International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 9, Issue 1, 2017

Original Article

RUELLIA TUBEROSA LINN. ACTS AS ANTI-FERTILITY AGENT THAT REDUCES SPERM COUNT, MOTILITY AND VIABILITY IN MALE SWISS ALBINO MICE (*MUS-MUSCULUS)*

PARDESHI M. H.[1,2], DESHMUKH A. A.[1,3], GAJARE K. A.[1,4*]

[1]Department of Zoology, Govt. Vidarbha Institute of Science and Amravati, [2]Department of Zoology, Arts Commerce and Science College, Maregaon, [3]Department of Zoology, Shivaji University Kolhapur, [4]Department of Zoology, The New College, Kolhapur
Email: kavita_kanu@rediffmail.com

Received: 01 Oct 2016, Revised and Accepted: 05 Dec 2016

ABSTRACT

Objective: Fertility control is an issue of global public health. Many of the contraceptives available today have one or the other side effects. Many plants and plant products are suggested as contraceptives in folk and traditional systems of medicine. However, that are least exploited in this regard. In the present investigation, root powder of *Ruellia tuberosa* was studied for its effect on male reproduction in mice.

Methods: The Swiss albino mice, *Mus musculus* of age three months were grouped into four, i)control group, fed on standard pellet, ii)experimental groups I and II received root powder of *Ruellia tuberosa* 50 mg/mouse/days for 15 d and 30 d respectively in the pellets, iii)positive control groups I and II received cotton seed oil 25 µl/mouse/day for 15 and 30 d and iv)recovery group received *Ruellia tuberosa* (50 mg/mouse/days) containing pellets for 15 d and later standard pellet for 15 d. Cauda epididymis sperm suspension was analyzed for sperm count, motility and viability.

Results: There was a highly significant decrease in sperm count, motility and viability ($p<0.001$) in experimental groups I and II and positive control groups I and II. The sperm count was reduced to 19.24±1.74 million/ml and 15.97±5.61 million/ml as compared to sperm count in control group (55.12±4.63 million/ml) in experimental groups. Partial reversal of the effect was noticed in a recovery group.

Conclusion: The results suggest that *Ruellia tuberosa* can be a potent member of reversible oral male contraceptives.

Keywords: *Ruellia tuberosa,* Sperm count, Sperm motility, Sperm viability

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DOI: http://dx.doi.org/10.22159/ijcpr.2017v9i1.16627

INTRODUCTION

Population explosion is the root cause of many socio-economical problems. Due to this, birth control is one of the prime important program**s**. Many contraceptive methods available are having their merits and demerits. Plant-based substances are not much exploited in this regard. Nature is the source of many chemical agents which can be used as drugs for thousands of years and an impressive number of modern drugs have been isolated from natural sources [1]. Many plant products are used by tribal and folk medicines as contraceptives. Many methods for birth control are targeted to women except a few. Orally administered men contraceptives are rare. The side effects and inconvenience of presently practiced male contraceptive methods prevent their universal acceptance [2, 3]. Development of additional plant based male contraceptive methods can provide tremendous Social and public health benefits. In this regard, root powder of *Ruellia tuberosa,* an annual herb, was studied for its effect on male reproduction.

Ruellia tuberosa (family-Acanthaceae) is commonly known as Minnie root or popping pod. *Ruellia tuberosa* is used in folk medicine due to its antidiuretic, antidiabetic, antipyretic, analgesic and antihypertensive properties [4]. On Grenada Island the tuberous roots, leaves and flowers are used in the treatment of common cold, fever and hypertension [5]. It is also included in a decoction for the treatment of male impotency in a drink called mamajuana in the Dominican Republic. Tuber powder (5-10 gm) is given with milk for treating abdominal pain after delivery [6]. However, the scanty information is available on its effects on the reproductive system.

MATERIALS AND METHODS

Collection and preparation of plant material

The roots of *Ruellia tuberosa Linn*. were collected from the campus of Government Vidarbha Institute of Science and Humanities, Amravati in the month of October and November and the material was identified by taxonomist of Botany Department Dr. Mrs. Prabha Y. Bhogaokar, Head, Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati.

