



ANTIFERTILITY EFFECT OF AQUEOUS LEAF EXTRACT OF *AEGLE MARMELLOS* ON MALE ALBINO RATS

K. SATHIYARAJ¹, A. SIVARAJ¹, G. MADHUMITHA², P.VINOTH KUMAR¹, A. MARY SARAL², K. DEVI³,
B.SENTHIL KUMAR^{1*}

¹PG & Research department of Zoology, C.Abdul Hakeem College, Melvisharam-632 509, Tamilnadu, India, ²Pharmaceutical Chemistry Division, School of Science, VIT University, Vellore, Tamilnadu, India, ³Department of Zoology, DKM College for Women, Vellore-632 001. Tamilnadu, India. E.mail: senthil_cahc@yahoo.co.in

ABSTRACT

The present study was carried out to evaluate the effective concentration of aqueous extract of *Aegle marmelos* leaves on male reproductive system of albino rats. The study was divided into three groups of six animals each. The first group (I) received distilled water serve as control. The second and third groups (II and III) of animals were administered the aqueous leaf extract daily at 250mg/kg body wt., and 350mg/kg body wt., respectively for a period of 45days. Significant decreases in the weights of testis, epididymes and seminal vesicle were observed. A dose related reduction in the testicular sperm count, epididymal sperm count and motility and abnormal sperm count were observed. The results showed that *Aegle marmelos* has effects on male rat reproduction, affecting the sexual behavior and epididymal sperm concentration.

Keywords: *Aegle marmelos*; epididymes; spermicidal; seminal vesicle.

INTRODUCTION

Population explosion is a leading cause of poverty and pollution in developing countries. Several potential approaches for 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 effects. The World Health Organization (WHO) has constituted a population control programme, which includes studies having traditional medical practices. Medicinal plants¹ products have a long history of indigenous use in India as well as other countries. Phytotherapy has a very long tradition, although proper scientific explanation is relatively new. In our country as well as in the world, there are several medicinal plants associated with antifertility properties^{1,2}. Although very few contraceptives have been developed from plant extracts, their potentiality has not been determined accurately, and their mode of action has been beyond our knowledge until now because there are many problems in assessing plant extract including batch to batch variation and a lack of definite active portion of the extract used for the development of herbal contraceptives. 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^{3,4}. 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.

Aegle marmelos, is a spiny tree belonging to the family Rutaceae. It is an indigenous tree found in India, Myanmar, Pakistan and Bangladesh. The leaves, roots, bark, seeds and fruits are edible and medicinal values. The leaves of *Aegle marmelos* are astringent, a laxative, expectorant and useful in treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhea, dysentery, heart palpitation, and asthmatic complications⁵, Cardio tonic effect in mammals⁶, regenerate damaged pancreatic beta cells and increased the activities of peroxides in the liver tissues⁷, potential antioxidant drug, which reduces blood sugar levels⁸. The ethonolic extract of *A.marmelos* leaf possesses anti spermatogenic activity⁹. The aqueous of the leaf has antimotility action on spermatozoa¹⁰.

The present study was carried out to test the efficacy antifertility activity of aqueous leaf extract of *Aegle marmelos* on male albino rats.

MATERIALS AND METHODS

Plant material

The leaves of *Aegle marmelos* (*Rutaceae*) were collected in and around Vellore District, Tamilnadu, India. The plant materials were cleaned with distilled water and shade dried at room temperature and authenticated (No.CAHC/07/2008) by Dr.B.Annadurai, Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamil Nadu., and voucher specimens were kept at the Department of Botany, C. Abdul Hakeem College, Melvisharam. The shade dried plant material was powdered by using electric blender.

Plants extract preparation

100gms of the dried powdered leaves of *Aegle marmelos* 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 10%.

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°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.

Experimental design

Group I: Control rats.

Group II: The rats were treated with *Aegle marmelos* aqueous leaf extract (250 mg/kg body wt.,) for 45 days.

Group III: The rats were treated with *Aegle marmelos* aqueous leaf extract (350 mg/kg body wt.,) for 45 days.

Estimation of sperm motility and count

The spermatozoa were obtained by making small cuts in caudae epididymis and vasdeferens placed in 1ml of

modified Krebs Ringer-bicarbonate buffer (pH 7.4). 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^{11,12}. To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air-dried and made permanent.

