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SPERMICIDAL ACTIVITY OF *NERIUM OLEANDER* FLOWER EXTRACT

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ABSTRACT

Fertility control is an issue of global and national public health concern. The traditional use of medicinal plants to treat different sorts of diseases, including fertility related problems is widespread throughout the world. The study investigated the spermicidal activity of *Nerium oleander* flower extract and the result showed a significant performance of germicidal activity. The sperm mortality was very high when the extract was extract out morphological abnormality employed on the sperm above 5% level and also the morphological abnormalities such as enlargement of sperm head, immobilization and increased mortality were also observed.

KEYWORDS: Flower, sperm, motility.

INTRODUCTION

The current world population is around 6.46 billion and that of India in particular is around 1.1 billion.[1] One of the critical problems of the developing countries like India is its geometrical increase in the human population. This population explosion will have negative impact on our economic policies and would be simultaneously misbalance our socioeconomic infrastructure. Thus the control of human fertility in the sense of its limitation is the most important and urgent requirement. In this search several potential approaches for induction of infertility have been investigated over a long period, including chemical, hormonal, and immunological approaches. However, no suitable method has emerged that is effective and free from side effect.[2,3] Hence, there is a need for development of new fertility regulating drug from medicinal plants because from times immemorial humans have relied on plant

products as sources of drugs and therapeutic agents. In recent times due to low toxicity and long standing experience of exposure, these drugs are used in ethnic medicine system.

MATERIALS AND METHODS

Flower material

The fresh flowers of *Nerium oleander* were collected from Thoothukudi and brought to the laboratory. The botanical identification of the flower was confirmed using Gamble Volume (I-III) and Flora of TamilNadu, India (I-III). Then the flowers were rinsed twice with distilled water and air dried on a clean sterilized paper sheet for one week at room temperature after that it was made into small pieces using sharp sterile scissors and powdered using sterile mortar and pestle.

PHYTOCHEMICAL STUDIES

The extract of the flower was done as per the procedure adopted by Deshpande.[4]

Screening of phytochemicals

Qualitative tests for the identification of various phytochemical constituents were prepared as per the standard procedures.[5-7]

SPERMICIDAL ACTIVITY

1. Test materials

Fresh sheep testes were obtained from the Slaughter house located in Thoothukudi in an aseptic way and brought to the laboratory in a sterile saline container. In the laboratory the cauda portion of epididymes was isolated, dissected out and minced in 0.9% saline solution (pH 7.5) and filtered through a piece of cheese cloth to get sperm suspension. Sperm count above 100-200 million/ml and viability above 60% with normal morphology, rapid and progressive motility was employed for the tests.

2. Preparation of flower extract

The dried flower material of *Nerium oleander*, were homogenized separately with the help of a mortar in physiological saline (pH 7.4). Homogenates were centrifuged at 10,000 rpm for 30 minutes. The pellet was discarded and the supernatant was preserved at $4^{O}C$ for experimental purposes. Using these stock, different concentrations of extracts were (1, 3, 5, 7 and 10%) prepared.

3. Immobilization assay

Different concentration of crude extracts of the plants were mixed with sheep epididymal sperm suspension (100 million/ml~200 million/ml) thoroughly in 1:1 ratio according to a modified method of Waller.[8] A drop of the mixture was placed immediately on a slide and at least five fields were microscopically observed under high power (40X) for assessment of sperm motility. The mixture was then incubated at $37^{O}C$ for 30 minutes and the above process was repeated.

4. EC50 determination

The effective concentration that caused 50% immobilization of highly motile cells (EC50) was determined by different dilutions of the extracts using physiological saline as the dilution medium.[9] Sperm suspension and respective plant extracts were mixed in 1:1 ratio. A drop of the mixture was placed immediately on a slide and five fields were observed microscopically under high power of microscope for the assessment of motility. The results observed were plotted in a graph and the 50% mortality was derived using the graph.

5. Nonspecific aggregation estimation

Different concentrations of extracts (ranging from 1,3,5,7 and 10%) were treated with sheep sperm suspension in 1:1 ratio and kept at $37^{O}C$ for 1 h. Then from the bottom of the microcentrifuge tube, one drop of the sediment sperm was placed on a slide and the percent aggregation was examined microscopically under 400X magnifications. Considering that the non-aggregated spermatozoa will remain in the supernatant, the latter was collected and the turbidity determined spectrophotometrically at 545 nm.[10]

RESULT AND DISCUSSION

The phytochemical analysis of the *Nerium oleander* flower in the present study showed the presence of rich quantity of alkaloid, catachin, coumarin, phenol, quinone, saponin, steroid, tannin, terpenoid, xanthoprotein, glycoside and fixed oil (Table-1).

Table 1: The presence or absence of various phytoconstituents in the *Nerium oleander* flower extract.