The cleaned roots were dried under shade at room temperature and powdered by the mechanical grinder and sieved through a muslin cloth. This powder was used for oral administration.

Experimental animals

The experimentation was carried out by due permission of the institutional animal ethics committee, Govt. Vidarbha Institute of Science and Amravati (1060/ac/07/CPCSEA). The breeding pairs of albino mice were procured from the Institute of Pharmaceutical Education and Research Wardha and reared in the departmental animal house in standard plastic cages with rice husk bed at the bottom. Three months healthy male Swiss albino mice weighing between 25-30 gm were used for the present investigation. Mice were divided into following groups:

1) Control group

Animals of this group were fed on standard laboratory animal feed, prepared in our laboratory(100 gm Wheat flour, 12 gm milk powder, 1 ml Cod liver oil, 2.4 ml Groundnut oil, 1 Multivitamin capsule and a pinch of Salt) 3 gm/mouse/day and water *ad libitum* [7].

2) Experimental group

I) The mice from the experimental group I, received a diet supplemented with root powder of *Ruellia tuberosa* 50 mg/mouse/day in 3 gm feed for 15 d.

II) Animals of experimental group II, were fed with diet containing root powder of *Ruellia tuberosa* at the dose of 50 mg/mouse/day in 3 gm feed for 30 d

3) Positive control group

I) Animals of positive control group I, were fed with Pellet containing crude cotton seed oil at the dose of 25 µl/mouse/day in 3 gm feed for 15 d.

II) Animals in positive control group II, were fed with a diet containing crude cotton seed oil at the dose of 25 µl/mouse/day in 3 gm feed for 30 d.

4) Recovery group

The animals of this group were fed with a diet containing root powder of *Ruellia tuberosa* at the dose of 50 mg/mouse/day in 3 gm feed for first 15 d and later on standard pellets (i.e. diet without root powder) for next fifteen days.

On completion of the treatment, the animals were weighed and then sacrificed by cervical dislocation and testis and the epididymis were removed, weighed and used for semen analysis.

Preparation of sperm suspension

100 mg of cauda epididymis was teased in 2 ml Ringer Tyrode solution (Ringer Tyrode solution: 0.8 gm NaCl, 0.02 gm KCl, 0.02 gm $CaCl_2$, 0.1 gm $NaHPO_4$, 0.005 gm NaH_2PO_4, 0.01 gm $MgCl_2$) and filtered through a piece of cheesecloth to get sperm suspension [8]. This sperm suspension was used for sperm function tests which are as follows-

Sperm count [9, 10]

Semen samples were analysed for sperm count using Neubauer's Chamber slide, as per the WHO manual 1992 and Prasad *et al.* 1972.

Sperm motility [9, 10]

Epididymal suspension was analysed for percent motility as per the WHO manual 1992 and Prasad *et al.* 1972. Percent motility was determined by counting both motile and immotile spermatozoa. Motile sperm count was also carried out.

Sperm viability test [9, 11]

0.2 ml of semen sample was mixed with 0.2 ml of 0.1% trypan blue, incubated for 15 min at 37 °C. A drop of the suspension was then placed on a pre-cleaned slide and observed for stained and unstained spermatozoa under 400X magnifications. Plasma membrane of the live spermatozoa is impermeable to trypan blue. Therefore live spermatozoa remain colourless while dead spermatozoa stained blue in colour. Percent viability and viable sperm count were carried out.

Statistical analysis

The results were expressed as mean±standard deviation. Statistical analysis was carried out by using one-way ANOVA.

