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Original Article

Studies on Antifertility Activity of Bark Aqueous Extracts of *Aegle marmelos* (L.) Corr. in male *Bandicota bengalensis* (Gray, 1835): In search for a potential Rodenticide

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Abstract

Rodents are considered as one of the major pests of agricultural crops and stored food grains. Rodents are highly adaptable and it is difficult to check their population effectively. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. There are several medicinal plants associated with reducing the male fertility potential in indigenous Indian medicine system. *Aegle marmelos* (L) Corr. commonly known as Bael, in Bengali, has various medicinal properties in the Ayurvedic system of medicine. Various parts of this plant (mainly the leaves, fruits, stem and roots) have been used in ethnomedicine for several medicinal properties. The present experiment is aimed at investigating the potential of bark extracts of *Aegle marmelos* (L) Corr. as an anti-fertility agent on male *Bandicota bengalensis* (Gray, 1835). Three various concentrations of aqueous extracts of barks were used for each group of male *Bandicota bengalensis* (Gray, 1835). The dose of 200mg/100ml, 400mg/100ml and 600mg/100ml barks aqueous was administered orally for 60 days through food to male *Bandicota.* It was found that the extracts had a considerable effect on reducing male fertility and there is positive correlation between dose increase and anti-fertility. Significant decrease in the weight of testis, decrease in number of offspring were observed. Histo-pathological studies of testis revealed elongated spermatids, degeneration of Sertoli cells. Prominent spaces were detected within the germinal epithelium signifying testicular cytotoxicity and necrosis. Many tubules showed lumen with reduced spermatozoa and Leydig cell. Our studies suggest that *Aegle marmelos* (L) Corr. bark aqueous extract may be used as potential rodenticide for controlling *Bandicota bengalensis* (Gray, 1835) population.

Keywords: *Aegle Marmelos;* Anti-Fertility; *Bandicota Bengalensis;* Population Control; Rodenticide.

Introduction

Rodents are considered as one of the major pests of agricultural crops and stored food grains. Rodents are highly adaptable, prolific breeders and can cope up with new environments, new foods and adjust to new associates with a striking swiftness, and hence, it is difficult to check down their population for longer periods either by any cultural methods or natural enemies or synthetic chemical agents (Prakash and Mathur, 1987). The lesser bandicoot rat, *Bandicota bengalensis* (Gray, 1835) is a predominant rodent pest species in India (Jain and Tripathi, 1988). It has turned commensal and inhabits godowns and other premises in metropolitan cities (Chakraborty 1992). The effects of rodent's damage cause huge amount of crop losses and food shortages (Fayenuwo et al., 2007) in some parts of the world.

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Use of chemical rodenticides, mainly anticoagulants is the most common and dominant approach of rodent control in agriculture, rural and urban environment (Prasad, 1999). But application of these rodenticides is limited due to the reported intoxication of domesticated as well as wild animals through direct consumption of baits (primary hazard) and/or secondary hazard resulting from eating of poisoned rodents by non-target predators and scavengers (Valchev *et al*., 2008).

Natural products are an excellent alternative to synthetic pesticides (Isman and Machial, 2006) as a means to reduce negative impacts to human health and the environment. Plants are rich sources of ecologically developed secondary metabolites, which are potent remedies for different ailments (Ganesh *et al,* 2011). The plant *Aegle marmelos* (L.) Corr. is indigenous to India, and belongs to the family Rutaceae. It is known as Bael' in Hindi and Bengali; and 'Bilwa' or 'Sriphal' in Sanskrit. The Bael tree was originated in India and is presently growing in most of the countries of Southeast Asia. In India, it grows wild, especially in dry forest, outer Himalayas, Shivaliks, South Indian plateau with altitudes ranging from 250-1200 m and also cultivated throughout Indian sub continent for its fruits. It prefers dry and sunny or warm parts of the hill slopes with well-drained loamy soil. The bael tree has great mythological significance and abounds in the vicinity of temples. The leaves of the tree are traditionally used as sacred offering to Lord Shiva, the God of health. It is one of the most useful medicinal plants of India. Its medicinal properties have been described in the ancient medical treatise in Charaka Samhita.

Various parts of this plant (mainly the leaves, fruits, stem and roots) have been used in ethnomedicine for several medicinal properties: they are said to be astringent, anti-diarrhoeal, anti-dysenteric, demulcent, anti-pyretic, anti-scourbutic, haemostatic, aphrodisiac and an antidote to snake venom (Kirtikar & Basu 1993). *Aegle marmelos* (L) Corr. has been widely used in indigenous systems of Indian medicine due to its various medicinal properties (Sahare *et al* 2008; Dhankhar and Ruhil, 2011). Extensive investigations have been carried out on different parts of *Aegle marmelos* (L) Corr. and as a consequences, varied classes of compound viz, alkaloid (halfordin, ethylcinnamamide, marmeline), phynylpropenoids (hydroxylcoumarins, phenylpropenes, lignans), terpenoids (limonene, α-phellandrene) are found (Sharma *et al* , 2007).

