of *Madhuca indica* leaves. But more research is required to determine whether *Madhuca indica* is effective as a spermicidal substance.

ACKNOWLEDGEMENT

The authors acknowledge UGC Fellowship for supporting this work.

References:

- Abraham SK, Sarma L, Kesavan PC. Role of chlorophyllin as an in vivo anticlastogen: Protection against gamma-radiation and chemical clastogens. Mutation Research. 1994; 322: 209–212.
- Jain S, Choudhary G. P, Jain D. K. Medicinal plants with potential anti-fertility activity: A review. International Journal of Green Pharmacy. 2015; 9 (4) | 223
- Badukale N A, Panchale W A, Manwar J V, Gudalwar B R, Bakal L, Phytochemistry, pharmacology and botanical aspects of *Madhuca indica*: A review. Journal of Pharmacognosy and Phytochemistry 2021; 10(2): 1280-1286
- Yuan H, Ma Q, Ye L, Piao G, The Traditional Medicine and Modern Medicine from Natural Products. Molecules 2016; 21, 559; doi:10.3390/molecules21050559
- Bala1 K *, Arya M, Katare D P, Herbal contraceptive: an overview. world journal of pharmacy and pharmaceutical sciences, 2014; Vol 3, Issue
- Abu A, Uchendu C, Ofukwu R, Sperm Immobilization Properties of Aqueous Ethanolic Extract of *Hymenocardia acida* Stem Bark. Macedonian Journal of Medical Sciences 2011; 4(3):261-264
- Lohiya N K, Kothari L K ,Manivannan B, Mishra P K, Pathak N. Human sperm immobilization effect of *Carica papaya* seed extracts: an in vitro study, :: Asian Journal of Andrology 2000 ; 2: 103-109

- PaulD, BeraS, JanaD, MaitiR, GhoshD, In vitro determination of the contraceptive spermicidal activity of a composite extract of *Achyranthes aspera* and *Stephania hernandifolia* on human semen, *Contraception*. 2006 Mar;73(3):284-8
- SouadK, AliS, MounirA, Mounir T M . Spermicidal activity of extract from *Cestrum parqui*, *contraception*. 2007 ;75(2):152-6.
- Dubey K, Sridhar C, Jayaveera K. N, Dubey M R. Sperm immobilization activity of aqueous, methanolic and saponins extract of bark of *Ziziphus*, *Pelagia Research Library Der Pharmacia Sinica*, 2011, 2 (2): 11-16
- NR Farnsworth., DP Waller. In: Zatuchni G.I, editor, *Research frontiers in fertility regulation*, 1982, 2:1-16. [12]
- M Ghosh, SP Babu, NC Sukul, SB Mahato, *Indian J. Exp. Biol.*, 1993, 31:604-606.
- Hyacinth A A, Terzungwe A, Owoicho O D and Mathias AA. Evaluation of spermicidal property of aqueous ethanolic extract of *Lawsonia inermis* linn. Leaves. *Annals of Biological Research*, 2012, 3 (8):3846-384
- Remya M, Sharma R. C, Shoaib H, Jyotirmoy R, Asad U Singh S. In vitro effect of *Aegle marmelos* on human sperm motility. *Journal of Medicinal Plants Research*. 2009, Vol. 3(12), pp. 1137-1139