Trends in Life Sciences
An International Peer-Reviewed Journal
www.sciencejournal.in

ANTISPERMATOGENIC EFFECT OF THE AQUEOUS ROOT EXTRACT OF *RUELLIA TUBEROSA* L. ON ALBINO RATS

Bhogaonkar P. Y., Kanerkar U. R., Indurwade N. H. * and Chondekar R. P. **
Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati (MS)., India.
*Dr. R. G. Bhoyar Institute of Pharmaceutical Education and Research, Wardha (MS)., India.
**Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS) ., India.
(**E-Mail: sam_chondekar@yahoo.com)

ABSTRACT
Aqueous extract of tuberous roots of *Ruellia tuberosa* L. administered orally at the dose of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight respectively for 21 days resulted in significant decreased sperm count in male albino rats. The results suggest that the aqueous extract of *R. tuberosa* produces antispermatogenic effect in male albino rats.

KEYWORDS: Antispermatogenic, *Ruellia tuberosa*, antifertility.

INTRODUCTION

India is first among the countries to adopt an official family planning programme, as early as 1950. However, fifty years later this has not prevented the population touching one billion marks. It is obvious that despite good intentions and concentrated efforts we have failed in controlling our population. Approximately 48.2% of couples of 15 to 49 years of age practice family planning methods in India; female sterilization accounting for 34.2% while, male sterilization declining from 3.4% in 1992-93 to 1.9% in 1998-99 (Sharma *et al.*, 2008). Indian male psyche to avoid sterilization underlines a strong need to develop effective and safe contraceptive for men. Current efforts in India to develop a male contraceptive are mainly directed towards – (i) development of antispermatogenic agents (Reddy and Rao 1972; Bajaj and Madan 1983; World Health Organization, 1990; Tyagi et al., 1999 a,b,c; Rajalakshmi, 2000), (ii) prevention of sperm maturation (however, no success is in sight in near future); (iii) prevention of sperm transport (Ahsan et al., 1980; Guha, 1999). The research mainly centres around synthetic drugs. Several plant products were tested for their antifertility activity at the University of Rajasthan of which extracts of *Carica papaya* seeds showed encouraging results to certain extent (Lohiya and Goyal, 1992; Lohiya et al, 2000a; Lohiya et al., 1999). *Ruellia tuberosa* a native of America was introduced as garden plant in India. Now it has naturalized and grows as garden weed. In Indonesia the herb is used for treatment of stones in bladder, leaf decoction is given in chronic bronchitis, because of its emetic properties it is used as substitute of ipecacuanha (Anonymous, 2004). In folk medicine of Taiwan it has been used as anti-diabetic, antipyretic, analgesic, anti-hypotensive, thirst quenching and antidotal (Chiu and Chang, 1995). Latin American healers use the herb to treat uterine fibroids (Balick *et al.*, 2000).

Recently it has been incorporated as a component in a herbal drink in Taiwan (Chen, 2005). Studies on biochemistry of the plant revealed that the leaves are endowed with antioxidant phytochemicals and moderate nutritive value (Manikandan and Doss, 2010). Ethanolic leaf extract was found to have ability to moderately repair kidney and liver damage (Manikandan and Doss, 2010a). Non-enzymatic antioxidants present in leaves are responsible for this damage repair (Manikandan and Doss, 2010b). Hypoglycemic as well antioxidant activity of whole plant was also demonstrated (Shahwar *et al.*, 2011). Antioxidant property of aerial parts was also proved (Kalia *et al.*,2011). *Ruellia prostrata* and *R. tuberosa* are tested for antifertility property in female rats. The result shows that *R. prostrata* exhibit antifertility activity to some extent, but *R. tuberosa* do not show such activity (Andhiwall *et al.*, 1985). Here an attempt was made to test *R. tuberosa* for antifertility in male rats.

