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Antifertility Efficacy of Aqueous Leaf Extract of Aegle Marmelos (Linn.) on Seminal Quality of Swiss Albino Mice

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Abstract: ***The effect of aqueous leaf extract of Aegle marmelos on epididymal sperm count, seminal pH, sperm motility, sperm mortality and abnormality of spermatozoa in male Swiss albino mice were studied. Mice were treated with 0.1ml of aqueous leaf extract daily up to 30 days and killed after 10, 20 and 30 days of exposure. Mice after 30 days treatment showed highly significant decrease in sperm count, seminal pH and sperm motility (P<0.001), while, highly significantly increase in sperm mortality and their abnormality (P<0.001) were observed compared to control groups of mice. These observations suggest that the aqueous leaf extract of Aegle marmelos cause antifertility effects among male mice.***

Keywords: Sperm count, Sperm Motility, Sperm Mortality, Seminal pH, Sperm Abnormality

1. Introduction

Population explosion is a serious problem for the developing countries. It is a challenging task for the people who are working on fertility control. There are various contraceptive devices are in practice among both sexes of human subjects but their side effects and failure rates are very high. Therefore, it is desired to derive such type of contraceptive agents which are cost effective, less failure rate, indigenous and without any side effects. In spite of considerable development in contraceptive methods, search for male antifertility agents in plants continue to be a potential area of investigation. Various plants have been known to possess antifertility activity but less studies have been made in this area of investigation. The main target sites for fertility regulation in male reproductive organs are testis and epididymis, where spermatogenesis occurs, and spermatozoa matures to develop fertilizing ability respectively.

Aegle marmelos belonging to family Rutaceae is commonly known as Bael having various medicinal properties. The Bael is one of the sacred tree of the Hindus. It is a deciduous tree, associated with divine rituals. This tree is found in India as well as Bangladesh, Egypt, Malaysia, Myanmar, Pakistan and Thailand. The aqueous leaf extract possesses activities like antibacterial (Rajsekaran, et. al. 2008), antihistaminic (Nigroho, A.E. et. al.2010), anti-inflammatory, antiseptic and analgesic (Rao, C.V. 2003), hepatoprotective (Singanan, V. 2007), insecticidal (Kumar, R. et. al. 2008), hypoglycemic and anti-oxidant (Upadhaya, et. al. 2004), anti-testicular activity (Das, U.K. et. al. 2006), anti-depressant (Kothari, S. 2010), wound healing (Jaswanth, A. et. al. 2000), anti-stress (Duraisami, R. et. al. 2010), antifertility (Remya, M. et. al. 2006; K. Sathyaraj et. al. 2010).

The present study is employed to understand the efficacy of *Aegle marmelos*on seminal quality of mice for fertility control.

2. Materials and Methods

The fresh mature leaves of *Aegle marmelos* were collected from Bhagalpur district of Bihar. The mature plant leaves were cleaned with tap water and dried in shade at room temperature. The shade dried leaves were crushed to powder by electric blender.

For the preparation of aqueous leaf extract of *Aegle marmelos* leaves. 100g of dried leaves powder were mixed with 1000ml of distilled water and the mixture was left in the container for overnight at room temperature. The residue was removed by filtration. The dose of male mice was given at the rate of 350mg/kg body weight (K. Sathyaraj, et. al. 2010) of leaf extract orally by gastric catheter.

Adult male Swiss albino mice of 25-30g body wt. were selected for the experiment and divided in to four groups. Each group containing 6 mice. One group of male mice were considered as control group of mice and other three groups were known as treated groups of mice (i.e. 10, 20 and 30 days). All the animals were kept in polypropylene cages under hygienic conditions in well ventilated room with 10 hrs.photoperiod at an ambient temperature 25± 2˚C under animal husbandry conditions.

Control group of mice were fed with 0.1ml distilled water orally with gastric catheter and treated groups were fed with 0.1ml (350mg/kg body wt.) of aqueous leaf extract of *Aegle marmelos* for 10, 20 and 30 days. All the mice were sacrificed after feeding by the method of cervical dislocation. After killing, both the cauda epididymis of each male mice were exposed and kept in watch glass and tinged with 2ml of normal saline. Both cauda epididymis is crushed and seminal content were sieved with metallic filter to avoid tissue debris in seminal content. Sperm counts were done after the method of Eliasson (1975), motility of spermatozoa were observed after the method of Tijee and Oentoeng (1968). pH of seminal plasma was measured with the help of pH indicator, which was procured from Merck Limited Worly, Mumbai.

Statistical Analysis
Student t- test was applied for the test of significance.