Serum testosterone

Serum levels of testosterone were assayed in duplicate using specific RIA method¹³. Serum samples were separated by standard procedure and stored at -20°C for subsequent analysis.

RESULTS

In the period the treatment with *Aegle marmelos* leaf extract, no significant clinical and behavioral changes were observed in both group II and group III animals. The treatment of rats with plant extract caused no effect on the body weight of the animals; weight gain was normal in all the experimental groups. The treatment with *Aegle marmelos* aqueous leaf extract treated rats caused a highly significant (P<0.001) decrease in the accessory sex organ weights, namely testis, epididymis and seminal vesicle in all treated groups. The group III animals the sex organ weights were highly reduced (P<0.001) when compared to the group II as well as group I (Normal) animals.

A highly significant decline (P<0.001) in serum testosterone was observed in both groups when compared to the group I control animals.

Table 1: Antifertility effect of *Aegle marmelos* aqueous leaf extract of male albino rats

Treatment	x		Testis	Epididymis	seminal vesicle
	Initial	final			
Group-I	185±2.55	207±4.02	990±10.2	485±6.2	510±2.73
Group II	187±3.74	205±5.5 ^b	841.2±4.35 ^a	4.23±1.24 ^a	466±3.51 ^a
Group III	192±2.6	202±2.5 ^b	634±5.4 ^a	374±5.2 ^a	428±2.3 ^a

Data are expressed as Mean ±S.E.M of 6 individual observations. ^a Significant (P<0.001). ^b non significant. Group II and Group III were compared to Group I (Control).

Table 2: It shows effect of *Aegle marmelos* aqueous leaf extract on body abd organ weight

Treatment	Body weight		Reproductive organ weight (mg/100g bd wt)		
	Serum testosterone	Total count	Motile	Abnormal	
				% HD	% Tail
Group I	4.67±0.27	7.8±2.62	94±1.93	1±0.01	1±0.01
Group II	2.38±0.44 ^a	5.7±4.41 ^a	68±6.10 ^a	12±1.2 ^a	11±1.02 ^a
Group III	0.98±0.14 ^a	3.9±4.62 ^a	38±7.11 ^a	20±4.2 ^a	22±4.7 ^a

Data are expressed as Mean ±S.E.M of 6 individual observations. ^a Significant (P<0.001). ^b non significant. Group II and Group III were compared to Group I (Control).

The sperm of the control rats had normal counts, motility, and morphology (Table:1). In *Aegle marmelos* aqueous leaf extract treated rats the cauda epididymidal sperm parameters showed evidence of dose dependent toxicity. The sperm counts were

significantly (P<0.001) decreased in group II and group III animals (Table-1). In group III animals the sperm count were very much reduced when compared to the group II as well as control animals. The sperm motility was very much inhibited both group II and

group III animals (Table:1). More than 50% of the sperm had abnormal morphologies of various kinds, which included globose head, coiling of tails, fusion of tails of two or more sperm etc., were observed. The plant extract intoxication exerted a significant ($P<0.001$) decrease epididymal sperm concentration and sperm progress motility. The live/dead sperm count was increased in both group II and group III animals. The reduction of sperm count and sperm motility were significantly ($P<0.001$) higher in plant extract 350 mg/kg body wt., (Group III) treated animals when compared to 250 mg/kg body wt., (Group II) and control animals.

DISCUSSION

Treatment with the extract (dose 250 mg/kg body weight and 350 mg/body weight for 30 days) was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicates that the drug caused structural and functional alteration in testes, epididymides and seminal vesicle. Interestingly, it was observed that sperm morphology remained unaltered in placebo- as well as drug treated animals. Depletion of sperm count in the drug treated animals suggests alteration in sperm production in the testes. Decrease in sperm motility suggests alteration of sperm maturation in the epididymides. Changes in both sperm count and motility resulted in complete infertility within 45days. This resulted in abnormal sperm functions which ultimately gave rise to complete male sterility.