RESULTS

Sperm count

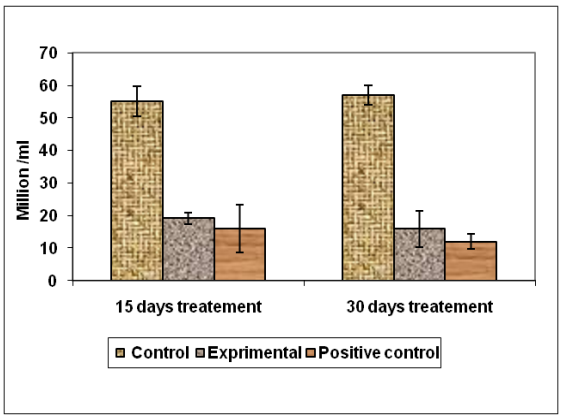
The sperm count was reduced to 19.24±1.74 million/ml after 15 d treatment of root powder of *Ruellia tuberosa* which was 55.12±4.63 million/ml in control group. The results were highly significant ($p<0.001$). Similarly, when the treatment was extended for 30 d, the sperm count was reduced to 15.97±5.61 million/ml. The results were highly significant ($p<0.001$) (table 1, Graph 1). It suggests the anti-spermatogenic property of *Ruellia tuberosa.* Abnormal sperms were not found.

There was 15.98±2.04 million/ml sperm count after 15 d treatment of cotton seed oil and 12.01±2.37 million/ml after 30 d treatment.

Table 1: Effect of *Ruellia tuberosa Linn*. on sperm count, motility and viability in male swiss albino mice (*Mus musculus)*

Animal groups	Treatment	Sperm count	% Sperm motility	Motile sperm count (Millions/ml)	% sperm viability	Viable sperm count (Millions/ml)
Control	15 d	55.12±4.63	59.2±2.59	32.61±2.82	77.6±3.44	42.26±4.53
	30 d	56.95±3.01	56.95±2.59	33.68±1.47	76.6±3.44	43.65±3.52
Experimental (Treatment:	15 d	19.24±1.74***	41.2±1.92***	7.92±0.66***	72.2±2.05*	13.90±1.45***
Root powder of *Ruellia tuberosa*)	30 d	15.97±5.61***	38.2±1.92***	6.16±2.42***	65±0.71**	10.39±3.66***
Positive Control (Treatment:	15 d	15.98±7.29***	24.4±0.55***	3.88±1.72***	60±1.22***	9.59±4.39***
Cotton seed oil)	30 d	12.01±2.37***	9.2±2.59***	0.94±0.48***	54.6±0.55***	6.56±1.33***

*= Significant ($p<0.05$) **=Moderately significant ($p<0.01$) ***=Highly significant ($p<0.001$), When compared with Control

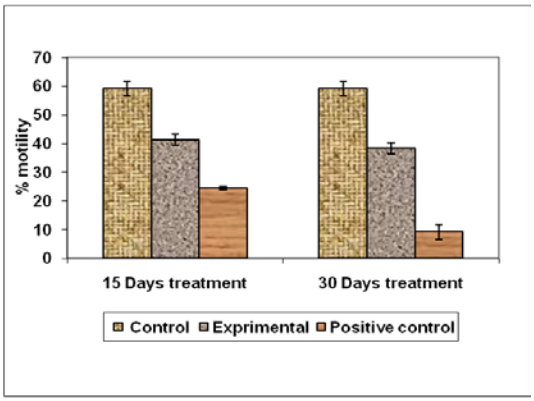


Graph No 1: Effect of *Ruellia tuberosa* on sperm count in albino mice

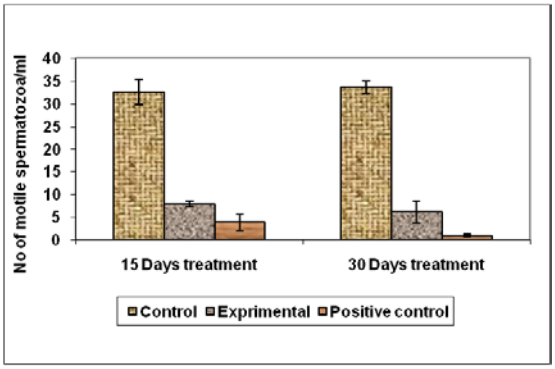
Sperm motility

The forward sperm motility was significantly reduced with the treatment of root powder of *Ruellia tuberosa* for 15 d and 30 d. The motile sperm count was 7.92±0.66 million/ml after 15 d and 6.16±2.42 million/ml after 30 d treatment of root powder of *Ruellia tuberosa*. This decrease was highly significant ($p<0.001$). It was 41.2±1.92% after 15 d treatment and 38.2±1.92 % after 30 d treatment (table 1, Graph 2, 3).