3. Result

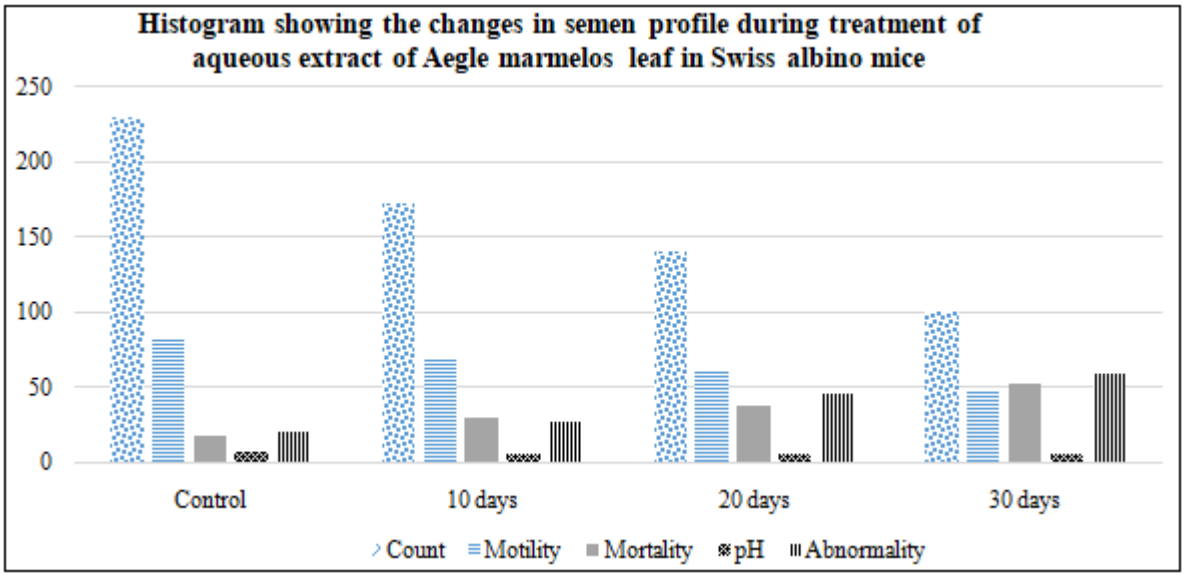
Dose and duration dependent oral feeding of aqueous leaf extracts to male mice cause significant decrease in sperm counts (P<0.001), sperm motility (P<0.001) and seminal pH of mice (P<0.001)than the control after10, 20 and 30 days of exposure. However mortality of spermatozoa shows significant increase (P<0.001) after 10, 20 and30 days treatment of aqueous extract of *Aegle marmelos* leaf than the control group of mice. Abnormality of spermatozoa also shows significant increase (P<0.001) than the control group of mice after 10, 20 and 30 days of treatment. Various types of abnormal spermatozoa were seenamong semen of treated group of mice likedouble headed, club shaped, banana shaped and without tail. As indicated in Table-1, Sperm counts, motility of spermatozoa and seminal pH showing highly significant decline during 10, 20 and 30 days of treatment. Mortality and abnormality of spermatozoa show highly significantincrease (P<0.001) after the treatment of 10, 20 and 30 days with aqueous extract of *Aegle marmelos* leaf.

Table 1

Table showing the effects of aqueous leaf extract of *Aegle marmelos* on seminal quality of mice.

Groups	Sperm Counts(X10^4 Sperms/ml)	Motility Percent	Mortality Percent	Seminal pH	Abnormality Percent
Control (6)	230± 2.004	82.5± 0.83	17.5± 0.83	7.26± 0.07	20± 0.63
10days treatment (6)	172.83± 1.11**	70.33± 1.37*	29.67± 1.37*	6.48± 0.05**	27± 1.06*
20days treatment (6)	139.67± 1.66**	62± 1.06**	38± 1.06**	5.95± 0.04**	45.83± 1.73**
30days treatment (6)	100± 2.11**	47.5± 0.83**	52.5± 0.83**	5.48± 0.03**	59.83± 1.53**

Data presented as Mean± SEM; *, ** shows significance at 0.01 and 0.001 levels with value in control. Number within parenthesis denote number of samples.



4. Discussion

This study shows that sperm count decreases significantly (P<0.001) in *Aegle marmelos* treated mice than the control groups of mice. Reduction in sperm counts indicate the interference in testicular spermatogenesis. As spermatogenic process is under direct influence of androgen, hence *Aeglemarmelos* leaf extract of *Aegle marmelos* causes significant decrease in androgen level (K. Sathyarj, et. al. 2010). Thus it can be concluded that the treatment of *Aegle marmelos* leaf extract may cause reduction in androgen level (Chauhan et. al. 2008) which in turn impedes the spermatogenesis among treated groups of mice. Such reduction in sperm counts leads to infertility among treated groups of mice.

As indicated in Table-1 sperm motility and seminal pH also decreased significantly (P<0.001) in *Aegle marmelos* leaf treated mice than control groups of mice. Sperm motility is one of the most important parameter for evaluation of fertility in male. Decrease in sperm motilitysuggests alteration of sperm maturation in epididymis (Ji. Zhou, Li Chenet. al. 2015) and it also decreases the chances of fusion of maleand female gamete in the fallopian tube (Lohiya& Goyal 1992), which depends on the androgen level (K. Sathyaraj et. al. 2010). Hence lower level of testosterone in *Aegle marmelos* treated mice decreases the motility of spermatozoa.

The pH of seminal plasma also decreased in treated groups of mice significantly (P<0.001) after 10, 20 and 30 days of treatment than the control groups of mice. Decreased pH of seminal plasma also lowers sperm motility and enhance the

mortality of spermatozoa which results into decrease in sperm count of treated mice (Ji Zhou, Li Chen et. al. 2015).

The mortality and abnormality of spermatozoa increased significantly (P<0.001) in the *Aegle marmelos* treated mice, which may be due to lower level of seminal pH and androgen(Chauhan et. al. 2008). Abnormal sperms are not able to fuse with the female gamete that leads to infertility in the *Aegle marmelos* treated mice (Lohiya& Goyal 1992).

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