There are several medicinal plants associated with male antifertility potential (Priya *et al* 2012). From Ayurvedic medicine, it has been claimed that the leaves of *Aegle marmelos* possess contraceptive efficacy (Bhattacharya 1982), and they are used for contraceptive purposes by men from different tribal areas of India. The ethanolic extract of Aegle marmelos leaf possesses antispermatogenic activity (Sur et al,. 1999) and aqueous extract of the leaf has anti-motility action on spermatozoa in rats (Sur et al., 2002). Sperm motility describes the ability of sperm to move properly towards an egg. This can also be thought of as the 'quality' of the sperm, which is a factor in successful pregnancies, as opposed to the 'quantity'. Sperm which do not properly 'swim' will not reach the egg in order to fertilize it. Sperm motility is an important factor in semen quality. Insufficient sperm motility is a common cause of sub fertility or infertility (Chauhan *et al*, 2007; 2009). Research on the contraceptive effects of *A. marmelos* (L) Corr. and its mode of action is scant (Remya *et al*, 2009). The present investigation has been carried out to determine the anti-fertility activity of *A. marmelos* (L) Corr. bark extract on male *Bandicota bengalensis* (Gray, 1835).

Materials and Methods

Bark of *Aegle marmelos* (L) Corr. were collected from local area. The bark was dried and pulverized in an electric grinder and bark powder was made and kept in refrigerator. The powder was dissolved in distilled water the following dose were prepared *viz.* 200mg / 100 ml (Dose I), 400mg / 100 ml (Dose II), and 600 mg / 100 ml (Dose III) and kept in separate container. 60 male *Bandicota bengalensis* (Gray, 1835) were taken and divided into 4 groups of 15 rodents each. The 3 groups were treated with 3 different doses (oral administration with food, three times a day). Another group was kept as control, where only food and distilled water was provided. After 60 days of the experiment, we observed the physical changes of those 4 groups. The testis were weighted and used for histological studies. For histology evaluation, testis was fixed in Bouin's fixative. Tissues were processed for wax embedding and embedded in paraffin, wax blocks were sectioned 7µm thick and stained with haematoxyline and eosin. Sperm morphology was observed under MSZ TR Stereo Zoom from the extract of epidedymis in the laboratory. 5 rodents from each group, the control as well as the treated, were kept with female *Bandicota bengalensis* (Gray, 1835) for the assessment of fertility

Results

Histopathology

In the control group there are no changes in sperm morphology and histology of testis as they are not treated with extract. The treated group shows following changes accordingly with doses of extracts. The changes are decreasing the weight of testis and depletion of the germinal layer. Prominent space detected within the germinal epithelium. There are reduction in the number of Sertoli cells were also observed. Histological examination of testes in control animal showed seminiferous tubules of the testes

possesses epithelia containing the sertoli cells and the germ cells at various stages, covering the complete process of spermatogenesis. Sertoli cells exhibited typical, irregular nuclei and well-defined cytoplasm, which appeared granular. The spermatogonia, oval in shape, were closely associated with the basal lamina. Spermatocytes showed various degrees of condensation of the nuclei and were closely associated with sertoli cell cytoplasm. The lumen contained mature spermatozoa, and the interstitium contained distinct Leydig cells (Figure 1). Group G I, G II and G III animals showed dose dependent defect on histopathology of testes. Figure 2 of Group G1 (Dose 1) shows mild to moderate effect. Large multinucleated cells were also present in a number of tubules in group G2 (Dose II) (Figure 3). In addition, prominent spaces detected within the germinal epithelium were consistent with structural disorganization in group G3 (Dose III) (Fig 4). The cytoplasm of all germ cells of group G1, G2 and G3 shows vacuoles and tubules suggesting degeneration of their germinal epithelium. Many tubules showed signs of restructuring lumen contained reduced spermatozoa, and the Leydig cells.

Effect on Reproduction

The testis weights were measured in the control and treated *Bandicota bengalensis*. The fertility of the male was also assessed from the pregnancy and birth

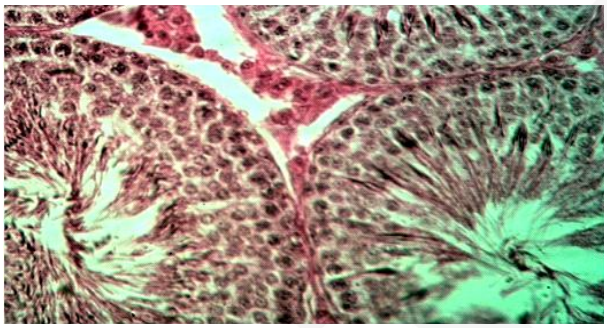

Fig. 1: T. S. of Testes of control shows normal normal Sertoli cells, germ cells and lumen contains mature spermatozoa