MATERIAL AND METHODS

Preparation of Extracts – Tuberous roots of *R. tuberosa* were collected from the campus garden of GVISH, Amravati washed thoroughly and shade dried. Dried material was powdered and extracted in distilled water with the help of Soxhlet's extraction assembly. 50 gm of powder was taken in about 100-150 ml distilled water and soxhlated for 24 hrs at 50^0C. After cooling, the extract was collected and evaporated on a water bath to dryness. The extracts were kept at room temperature. The extracts were completely dissolved in double distilled water and solution obtained was used for treatment. Male albino rats (Wistar strain) of age between 11-14 weeks were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad and allowed to acclimatize in the animal house. The animals were maintained and housed in wire mesh cages under standard environmental conditions. They were feed with pellet diet and water *ad libitum.* The animal room was well ventilated with a temperature range of 25-27^oC under day/night 12-12 hour photoperiod. All experiments were carried out in quiet laboratory settings with ambient illumination and temperature close to those of the animal house.

Experimental Studies

Male albino rats of proven fertility were divided into following 4 groups of 6 each:
Group I - Control : Distilled water (Vehicle)
Group II - administered with aqueous extract (50mg/kg) body weight for 1-21 days.
Group III- administered with aqueous extract (100mg/kg) body weight for 1-21 days.
Group IV- administered with aqueous extract (150mg/kg) body weight for 1-21 days.

The extracts were fed orally with catheter; control animals were given only the vehicle. The duration of the experiment was 21 days. Sperm count was done using the method prescribed by Mukherjee (1988). Rats were weighed before and after treatment. At the end of the experimental period the rats were fasted overnight. All animals in the above four groups were sacrificed on the 22nd day. The left and right epididymis were isolated and freed from adjoining fat. The cauda portion was cut off so as to separate it from the caput epididymis portion and 5 ml of saline solution was aspirated into each cauda epididymis and the aspirate from both the cauda was collected together to give a sperm suspension.

This suspension was diluted in ratio of 1:20 with 9% saline solution. This diluted suspension was then used for the sperm count on a Neubauer haemocytometer.
Sperm count/ ml = N x 50,000
(N = Number of sperms counted in a square on haemocytometer scale)

No. of sperms/ml of Diluted suspension = $\frac{\text{Sperm count} \times 20}{4 \times 0.1} \times 1000$

Statistical Analysis

The results were analyzed using Microsoft Excel 2007. A one-way ANOVA was employed for comparison among the four groups.

RESULTS AND DISCUSSION

Aqueous extract of root tubers was fed orally to fertile male rats for 21 days before taking the sperm count. Treatment with *Ruellia tuberosa* tuber extract significantly reduced sperm density from 27.63million/ml in control to 23.88 million/ml, 17.10 million/ml and 12.65 million/ml showing gradual increase in average antispermatogenic activity (13.52%), (38.08%) and (54.20%) in groups treated with the tubers. The decrease was statistically significant as was concluded with the values of F (6532.45) and C.D. (0.90) at 5%.

Table I: Antispermatogenic effect of Ruellia tuberosa L.

Sr. No.	Group and Treatment	Number of Males Used	Body Wt. (gm)	Sperm Density in Cauda Epididymis (Million/ml)	Average Sperm Density in Cauda Epididymis (Million/ml)
1	Control (Vehicle)	6	150-200	26.5, 26.7, 27.8, 27.8, 28.6, 28.4	**27.63 ± 1.1**
2	Aqueous Extract (50mg/Kg)	6	150-200	23.5, 23.6, 23.8, 23.9, 24.2, 24.3	**23.88 ± 0.4**
3	Aqueous Extract (100mg/Kg)	6	150-200	16.8, 16.9, 17.0, 17.2, 17.3, 17.4	**17.1 ± 0.3**
4	Aqueous Extract (150mg/Kg)	6	150-200	**12.3, 12.4, 12.7, 12.8, 12.8, 12.9**	**12.6 ± 0.6**

The activity of drug is dose dependent. Individual response with 50 mg/kg body weight showed somewhat wider range; with increase in dose the range becomes narrower and with 150 mg/kg body weight the individual response is almost uniform. The maximum antispermatogenic activity produced was found to be 54.58% (Graph) i.e. the efficacy is little more than 50%. Eight compounds isolated from ethanol extract of *R. tuberosa* – these cirsimaritin, cirsimarin and cirsiliol 4'-glucoside were found to be cytotoxic (Lin *et al.*, 2006). Probably they might be affecting the development of sperm mother cells.

