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Antifertility Activity of Hexane and Ethyl Acetate Extracts of Aerial Parts of *Tragia involucrata*. Linn

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ABSTRACT

Many people in rural Bangladesh use a traditional contraceptive pill, "Shanti Bori" to control fertility in which *Tragia involucrata* is one of the components. The aim of the present study was to evaluate the validity of antifertility effect of this plant. The hexane (HE) and ethyl acetate extracts (EAE) of the aerial parts of *T. involucrata* have been evaluated for the anti-fertility activity in proven fertile female rats at dose of 200 mg kg^{-1} body weight. Preliminary phytochemical analysis showed the presence of phytosterols, triterpenes and flavonoids in the extracts. HE and EAE showed significant anti-fertility activity. It was found that the extracts reduced the number of litters born significantly at the dose of 200 mg kg^{-1} body weight. HE and EAE exhibited 81 and 50% antiimplantation potency respectively at the tested dose. The extracts also showed the estrogenic activity and potentiated the action of the standard drug ethanyl estradiol. All these observations suggest that extracts have antiimplantation as well as the abortifacient activity and are safe at the effective antifertility doses employed in this study.

Key words: *T. involucrata*, estrogenic activity, flavonoid, terpenoid, antiimplantation

INTRODUCTION

Population explosion has created grave setback in the economic growth and all-round human development in developing countries. Current pandemic population explosion demands an immediate betterment of new potential contraceptives (Ghosh and Bhattacharya, 2004; Jha *et al*., 2009). Easy availability and acceptability of contraceptive help the poorer countries to cut down the population growth and all other nations to avoid maternal morbidity and mortality arising from unplanned pregnancies. Hormonal steroids or various forms of barrier designs are predominantly used contraceptives methods. Studies of many years have highlighted the unmet demand for safe, inexpensive and acceptable contraceptives to avoid unwanted pregnancies and resultant abortions (Aitken *et al*., 2008). Natural products were used for fertility regulation since ancient time. The plant preparations are reported in many literatures of indigenous medicines to treat various kinds of reproductive related complications. Even though plants are the source of ancient and modern medicines, mass screening of the plants in the search for new drugs is an expensive and inefficient task while the traditional knowledge offered a better result (Badami *et al*., 2003; Vasudeva and Sharma, 2006; Patwardhan and Vaidya, 2010). It is likely that an herbal contraceptive would

enable couples managing their fertility and may probably increase the number of people to opt for family planning. Familiarity rural people have with traditional medicine, fewer side effects, ready availability from the local sources, cheap, non hormonal and acceptability among the most woman in the society and protection of privacy are the other added advantages of such contraceptives. (Gbotolorun *et al*., 2008). Modern scientific studies have authenticated the effects of different traditionally used herbs and herbal products in the reproductive systems of male and female experimental animals (Monsefi *et al*., 2006; Sarathchandiran *et al*., 2007a, b; Ravichandran *et al*., 2007; Mathur *et al*., 2010). *Tragia involucrata* (Euphorbiaceae) is one such plant with a traditional history of medicinal use. It is a small shrub widely found in the Indian subcontinent (Dhara *et al*., 2000).The efficacy of this plant is well known in Indian traditional medicine and it is used for treatment of eczema, wounds and headache (Samy *et al*., 1998). Aqueous extract of leaves and methanol fractions from roots have been obtained from *T. involucrata* and were reported to display anti-inflammatory effect (Dhara *et al*., 2000; Samy *et al*., 2006). *In vitro* antibacterial properties of different compounds isolated from the leaves of this plant studied against few microorganisms (Samy *et al*., 2006). Chowdhury *et al*. (1984) studied the anti-fertility activity of "Shanti Bori", a traditional contraceptive pill used in rural Bangladesh in which *T. involucrata* is one of the components. But the basis for the activity of this plant has not been thoroughly investigated. Hence the present investigation reports the in vivo anti-fertility activity of hexane (HE) and ethyl acetate extracts (EAE) of aerial parts of *T. involucrata* on female rats.

MATERIALS AND METHODS

Plant material: The aerial parts of the plants were collected in the month of October and November 2004 from Bangalore and the herbarium was authenticated by NISCAIR, New Delhi. Herbarium was deposited in GKVK, Bangalore, (Herbarium No. 3687).

