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COMPARATIVE STUDY ON THE SPERMICIDAL ACTIVITY OF DATURA METAL AND ALLAMANDA CATHARTICA FLOWER EXTRACTS

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ABSTRACT

A comparative study was planned to investigate the spermicidal activity of the extracts of *Datura metal* and *Allamanda cathartica* flowers. The *Datura metel* extract was identified as the best one and hence it was used for further indepth spermicidal activity. The extract was administered to Rates at different doses and the reproductive activity of the rat was assessed. The extract showed remarkable alterations on the morphology and physiology of the sperm cells. There was a notable reduction in the sperm count, reduction in the size of testes, epididymis and production of testosterone.

KEYWORDS: *Datura metal*, *Allamanda cathartica*, Epididymes, Spermicidal, Seminal vesicle.

INTRODUCTION

Rising human population throughout the world more particularly in developing and under developing countries has detrimental effects on the life supporting system on the earth. Fertility regulation comprising contraception and management of infertility forms an important component of reproductive health. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception among women, possibilities on men are still lacking or limited. With recent progress towards a better understanding of male reproductive physiology there is a need to develop new contraceptive modalities for male. Among the products presently available for birth control, spermicides are a mean that can be totally controlled by the woman and are very reliable compared to other contraceptives in common use. However, they cause irritation in the

vaginal epithelium due to their tensoactive effect on cellular membranes which might enhance the risk of acquiring sexually transmissible diseases.

In searching for new alternatives, it was observed that a wide variety of plants have spermicidal activity. Hence it is interesting to consider potential contraceptives of vegetable origin. Recently efforts are being made to explore the hidden wealth of medicinal plants for contraceptive use. There has been a steady accumulation of information regarding the screening of plants having antifertility efficacy. The folklore information and the ancient literature about the plants and herbs can help the antifertility program. In the recent past a number of plants have been identified and evaluation of extracts and active principles from different parts such as, stem, barks, seeds, roots, leaves, flowers and so on have been used by various researchers. These reports have been exhaustively reviewed by several authors.[1-6] However, the search for an orally active, safe and effective plant preparation or its compound is yet to be needed for fertility regulation due to incomplete inhibition of fertility or side effects.

Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine. A large number of plant species with anti-fertility effects have been screened in China and India beginning about 50 years ago and were subsequently fortified by national and international agencies.[7,8]

MATERIALS AND METHODS

Flower material

The fresh flowers of *Datura metal* and *Allamanda cathartica* were collected from Thoothukudi and brought to the laboratory. The botanical identification of the flowers 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 by simple extraction method.[9]

Screening of phytochemicals

Qualitative tests for the identification of various phytochemical constituents were prepared as per the standard procedures.[10-12]

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 *Datura metal* and *Allamanda cathartica* 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.[13] 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)[14] was determined by different dilutions of the extracts using physiological saline as the dilution medium. 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 hr. Then from the bottom of the microcentrifuge tube, one drop of the sedimented 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.[15]

Antifertility Effect of *Datura Metel* Aqueous Flower Extract on Male Albino Rats

1. Flower extracts preparation

100gms of the dried powdered flower of *Datura metel* was taken and mixed with 500ml of distilled water and magnetically stirred in a container for overnight at room temperature. The residue was removed by filtration and the aqueous extracts were lipolization and concentrated under vacuum to get solid yield of 10%.

2. Animals

Adult male Wistar rats weighing around 180-200g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each) at an ambient temperature of 25 ± 2^{0}C and 55- 65% relative humidity 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

3. Experimental design

Group I: Control rats.

Group II: The rats were injected daily in the morning with *Datura metel* aqueous extract (500 mg/kg body wt.) for 30 days. The solid extract was dissolved in deionized distilled water in an appropriate amount and injected. It was freshly prepared every day. After 30 days the animals were narcotized and the testes along with the epidiymis was carefully removed from the body in an aseptic way using sterile instruments.

Group III: The rats were treated with *Datura metel* aqueous extract (700 mg/kg body wt.) for 30 days.

4. Estimation of sperm motility and count

The spermatozoa was obtained by making small cuts in caudae epididymis and vasdeferens were placed in 1ml of modified Krebs Ringer-bicarbonate buffer (pH 7.4) and the sperm suspension was evaluated for sperm content, percent motility. The percent motility was determined by the progressive and non-progressive movements of sperms observed under a compound microscope. The sperm count was determined under a Neubauer haemocytometer.[16,17] To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air dried and made permanent.

5. Serum testosterone

Serum levels of testosterone were assayed in duplicate using specific RIA (Radioimmuno assay) method. Serum samples were separated by standard procedure and stored at -20^{o}C for subsequent analysis.

RESULT AND DISCUSSION

The results of phytochemical analysis of the *Datura metal* flower in the present study showed that the flower are rich in alkaloid, coumarin, phenol, quinone, saponin, steroid, tannin, glycoside and fixed oil. The phytochemical analysis of *Allamanda cathartica* shows the flowers are rich in alkaloid, catachin, coumarin, phenol, quinine, saponin, steroid, terpenoid, glycoside and fixed oil.