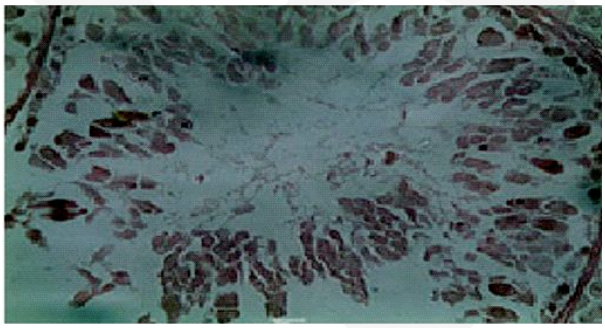

Fig. 2: T. S. of testis of Group 1 treated with 200mg/100ml (Dose 1) showing Sertoli cells started to vacuolize

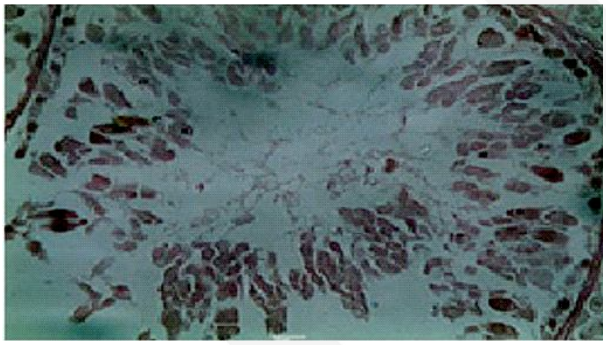

Fig. 3: T. S. of testis of Goup 2 Treated with 400mg/100ml (Dose II), quality of sperm is reduced, elongated spermatid

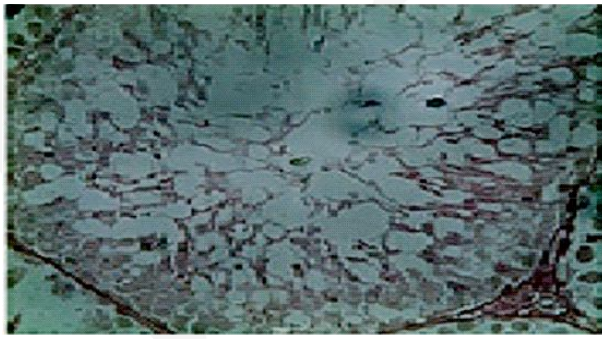

Fig. 4: T. S. of testis of Group 3 Treated with 600mg/100ml (Dose III) showing depletion in germinal layer resulting less production of spermatids

Table 1: Testes weight in different dose of mature *Bandicota bengalensis*

Control	1.82 ± 0.02 gm/100 gm body weight
Dose I (200mg/100 ml)	**0.72 ± 0.04 gm/100 gm body weight**
Dose II (400mg/100 ml)	0.51 ± 0.01 gm/100 gm body weight
Dose III600 mg/100 ml)	0.37 ± 0.12 gm/100 gm body weight

Table 2: Changes in fertility of *bandicota bengalensis* in treated and untreated

Treatment	Number of offspring produced/female/reproductive cycle
Control	7.4± 2.1
Dose I (200mg/100 ml)	2.1± 1.1
Dose II (400mg/100 ml)	00
Dose III (600 mg/100 ml)	00

rate of the partner female. The observations are shown in the table:

Discussion

Our research showed that oral administration of *Aegle marmelos* (L) Corr. barks aquous extract lead to dose dependent defects in the testicular spermatogenesis which leads to production of defective sperms. The results of the present study indicate that the bark extracts of *A. marmelos* (L) Corr. have a considerable effect on fertility. It has already been reported that aquous extracts of *A. marmelos* (L) Corr. decreases the motility of rat sperms in vitro (Agrawal *et al*, 2012). An earlier study on 50% ethanolic extracts of the leaves reported the potential of the same in suppressing the fertility of male albino rats (Sur *et al*, 1999). Phytochemical analysis of the bark extracts by TLC have revealed the presence of skimmaianine, fagarine, marmine (Uttam, K.D. *et al*, 2006). Studies have also indicated the presence of alkaloids, tannins, terpenoids, volatile oil, glycosides, phenolic group in aquous extracts of A. *marmelos* (L) Corr. This leads to the conclusion that the activity shown by the extracts could be because of the presence of the above mentioned secondary metabolites in them.

Conclusion

The findings of the present study clearly indicate that the bark extracts of *Aegle marmelos* (L) Corr. have a negative effect on the fertility of *Bandicota bengalensis* (Gray, 1835). The decrease the weight of testis and depletion of the germinal layer could be a possible explanation of reduced fertility. The future use of *A. marmelos* (L) Corr. as rodenticide on a commercial basis deserves further investigation.

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