The 100 g of the dried powder of *T. involucrata* was extracted in a soxhlet extractor with (500 mL) hexane and (500 mL) methanol. The mark was pressed and the expressed solvent was mixed with the main extract. The extract was concentrated to constant weight in a rotary shaker evaporator. The hexane fraction (1.78 g) was defatted and labeled hexane extract {(0.65 g) (HE)}. The concentrated methanol extract (2.64 g) was dissolved in 80% methanol (200 mL) with stirring and filtrated. The filtrate was extracted successively with n-hexane (500 mLX 3), dichloromethane (500 mLX2) and ethyl acetate (500 mLX4). The ethyl acetate fraction was concentrated and the solvent was evaporated to dryness. The residue (0.29 g) was labeled ethyl acetate extract (EAE). HE and EAE were prepared in tween 80 (1%) and propylene glycol: water (1:4) respectively and were administered at a dose of 200 mg kg^{-1} body weight orally.

Experimental animals: Wistar rats of either sex (150-200 g) were used for evaluating anti-fertility activities. They were housed in polypropylene shoebox type cages with stainless steel grill top and bedded with rice husk. The animals were provided with pelleted diet (Goldmohur, Lipton India) and water *ad libitum*. They were allowed a one-week acclimatization period before the experimental session. All the experimental protocols were met with the approval of Institutional animal ethics committee.

Anti-fertility activity: Antiimplantation activity was determined as explained by Khanna and Chaudhary (1968). The pregnant rats were divided into three groups containing six rats in each

group. Group I served as control and received the vehicle for seven days. Groups II and III received suspension of HE and EAE at the dose of 200 mg kg^{-1} p.o respectively for seven days starting from the day one of pregnancy. Number of litters born was noted after delivery.

Estrogenic and anti-estrogenic activity: The HE and EAE showed significant anti-implantation activity at 200 mg kg^{-1} body weight compared to control. Hence, they were subjected to a detail investigation for estrogenic and anti-estrogenic activity. Young female rats (20-22d, 250-300 g) were divided into six groups of six animals in each group. Group one served as control and received vehicle solution p.o for seven days. Group II served as standard and received ethinyl estradiol (1μg/rat/day) in olive oil for seven days. Group III received suspension of HE (200 mg kg^{-1} p.o) and Group IV received suspension of EAE (200 mg kg^{-1} p.o) for seven days. Groups V, VI received ethinyl estradiol along with the suspensions of HE and EAE (200 mg kg^{-1} p.o) respectively for seven days. The diameter of uterus, thickness of endometrium and height of endometrial epithelium were measured in randomly selected tissues sections.

Statistical analysis: The results were expressed as Mean±SEM. The significance was evaluated by student t-test compared with control and $p<0.05$ implied significance (Woodson, 1989).

RESULTS

Post- coital antifertility activity: The results of antiimplantation activity of hexane and ethyl acetate extracts of aerial parts of *T. involucrata* was expressed as the percentage of rats without implantation on day 10 of pregnancy (Table 1). HE and EAE at 200 mg kg^{-1} body weight showed significant antiimplantation activity (versus control $p>0.05$).Both the extracts reduced the number of litters born significantly confirming the antifertility potential of the extracts. HE and EAE showed 81 and 50% anti-implantation activity with mean values of litters born 0.83±0.67 and 4.12±0.78, respectively.

Estrogenic and anti-estrogenic activity: The estrogenic effect of HE and EAE is shown in Table 2. Oral administration of these extracts at 200 mg kg^{-1} body weight resulted in a significant

Table 1: Antiimplantation activity of HE and EAE

Treatment	Dose(mg kg^{-1})	Rats with implantation on day 10	Mean No. of implants±SE	Rats delivered	%anti-implantationactivity
Control	----	6	7.16±0.53	6	Nil
HE	200	2	0.83±0.67	1	81
EAE	200	4	4.12±0.78	3	50

Note: anti-implantation activity, $p<0.05$ vs. control, n = 6

Table 2: Estrogenic and anti-estrogenic activity of HE and EAE

Treatment	Dose (mg kg^{-1})	Uterine weight (mg/100 g body weight)	Vaginal cornification
Control	---	52.1±0.98	Nil
Ethinyl estradiol	1 μg/rat/day s.c	192.0±0.83	+++
HE	200	169.0±2.80	++ to+
EAE	200	102.0±1.93	++ to+
HE+Ethinyl estradiol	200+1 μg	239.0±2.87	+++
EAE+Ethinyl estradiol	200+1 μg	230.0±2.87	+++

Note: estrogenic activity, $p<0.05$ vs. control, n = 6

Table 3: Histological changes in the uterus and endometrium after treatment with the HE and EAE

Treatment	Dose(mg kg^{-1})	Diameter of the uterus (μm)	Thickness of endometrium (μm)
Control	------	305±10.00	72.0±3.97
Ethinyl eatradiol	1 μg/rat/day	670±18.33[a]	270±3.21
HE	200	653±17.13	220±6.09
EAE	200	502±8.15[a]	199±9.06
HE+Ethinyl eatradiol	200+1μg	731±11.52	456±2.74
EAE+Ethinyl eatradiol	200+1μg	698±10.63[a]	389±1.14