Table II: Percent antispermatogenic effect of Ruellia tuberosa on individual male rats

Treatment Group Animals	Percent Sperm Count After Treatment			
Sr. No.	**Control**	**50 mg/kg body wt.**	**100 mg/kg body wt.**	**150 mg/kg body wt.**
1	0.000	11.320	36.600	53.580
2	0.000	11.610	36.700	53.560
3	0.000	14.390	38.850	54.320
4	0.000	14.030	38.130	53.960
5	0.000	15.380	39.510	55.240
6	0.000	14.440	38.730	54.580

Table III: Stastical Analysis

Groups	Count	Sum	Average	Variance
Control	6.000	0.000	0.000	0.000
50 mg/kg body wt.	6.000	81.169	13.528	2.762
100 mg/kg body wt.	6.000	228.529	38.088	1.427
150 mg/kg body wt.	6.000	325.238	54.206	0.419

Source of Variation	Df	ss	mss	F
Replicate	5	14.90716	2.981432	5.494141
Treatment	3	10634.64	3544.88	6532.454
Error	15	8.139849	0.542657	
Total	23	10657.69		
S.E.	0.425306			
C.D. 5 %	0.905903			
C.D. 1 %	1.254654			

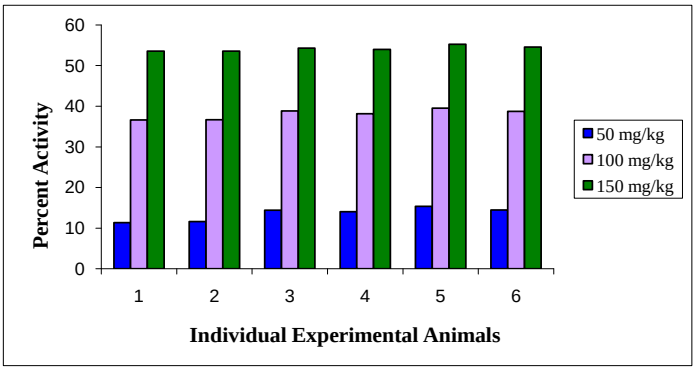

Figure 1. Antiseprmatic activity

CONCLUSION

Further study is necessary before drawing any conclusion. However, about 54% antispermatogenic activity makes *R. tuberosa* a strong candidate to be worked out further to develop male contraceptive.

ACKNOWLEDGEMENT

Authors are thankful to Director, Govt. Vidarbha Institute of Science & Humanities, Amravati and Sudhakarrao Naik Institute of Pharmacy, Pusad (District: Yavatmal) for providing necessary facilities for the work.

REFERENCES

Ahsan R.K., Kapur M.M., Farooq A. and Laumas K.R. (1980). Further studies of an intravasal copper device in rats, *J. Reprod. Fertil*. 59 (1980) 341-345.

Andhiwal C.K., Chandra Has and Varshney R.P. (1985). Antifertility screening of *Ruellia prostrata* Poir and an Ayurvedic preparation.*Curr.Sci.* 54 (19): 995-997.

Anonymous. (2004). *The wealth of India* - Raw Materials. Supplement R-Z. Council of Scientific and Industrial Research, New Delhi, 9 (2004) 90.

Bajaj J.S. and Madan R. (1983). Regulation of male fertility: in *Research on the regulation of human fertility* (eds) E Diczfalusy and A Diczfalusy (Copenhagen: Scriptor), 2 729-767.

Balick M.J., Kronenberg F., Ososki A.L., Reiff M., Fugh-Berman A., O'Connor B., Roble M., Lohr P., and Atha D. (2000). Medicinal plants used by latino healers for women's health conditions in New York city, *Economic Bot.*54 (3):344-357.

Chen F.A., Wu A.B., Shieh P., Kuo D.H. and Hsieh C.Y. (2006). Evaluation of the antioxidant activity of *Ruellia tuberosa. Food Chem*. 94 (2006) 14-18.

Chiu N.Y. and Chang K.H. (1995). The illustrated medicinal plants of Taiwan. *Mingtong Medical J*. 226 (1).