Among the plant based contraceptives, inhibition of male fertility after administration of natural substances has been related to decreased spermatozoa density¹⁴. Also, for male contraception, it is not necessary to stop spermatogenesis, but rather to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm¹⁵. By the treatment employed in this study, no toxic effect was produced in the pituitary gland, which is directly involved on the development and functioning of the male reproductive system¹⁶ nor in the reproductive organs.

In conclusion, our results in suggest that *Aegle marmelos* leaf extract treatment and durations employed in the present study causes marked alterations in the male reproductive organs and that the alterations are reversible after cessation of treatment. Treatment also had a reversible effect on suppression of fertility in males. Further, no toxic effects could be detected in treated rats.

REFERENCES

1. a) Hiremath SP, Badani S, Hunasagatta SK, Patil SB: Antifertility and hormonal property of flavones of strigaoroleanchioider. Eur J Pharmacol. 2000; 391:193-7; b) Madhumitha G, Saral AM: Free radical scavenging assay of

thetvetia neriifolia leaf extracts. Asian Journal of chemistry. 2009; 21: 2468-2470; b) Madhumitha G, Saral AM: Antimicrobial activity of successive extracts of thevetia neriifolia. Asian Journal of chemistry. 2009; 21: 2471-2472.

2. Hiremath SP, Rudresh K, Badani S, Patil SB, Patil SR: Post-coital antifertility of *Aealypha indiac L.* J Ethnopharmacol 1999;67:226-8.
3. WHO. Reproductive health research at WHO: a new beginning, Biennial Report 1998-99, Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, Geneva, 2000.
4. Lohiya NK: Plant products for contraception: How to make it a reality? In: Puri, CP., (Edn.), ISSRF News letter, Vol. 5, Indian society for te study of reproduction and fertility, Mumbai. 2000; 9-12.
5. Kirtikar KR, Basu BD: Indian medicinal plants IInd Edn. Periodical Experts Books Agency. New Delhi, 1993; 499 – 505.
6. Nadakarni AK: Indian materia medica, IIIrd Edn. Popular press, Mumbai, India, 2000; 45 - 49.
7. Rajadurai M, Padmanabhan M, Prince PSM : Effect of *Aegle marmelos* leaf extract and α tocopherol on lipid peroxidation and antioxidants in isoproterenol induced myocardial infarction in rats. Indian. J. Exp. Biol. 2005; 36: 60-64.
8. Sabu MC, Ramadasan K: Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Indian.J.Physiol. Pharmacol. 2004; 48: 81-88.
9. Sur TK, Pandit S, Pramanik T : Antispermatogenic activity of leaves if *Aegle marmelos*, Corr. In albino rats: A preliminary report. Biomedicine. 1999; 19: 199-202.
10. Sur TK, Pandit S, Paramanik T, Bhattacharyya D : Effect of *Aegle marmelos* leaf on rat sperm motility: An in vitro study. Indian J. Pharmacol. 2002; 34: 276-277.
11. Srikanth V, Malini T, Arunakaran J, Govindarajulu P, Balasubramanian K: Effects of ethanol treatment on epididymal secretary products and sperm maturation in albino rats. J Pharmacol Exp Ther. 1999; 288: 509-15.
12. Zaneveld LJD, Polakoski KL: Collection and physical examination of the ejaculate. In: Hafez ESE, editor. Techniques of human

- andrology. Amsterdam (Holland): North Biomedical Press. 1977; 147-56.
13. WHO. Method manual. Programme for the provision of matched assay reagents for the radioimmunoassay of hormones in reproductive physiology fifth editor. Geneva: World Health Organisation: 1987.
 14. Sharma N, Jacob D: Antifertility investigation and toxicological screening of the petroleum ether extract of the leaves of *Mentha arvensis* L. in male albino mice. *J Ethnopharmacol.* 2001; 75:5-12.
 15. Nikkanen V, Soderstrom KO, Tuusa S, Jaakkola UM: Effect of local epididymal levonorgestrel on the levorgestrel on the fertilizing ability of male rat, a model for post-testicular contraception 2000; 61: 4001-6.
 16. Mahony MC, Hodgen GD: Toxic effects on the hypothalamus anterior pituitary-gonadal axis, control on the male and female reproductive system, and related issues. In: Witorsch RJ, editor. *Reproductive toxicology*. 2nd. New York: Raven press, 1995; 195-213.