[a] $p<0.05$ vs. control, n = 6

increase in uterine weight in animals (versus control $p<0.05$). The uterotrophic capacity of the extracts as shown by the weight of the uterus was about 88% for HE and 53% for EAE, respectively of that of the standard drug ethinyl estradiol. Simultaneous administration of ethinyl estradiol with the extract showed the uterotrophic response significantly higher than that produced by ethinyl estradiol alone. There fore these extracts exhibited the estrogenic activity but no anti-estrogenic activity. The treated animals were showing the characteristics of proestrous/oestrous uterus. Spindle shaped cells with basal nuclei was observed on the endometrial epithelium. The extracts induced the opening of vagina in treated rats. The extent of cornification in vaginal smears was significantly higher than the controls, but lesser than those of ethinyl estradiol treated animals.

Histological studies: The histological studies (Table 3) revealed the significant increase in the diameter of the uterus, height of the endometrial epithelium and thickness of endometrium ($p<0.05$) compared with control rats. There were no significant physiological and histological changes in the control and treated animals suggesting the non-toxic nature of these extracts to the experimental animals.

DISCUSSION

In the present study, the hexane and ethyl acetate extracts of aerial parts of *T. involucrata* were tested for the antifertility activity at the dose of 200 mg kg^{-1} body weight in fertility proven female rats. HE and EAE showed significant anti-implantation activity when compared with the control rats. The loss of implantation caused by the HE and EAE may be due to anti-zygotic, blastocytotoxic or anti-implantation activity of these compounds (Hafez, 1970). Treatment of the animals with the extracts, resulted in a significant decrease in the number litters compared to control. This trend reveals the abortifacient nature of these extracts. The litters of the extract treated rats did not show any physical deformity and litters grew up to normal adult stage. Hence the extracts treatment does not show any teratogenic effect (Badami *et al.*, 2003).

It appears that HE and EAE have estrogenic activity at the dose of 200 mg kg^{-1} body weight as evident from the significant increase in the diameter of the uterus, height of the endometrial epithelium and thickness of endometrium in extract treated animals compared with control. These extract did not exhibit any antiestrogenic activity. Proper equilibrium between estrogen and progesterone is essential for implantation and any disturbance in the level of these hormones may affect the fertility (Pshychoyos, 1996). The present investigation clearly supports an unfavorable uterine milieu as evident from the histological examination. Therefore, the estrogenic activity of these extracts may be responsible for the observed antiimplantion effect, resulting in the expulsion of ova from the tube, disrupting the luterophic activity of the blastocyst. The extracts showed 88 and 53% of estrogenic activity that of ethinyl estradiol and thus may reduce the unwanted side

effects caused by estrogens (Vasudeva and Sharma, 2007). There are many reports about the plant products showing estrogenic activity with antiferitility effects. The present study is comparable with the studies made by Vasudeva and Sharma (2006) and Badami *et al.* (2003) who had reported antifertility effect with similar observation in female rats on treatment with the methanol extract of *Achyranthus aspera* and the ethanolic extract of roots of *Derris brevipes,* respectively. The qualitative and TLC analysis of the extracts showed the presence of steroids and terpenoids in HE and polyphenos in EAE. There are many reports regarding the anti-fertility activity of Phytosterols triterpenes and polyphenols present in plant extracts (Brain and Ross, 1977; Kumar *et al.*, 1989; Gebirie *et al.*, 2005). Hence in the present study, the phytosterols triterpenes and polyphenols present in these extracts may be responsible for the observed anti-fertility effect.

CONCLUSION

The results of the present study provide the evidence for the antifertility activity of HE and EAE of *T. involucrata* as claimed in the traditional use. The terpenoids, phytosterols and flavonoids present in the extracts may be responsible for their activity. Further studies are going on this laboratory to find out the active principle(s) and the exact mechanism/s of action.

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REFERENCES

Aitken, R.J., M.A. Baker, G.F. Doncel, M.M. Matzuk, C.K. Mauck and M.J.K. Harper, 2008. As the world grows: Contraception in the 21st century. J. Clin. Invest., 118: 1330-1343.

Badami, S., R. Aneesh, S. Sankar, M.N. Sathishkumar, B. Suresh and S. Rajan, 2003. Antiferitility activity of *Derris brevipes* variety *coriacea*. J. Ethnopharmacol., 84: 99-104.

Brain, K.R. and R.G. Ross, 1977. An Introduction to Phytopharmacy: Steroid Hormones. Pitmen Medical Publishing Co. Ltd., Kent TNI 2QD.