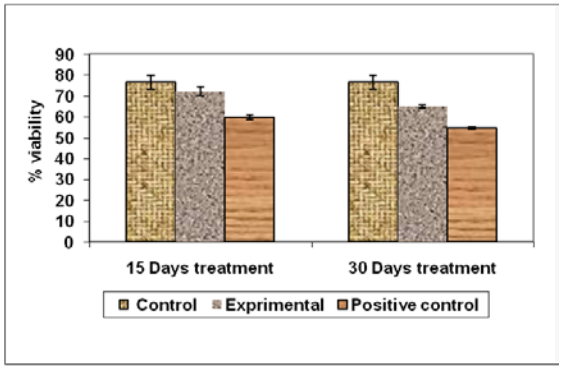
The sperm motility was found to be reduced in the positive control group also. The number of motile sperms was 3.88±1.72 million/ml after 15 d treatment and 1.06±0.25 million/ml after 30 d treatment. The result was highly significant ($p<0.001$) as compared to experimental and control groups.



Graph No 2: Effect of *Ruellia tuberosa* on percent sperm motility in albino mice



Graph No 3: Effect of *Ruellia tuberosa* on motile sperm count in albino mice



Graph No 4: Effect of *Ruellia tuberosa* on percent sperm viability in albino mice

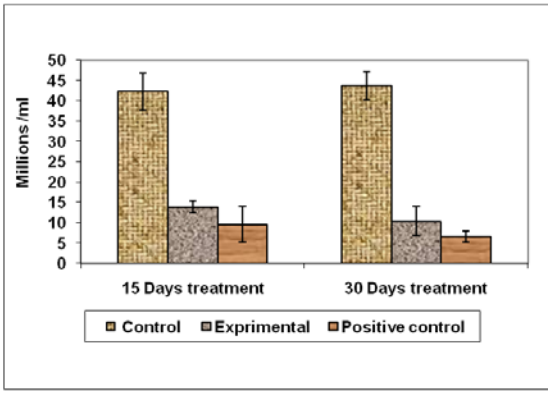
Sperm viability

In experimental group13.90±1.45 million/ml sperms were viable after 15 d treatment, which was further reduced to 10.39±3.6 million/ml after 30 d of treatment. The results were significant ($p<0.01$).

In the positive control group, the viability was 9.59±4.39 million/ml and 6.56±1.33 million/ml after 15 and 30 d of treatment respectively (table 1, Graph 4, 5).

Recovery group

Recovery of the sperm count and motility was studied after 15 d of termination of the treatment of *Ruellia tuberosa*. The sperm count and motility were increased in this group. The sperm count was 27.23±0.7 millions/ml in which 41.5% sperms were having forward motility. The results were significant at $p<0.05$ and $p<0.001$ respectively for sperm count and forward motility. The results are shown in table 2, Graph 6, 7, 8.