Phytochemicals	*Nerium oleander*	Phytochemicals	*Nerium oleander*
Alkaloid	+	Saponin	+
Anthraquinone	-	Steroid	+
Catachin	+	Tannin	+
Coumarin	+	Terpenoid	+
Flavonoid	-	Xanthoprotein	+
Phenol	+	Glycoside	+
Quinone	+	Fixed oil	+

+ present

- absent

Treatment of the sheep sperms with the flower extract of the *Nerium oleander* showed excellent and effective results in killing and immobilizing the status of the sperms. 50% motility was observed in the concentration of 4.2% of the extract (Figure – 1) and it was almost touched 90-100% at 7 and 10% of the extract respectively. The treated sperms with the flower extract showed that the extract caused structural and functional abnormalities on the sperms. While the live sperm collected from the epididymal region of the control sheep showed normal counts, motility and morphology. The sperms which were treated with the extract of the flower of *Nerium oleander* showed the evidence of dose dependent toxicity. The mortality of the sperm was gradually increased to a high level when the concentration of the extracts increased. The treatment of sperm cells with the extract of *Nerium oleander* (Table-2) caused a highly significant decrease in the count, morphological change and also immobility.

Fig - 1 EC 50 observed for Nerium oleander

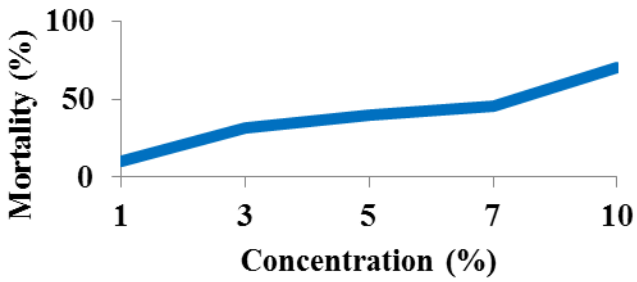

Table 2: Total sperm count of sheep treated with different concentrations of the *Nerium oleander* extract. Values are the mean of three observations and ±SD. Values indicated in the parenthesis are the percent reduction in the sperm count.

Concentration %	Total live count m/ml	Motile %	Abnormalities observed %			Mortality %
			Head	Tail	Agglutination	
Control	8.8±0.1	96.0	-	-	-	4.0
1	7.86±0.05 (10.60)	89.77	-	-	-	10.23
3	6.13±0.15 (30.30)	68.18	-	-	-	31.82
5	5.33±0.05 (39.39)	60.22	-	-	-	39.78
7	4.76±0.15 (45.83)	54.54	-	1	3	45.46
10	2.46±0.15 (71.96)	29.54	-	3	2	70.46

The sperm motility was very much inhibited by the extract of flower when the concentration of the extract exceeds above 5% level. When the concentration of the extract exceeds above 5% level the sperm cells had developed some abnormal morphologies like enlargement of head, damages in the head, coiling of tails, fusion of two or more sperm cells (agglutination) and so on resulted in immobility. The survival rate of the sperm cells when applying the flower extract was time dependent. When the time as well as the concentration of the extracts applied on sperm cells, the mortality rate of the sperm cells increased significantly (Table-3) as well as the morphology, anatomy and physiology of the sex organs of the rates. Depletion of live count and the development of abnormalities and clumping of sperm cells suggested clearly that the extracts had some spermicidal activity.

Table 3: Time dependent survival rate of sperm at different concentration of *Nerium oleander* flower extracts. Values are the mean of three observations ± SD. Percent mortality of sperms indicated in parenthesis.

Flower extract	5mins	10mins	15mins	20mins	25mins
Control	8.66±0.15	7.86±0.15	6.96±0.15	6.5±0.1	6±0.1
5%	8±1 (7.62)	7±1 (10.94)	6.33±1.52 (9)	4±1 (38.46)	1.66±1.52 (72.22)
10%	6.6±0.2 (23.78)	6.13±0.15 (22.01)	5.66±0.15 (18.67)	3.56±0.15 (45.23)	2.9±0.1 (51.66)

Among the plant based contraceptives, inhibition of male fertility after administration of natural products has been related to decreased spermatozoa density. Previous workers reported that several plants has spermicidal potentials and effectively kill the sperms [*Gossypium herbaceum* on human,[11] *Andrographis paniculata* on rats,[12] *Calotropis procera* on mice,[13] *Curcuma longa* in rats,[14] *Achyranthes aspera* in rat,[15] *Aegle marmelos*

on rat,[16] *Albizia procera* in rat,[17,18] and *Alstonia scholaris* on rats.[17,18] For male sterility it is not necessary to stop spermatogenesis, but rather to reduce the fertilizing ability of the spermatogenesis, by causing changes in the morphology, and functions of the sperm such as motility.[20-22]

CONCLUSION

The study concluded that the extracts of *Nerium oleander* has some spermicidal effect such as immobilizing the sperms, clumping of sperms and so on and this might be due to the phytoconstituents such as saponins[18] and steroids[23] present in it. In the present study only the crude aqueous extract was employed. The actual component responsible for the antifertility action was not recognized. Identifying the actual component responsible for the spermicidal action will open a new avenue in the field of medical sciences and human welfare.

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