Chowdhury, A.A.K., R.A. Khaleque and S.K. Chakder, 1984. Antifertililty activity of a traditional contraceptive pill comprising of *Acacia catechu, A. Arabica* and *Tragia involucrata*. Indian J. Med. Res., 80: 372-374.

Dhara, A.K., V. Suba, T. Sen, S. Pal and A.K.N. Chaudhuri, 2000. Preliminary studies on the antiinflammatory and analgesic activity of the methanolic fraction of the root extract of *Tragia involucrate* Linn. J. Ethnopharmacol., 72: 265-268.

Gbotolorun, S.C., A.A. Osinubi, C.C. Noronha and A.O. Okanlawon, 2008. Antifertility potential of Neem flower extract on adult female Sprague-Dawley rats. Afr. Health Sci., 8: 168-173.

Gebirie, E., E. Makonnen, A. Debella and L. Zerihun, 2005. Phytochemical screening and pharmacological evaluations for the antifertility effect of the methanolic root extract of *Rumex steudelii*. J. Ethnopharmacol., 96: 139-143.

Ghosh, K. and T.K. Bhattacharya, 2004. Preliminary study on the antiimplantation activity of compounds from the extracts of seeds of *Thespesia populnea*. Ind. J. Pharmacol., 36: 288-291.

Hafez, E.S.E., 1970. Reproduction and Breeding Techniques for Laboratory Animals. Lea and Febiger, Philadelphia, PA.

Jha, R.K., P.K. Jha, S.V.S. Rana and S.K. Guha, 2009. Spermicidal action of styrene maleic anhydride polyelectrolyte in combination with magnetic and electrically conductive particles. Int. J. Pharmacol., 5: 1-12.

Khanna, U. and R.R. Chaudhary, 1968. Antifertility screening of plants. Part I. Investigation of *Butea monosperma* (Lam) Kutze. Indian J. Med. Res., 56: 1575-1579.

Kumar, G.P., M. Laloraya and M.M. Laloraya, 1989. The effect of some of the polyphenolic compounds on sperm motility *in vitro*: A structure-activity relationship. Contraception, 39: 531-539.

Mathur, N., G.C. Jain and G. Pandey, 2010. Effect of *Tecoma stans* leaves on the reproductive system of male albino rats. Int. J. Pharmacol., 6: 152-156.

Monsefi, M., M. Ghasemi and A. Bahaoddini, 2006. The effects of *Anethum graveolens* L. on female reproductive system. Phytother. Res., 20: 865-868.

Patwardhan, B. and A.D. Vaidya, 2010. Natural product drug discovery: Accelarating the clinical candidate development using reverse pharmacology approaches. Indian J. Exp. Biol., 48: 220-227.

Pshychoyos, A., 1966. Recent Research on Egg Implantation. CIBA Foundation Study Group, London.

Ravichandran, V., B. Suresh, M.N. Satishkumar, K. Elango and R. Srinivasan, 2007. Antifertility activity of hydroalcoholic extracts of *Ailanthus excels* (Roxb): An ethno medicines used by tribals of Nilgiris region in Tamilnadu. J. Ethno. Pharmacol., 112: 189-191.

Samy, R.P., S. Ignacimuthu and H. Sen, 1998. Screening of 34 Indian medicinal plants for antibacterial properties. J. Ethnopharmacol., 62: 173-182.

Samy, R.P., P. Gopalakrishnakone, P. Houghton, M.M. Thwin and S. Ignacimuthu, 2006. Effect of aqueous extract of *Tragia involucrata* Linn. on acute and subacuteinflammation. Phytother. Res., 20: 310-312.

Sarathchandiran, I., R. Manavalan, M.A. Akbarsha, B. Kadalmani and P.K. Karar, 2007a. Studies on spermatotoxic effect of ethanolic extract of *Capparis aphylla* (Roth). J. Boil. Sci., 7: 544-548.

Sarathchandiran, I., R. Manavalan, M.A. Akbarsha, B. Kadalmani and P.K. Karar, 2007b. Effects of ethanolic extract of *Capparis aphylla* (Roth.) on testicular steroidogenesis in rats. J. Boil. Sci., 7: 582-584.

Vasudeva, N. and S.K. Sharma, 2006. Post-coital antifertility activity of *Achyranthus aspera* Linn. root. J. Ethnopharmacol., 107: 179-181.

Vasudeva, N. and S.K. Sharma, 2007. Estrogenic and pregnancy interceptor effects of *Achyranthes aspera* Linn. root. Afr. J. Trad. CAM., 4: 7-11.

Woodson, R.F., 1989. Statistical Methods for the Analysis of Biomedical Data: Probability and Mathematical Statistics. Wiley Press, Chichester.