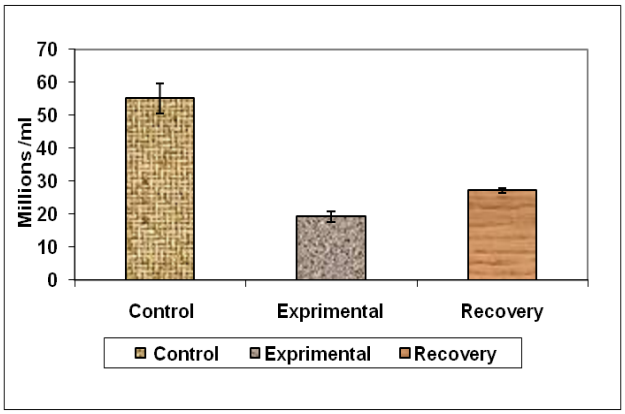


Graph No 5: Effect of *Ruellia tuberosa* on viable sperm count in albino mice

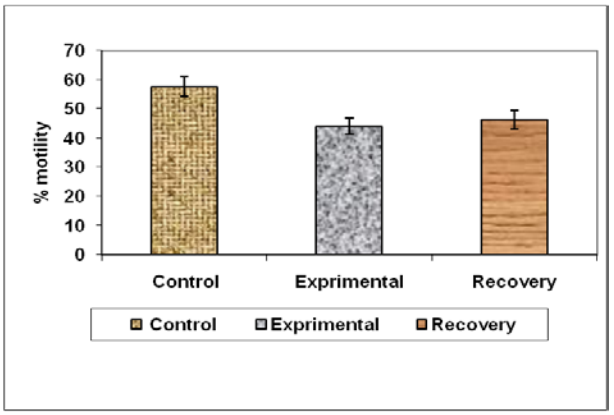
Table 2: Effect of root powder of *Ruellia tuberosa* on sperm count and motility after discontinuation of treatment

Animal groups	Sperm count (Millions/ml)	Motility (%)	Motile sperm count (Millions/ml)
Control	55.12±4.63	57.6±3.36	31.71±2.62
Experimental (Treatment: Root powder of *Ruellia tuberosa*)	19.24±1.74***	44.0±2.74**	8.45±0.74***
Reverse treatment (15 d treatment followed by 15 d normal diet)	27.23±0.70***	46.25±3.1**	12.97±0.30***

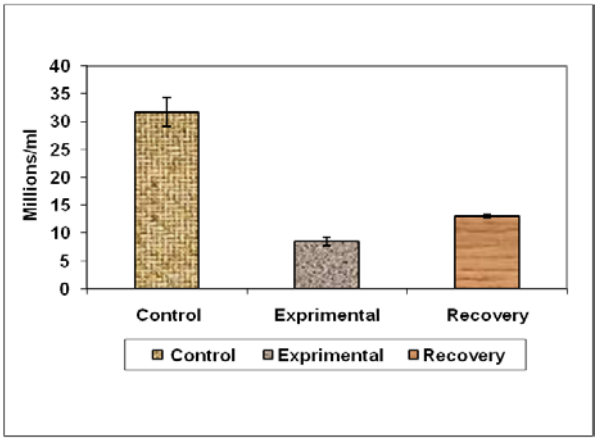
*=Statistically significant ($p<0.05$)**= moderately significant ($p<0.01$) ***=Highly significant ($p<0.001$)When compared with Control



Graph No 6: Effect reversal of the treatment of *Ruellia tuberosa* on sperm count in albino mice



Graph No 7: Effect reversal of the treatment of *Ruellia tuberosa* on percent sperm motility in albino mice



Graph No 8: Effect reversal of the treatment of *Ruellia tuberosa* on motile sperm count in albino mice

DISCUSSION

The sperm count lowering ability suggests that the root powder of *Ruellia tuberosa* has antifertility action. The results were comparable with the results obtained from the treatment of cotton seed oil (Graph 1). The decreased sperm count may be due to decreased cell division i.e. the rate of spermatogenesis was seemed to be affected. Lohiya *et al.* showed chloroform extract of *Carica papaya* seeds induce azoospermia in rabbits [12, 13] and gradually decrease in sperm concentration in langur monkeys [14]. Ethanolic extract of *Lagenaria breviflora* was found to reduce testicular mass and sperm count by inhibiting spermatogenesis in Wistar rats [15]. Similar results were shown by Parveen *et al.* [16] due to the treatment of *Quassia amara* and by Chinoy and Padman [17] after the treatment of *Carica papaya*. All these workers reported that antifertility activity is associated with low sperm count. Thus, root powder of *Ruellia tuberosa* also has antifertility property as it lowered the sperm count in 15 and 30 d. Prolonged treatment may be more effective. Abnormal sperms or immature sperms were not found in epididymal sperm suspension after the treatment. This indicates spermiogenesis was not affected.