Guha S.K. (1999). Non-invasive reversal of intraluminal vas deferens polymer injection induced azoospermia technology. *Asian J. Androl*. 1 (1999) 131-134.

Kalia A.N, Roopa, Borar S and Thakral J. (2011). Antioxidant potential fractionation from methanol extract of aerial parts of *Ruellia prostrata* Poir (Acanthaceae). *Int. J. Pharm. Sci. Res*. 2 (4): 1015-1022.

Lin C.F., Huang Y.L., Cheng L.Y., Sheu S.J. and Chen C.C. (2006). Bioactive flavonoids from *Ruellia tuberosa*. *J. Chin. Med*. 17 (3):103-109.

Lohiya N.K. and Goyal R.B. (1992). Antifertility investigations on the crude chloroform extract of *Carica papaya* Linn. seeds in male albino rats. *Indian J. Exp. Biol*. 30: 1051-1055.

Lohiya N.K., Kothari L.K., Manivannan B., Mishra P.K. and Pathak N. (2000a). Human sperm immobilization effect of *Carica papaya* seed extracts: an in vitro study, *Asian J. Androl*. 2: 103-109.

Lohiya N.K., Pathak N., Mishra P.K. and Manivannan B. (1999). Reversible contraception with chloroform extract of *Carica papaya* Linn. seeds in male rabbits, *Reprod. Toxicol*. 13 59-66.

Manikandan A. and Doss D.V.A. (2010). Evaluation of biochemical contents, nutritional value, trace elements, SDS-PAGE and HPTLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). *J. Chem. Pharm. Res.* 2 (3) (2010) 295-303.

Manikandan A., Doss D.V.A. (2010a). Effect of 50% Hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on AST, ALT, ACP and ALP levels in serum, liver and kidney of alloxan induced diabetic rats, *Annals Pharmacy Pharmaceut. Sci.* 1 (2) (2010a) 142-146.

Manikandan A., Doss D.V.A. (2010b). Effect of 50% Hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on non-enzymic antioxidants and other biochemical parameters in liver, kidney, serum of alloxan induced diabetic Swiss albino rats. *J Biomed. Sci. Res.* 2 (3) (2010b): 190-201.

Rajalakshmi M. (2000). Induction of azoospermia by hormonal methods in non-human primates; in *Current concepts in fertility regulation and reproduction (eds)* C P Puri and P F A Van Look (New Delhi: Wiley Eastern) 117-132.

Reddy P.R.K. and Rao J.M. (1972). Reversible antifertility action of testosterone propionate in human males. *Contraception.* 5 (1972) 295-301.

Sharma R.S., Rajalakshmi M. and Jeyaraj D.A. (2001). Current status of fertility control methods in India. *J. Biosci.* 26(4):391-405.

Shahwar D., Ullah S., Ahmadb M., Ullah S., Ahmad N. and Khan M.A. (2011). Hypoglycemic activity of *Ruellia tuberosa* Linn (Acanthaceae) in normal and alloxan-induced diabetic Rabbits, *Ir. J. Pharmaceutical Sci.* Spring: 7 (2):107-115.

Tyagi A., Rajalakshmi M., Antony Jeyaraj D., Sharma R.S. and Bajaj J.S. (1999b). Effects of long-term use of testosterone enanthate. II. Effects on lipids, high and low density lipo-protein cholesterol and liver function parameters. *Int. J. Androl*. 22 (1999b) 139-147.

Tyagi A., Rajalakshmi M., Antony Jeyaraj D., Sharma R.S. and Bajaj J.S. (1999c). Effects of long-term administration of testosterone enanthate on glucose metabolism in rhesus monkeys. *Contraception.* 59 (1999c) 333-337.

Tyagi A., Rajalakshmi M., Bajaj J.S. and Mohan Kumar V. (1999a). Effects of long-term treatment with testosterone enanthate in rhesus monkeys. I. Pharmacokinetics of testosterone, testicular volume and liver metabolism of testosterone. *Int. J. Androl*. 22 139.

World Health Organization (1990). Task force on methods for the regulation of male fertility 1990. Contraceptive efficacy of testosterone-induced azoospermia in normal men. *Lancet.* 336:955-959.