Table 1: The presence or absence of various phytoconstituents in the studied flower extract.

Phytochemicals	***Datura metel***	***Allamanda cathartica***	**Phytochemicals**	***Datura metal***	***Allamanda cathartica***
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 extracts of the studied flowers showed excellent and effective results in killing and immobilizing the status of the sperms. The treated sperms with the flower extracts showed that the extracts caused structural and functional abnormalities on the sperms. Depletion of live count and the development of

abnormalities and clumping of sperm cells suggested clearly that the extracts had some spermicidal activity.

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 extracts of the flower of *Datura metel*, and *Allamanda cathartica* showed the evidence of dose dependent toxicity.

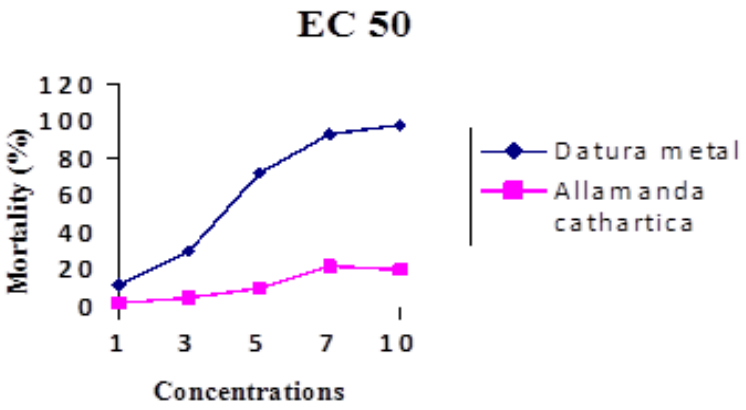

Figure 1: Showing the EC 50 observed for different flower extracts.

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 *Datura metel* (Table-2) caused a highly significant decrease in the count, morphological change and also immobility. 50% motility was observed in the concentration of 2.5% of the extract (Figure – 1) and it was almost touched 90-100% at 7 and 10% of the extract respectively.

Table – 2: Total sperm count of sheep treated with different concentrations of the flower extract *Datura metel.* 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	7.5±0.1 (14.77)	88	-	-	-	12
3	5.8±0.1 (34.09)	70	1	-	-	30
5	2.433±0.15 (72.34)	27.28	1.6	13	21	72.72
7	0.66±0.05 (92.42)	6.81	1.5	16	33	93.19
10	0.13±0.05 (98.48)	1.14	-	-	-	98.86

The flower extract of *Allamanda cathartica* showed very poor performance in the inactivation of sperm cells of sheep (Table-3). It did not show 50% mortality even in the higher concentration (Figure 1) studied. Among the both extracts studied the *Datura metel* extract exhibited best performance and the *Allamanda cathartica* exhibited least performance.

Table 3: Total sperm count of sheep treated with different concentrations of the flower extract *Allamanda cathartica.* 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	8.6±0.1 (2.27)	97.73	-	-	-	2.27
3	8.46±0.15 (3.78)	94.32	-	-	-	5.68
5	7.73±0.15 (12.12)	89.77	-	-	-	10.23
7	6.73±0.11 (23.48)	77.27	-	2	3	22.73
10	7.16±0.15 (18.56)	79.55	-	4	1	20.45

The sperm motility was very much inhibited by the extracts of flowers 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 occurrence of such kind of abnormality was significantly high in the extracts of *Datura metel* and it was decreased in the *Allamanda cathartica* (Table-3&4). The survival rate of the sperm cells in both flower extracts were time dependent. When the time as well as the concentration of the extracts applied on sperm cells, the mortality rate of the sperm cells also increased significantly (Table-4).

Based on the results obtained from the above experiment, the best extract was identified as the *Datura metel* and hence it was used for further indepth spermicidal activity. The extract was administered to the rats at different dose and its reproductive activity was assessed including its effect on the physical and morphological alteration on the sperm cells.

Table 4: Time dependent survival rate of sperm at different concentration of different flower extracts. Values are the mean of three observations ± SD. Percent mortality of sperms indicated in parenthesis.

Flower extract	Concentration	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
Datura metel	5%	5±1 (42.26)	5.33±1.52 (32.14)	4±1 (42.52)	3±1 (53.84)	1±1 (83.3)
	10%	5.43±0.15 (37.29)	4.63±0.15 (41.09)	3.2±0.1 (54.02)	2.16±0.15 (66.76)	0.83±0.15 (86.16)
Allamanda cathartica	5%	7.66±1.52 (11.47)	7±1 (10.94)	6±1 (13.79)	4.33±1.52 (33.33)	2.66±1.52 (55.55)
	10%	6.1±0.1 (29.56)	6.66±0.15 (15.26)	5.63±0.15 (19.10)	3.9±0.1 (40)	2.06±0.15 (65.66)