The reduced number of forward motile sperms emphasised the contraceptive ability of the root powder of *Ruellia tuberosa* (Graph 3), because such a lower percentage of motile spermatozoa affect fertility. Inadequate concentration, sluggishly motile or immotile spermatozoa could not penetrate the cervical mucus and thus failed to fertilise the ova [18, 19]. There are many evidences showing low motility resulted into infertility. *Colebrookia oppositifolia* leaf extract was found to reduce sperm count and motility [20]. Gupta *etal*. [21] have also demonstrated the contraceptive efficacy of *Strychnospotatorum* seed extract in male albino rats in which sperm motility was found to be reduced in treated group. Treatment of ethanolic extract of *Lagenaria breviflora* R. caused lower sperm motility in rats [15] (Saba *et al.* 2009). Sathiyaraj *et al.* [22] demonstrated that antifertility was caused by inhibition of sperm motility due to the treatment of aqueous leaf extract of *Andrographis paniculata* in male albino rats. Reduced sperm motility and motile sperm count up to 10.82% of the normal sperm count on the treatment of root powder of *Ruellia tuberosa* also suggests its contraceptive efficacy. The reduction in the sperm motility in epididymal sperms is important with regard to low chances of fertilisation [23]. Khan and Awasthy [24] suggested that maintaining the microenvironment of the epididymis is important for sperm maturation. Inhibition of sperm motility during the treatment of root powder of *Ruellia tuberosa* suggests that the epididymis may be one of the targets of the drug.

Treatment of root powder *Ruellia tuberose* found to affect viability (Graph 5). Viability tests are related to sperm membrane integrity. It is a test to study the intactness of sperm membrane. Qiu *et al.* [25] showed membrane damage in 100% human spermatozoa when treated with a crude extract of *Polygala tenuifolia*. More than 50% reduction in the sperm viability occurred in sperms treated with *Allium sativum L*. extract [8]. Chloroform extract of *Butea monosperma* (Lam) caused irreversible damage to sperms and reduction of sperm viability [26]. Many antifertility plants and plant products were demonstrated to damage sperm membrane. One of the mechanisms for loss of motility of spermatozoa may be loss of membrane integrity.

Farnsworth and Waller [27] have screened a large number of plants for spermicidal property. They reported that the majority of plant derived spermicides were triterpene saponins of several structural types, flavonoids and phenol components. Arirudran *et al.* [28] found presence of tannin, flavonoids, steroid, triterpenoid and phenol in phytochemical analysis of *Ruellia tuberosa*. Chothani *et al.* [29] also showed presence of saponin, alkaloids, triterpenoids, phenolic, sterols and flavonoid in phytochemical analysis of crude *Ruellia tuberosa* extract. These components might be playing an important role in spermicidal action of root powder of *Ruellia tuberosa*.

TPR (tannins from Pomegranate rind) could congeal seminal plasma proteins and inhibit mobility, react with sperm membrane protein for decreasing stability and integrity [30]. Tannins are also found to be present in *Ruellia tuberosa* therefore the membrane damage and loss of sperm motility caused by *Ruellia tuberosa* may be due to similar mechanism as shown by Zhou *et al.* [30].

There was increased sperm count, sperm motility and motile sperm count after reversal of the treatment of root powder of *Ruellia tuberosa* within 15 d. These results indicate that root powder of *Ruellia tuberose* affects process of spermatogenesis and sperm maturity in epididymis, but it does not damage the structural components of the organs. Therefore the treatment of *Ruellia tuberosa* is reversible. Thus, *Ruellia tuberosa* can be a potent antifertility agent with reversible action.

CONCLUSION

The root powder of *Ruellia tuberosa* reduced sperm count and sperm motility. It was responsible for sperm membrane damage. These evidences suggest that root powder of *Ruellia tuberosa* has a potential to act as a male contraceptive. An effective, reversible, oral male contraceptive can be formulated from the rootpowder of *Ruellia tuberosa.*

CONFLICT OF INTERESTS

Declared none

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How to cite this article

- Pardeshi MH, Deshmukh AA, Gajare KA. *Ruellia tuberosa linn*. acts as an anti-fertility agent that reduces sperm count, motility and viability in male swiss albino mice (*mus-musculus)*. Int J Curr Pharm Res 2017;9(1):105-109.