The rats treated with the *Datura metel* extracts dose (500mg/kg of body wt and 700mg/kg of body wt) for 30 days showed a very good alteration in the reproductive system resulted in the sign of functional male sterility. During treatment no significant clinical and behavioral changes were observed in both the control and treated animals. The extract caused no effect on the body weight of the animal; the weight gain was normal and it was as in the case of control animal. But the *Datura metel* flower extract treated rats showed a significant ($p<0.05$) decrease in the weight of the reproductive organ and accessory organs namely testis (10.50 and 36.50), epididymis (11.72 and 35.57%) and seminal vesicles (18.26 and 31.94%) respectively in both groups than the control. In the group III animals which were treated with high dose of the extract the sex organs weight were reduced to a significant level ($p<0.05$) when compared to the group II as well as group I, the control animal. A steep fall in serum testosterone was also observed in both groups of treated animal when compared to the control (Table -5). The level significantly showed a decline in both the treated group and the control (45.2 and 77.5% respectively).

The sperm of the control rats had normal count, mortality and morphology. But in the treated rates the epididymal sperm parameters showed remarkable alterations which were dose dependent in nature. The sperm counts were significantly ($p<0.05$) decreased in group II and group III animals. 42.79% and 61.26% reduction in sperm count was observed in group II and III respectively than the control. The sperm motility percentage was also drastically reduced to 32.98% and 69.47% respectively in case of group II and group III animals than the control animal (Table – 5).

Table 5: Sterility effect of *Datura metel* flower aqueous extract on the male albino rats. The values are the mean of three observations and ± SD. The values are significant at p<0.05 level.

Treatment	Body weight		Reproductive organ weight (mg/100g bd wt)			Serum testosterone ng/ml	Total count m/ml	Motile %
	Initial	Final	Testis	Epididymis	Seminal vesicle			
Group I control	183.33 ±2.08	201 ±2	806.33 ±59.18	432.33 ±2.51	480.33 ±1.52	4.27 0.02±	7.4 ±0.1	95 ±1
Group II (500mg/kg body wt.)	186 ±1 (1.63)	204 ±1 (1.49)	721.33 ±1.52 (10.50)	381.33 ±1.52 (11.72)	392.33 ±2.51 (18.26)	2.34 ±0.02 (45.19)	4.23 ±0.15 (42.79)	63.66 ±1.52 (32.98)
Group III (700mg/kg body wt.)	193.33 ±1.52 (5.64)	206.66 ±1.52 (2.81)	511 ±1 (36.60)	278.33 ±1.52 (35.57)	326.66 ±1.52 (31.94)	0.97 ±0.02 (77.28)	2.86 ±0.15 (61.26)	29 ±1 (69.47)

The extract treated male rats clearly indicated that, the extract caused structural and functional alterations in the reproductive system. There was not much change observed in the sperm morphology both in control and treated animals. The extract functioned as a placebo in both the groups of animals. It showed no side effects in the animal. The depletion in the size of the testes and epididymis in the treated animals resulted in the reduction in the sperm production of the animals. As the dose of the extract increased, further fall was observed. Decrease in sperm motility indicated alterations in sperm maturity in the epididymis.

The changes observed in both sperm count and motility resulted in complete infertility in the animals. This resulting abnormal sperm functions ultimately gave rise to complete male sterility in due course. Testosterone is one of the vital sex hormone essential for the normal functioning of the reproductive structures and sexual behavior. The steep fall in the level of testosterone in the present study in treated animals (45.19% in group II and 77.28% in group III) may be one of the vital causative factors in the fall in sperm count resulting in sterility (Table -5).

Among the plant based contraceptives, inhibition of male fertility after administration of natural products has been related to decreased spermatozoa density was reported by several workers in several plants (*Gossypium herbaceum* Linn. on human,[18] *Andrographis paniculata* Wall. Ex Nees on rats,[19] *Calotropis procera* (Ait.) R.Br. on mice,[20] *Curcuma longa* Linn. in rats,[21] *Achyranthes aspera* Linn**.** in rat,[22] *Aegle marmelos* Corr.ex Roxb on rat,[23] *Albizia procera* Roxb. Benth. in rat[24,25] and *Alstonia scholaris* R.Br. on rats[25,26]). 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.[27-29]

In the present study also the application of the *Datura metel* extract on the animal caused marked alterations in the male reproductive system of rat. But it did not cause any behavioral changes on the animal.[29]

Only crude aqueous extract was employed in the study and hence the actual component responsible for the antifertility action has to be recognized and then only it could be tried on humans. The phytocomponents such as the terpenoid, saponin, steroid, coumarin, glycoside, flavonoid and other phenolic components present in the flowers of *Datura metel* and *Allamanda cathartica* may act as the sperm suppressor components.[30-32] Gupta reported that the component saponins had the antifertility effect when injected on rat.[24] Raghavendra stated that the component steroid has antifertility activity when given to rats. To evolve a valuable and promising component from this flower further indepth study is needed.[33]

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