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Effects of *Aegle marmelos* leaf extract on seminal electrophoretic proteins of mice (*Mus musculus*) in relation to antifertility

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ABSTRACT

Aegle marmelos leaf extract induce the anodic protein concentration in semen of treated group of mice which increased highly significantly (P<0.001) than the control. However, cathodic protein concentration increased insignificantly than the control. Due to increase in anodic protein concentration, total electrophoretic protein concentration of treated group of mice also increased highly significantly (p<0.001) after 10 to 50 days of treatment than the control. Aqueous leaf extract of *Aegle marmelos* selectively modulate seminal proteins. Significant increase in anodic protein concentration in semen of *Aegle marmelos* treated mice may add more negative charges on sperm surface membrane which affect capacitation and fertilizing ability of spermatozoa that may cause infertility among treated group of mice.

Keywords: Antifertility, Seminal protein, Electrophoresis, Relative mobility.

INTRODUCTION

Rising human population throughout the world particularly in developing and underdeveloped countries has detrimental effects on the life supporting system on earth (Gupta and Sharma, 2006). Over population effects on economy, environmental degradation, depletion of natural resources, rise in unemployment. Modern reproductive biomedicine has provided several preventive and effective methods of contraceptives for fertility control in male and female but none of which is very safe and without any side effects (Deshpande *et al* (1980). There are several types of contraceptives which are providing a significant amount of protection from sexually transmitted diseases and unwanted pregnancies (Tshikalange *et al*, 2005). It is very difficult task to provide synthetic medicine to poor and illiterate people. Thus traditional medicine is often the only affordable and accessible form health care. Therefore, the use of

plants products as antifertility agent cause minimal side effects as compared to currently available contraceptive methods (Umadevi *et al*, 2013).

Various plants have been known to possess antifertility activity but less studies has been made scientifically. Such as *Azadirachta indica, Abrus precatorious, Allium sativa, Allium cepa, Bougainvellia spectibilis, Carica papaya, Ocimum sanctum, Piper betel* etc. *Aegle marmelos* is very common medicinal plant which is also known as 'Bael' (Singh *et al*, 1981). It belongs to family Rutaceae, family of citrus fruit. It is a sacred tree, leaves are used to worship lord Shiva and Parvathi in Indian temples since ancient time. *Aegle marmelos* possesses various medicinal properties and used to treat various diseases (Ganesh *et al*, 2011) such as anti-bacterial (Rajasekaran *et al* 2008), anti-fungal (Anandhan *et al*, 2015), insecticidal (Sharma *et al*, 2017), anti-diabetic (Upadhya *et al*, 2004), immunomodulatory (Gupta *et al*, 2016), anti-microfilarial **(**Sahare *et al*, 2008), anti-depressant (Duraisami *et al*, 2010).

Seminal proteins play a major role in the development and capacitation of spermatozoa for fertilization process in the female reproductive tract. Any alteration in the seminal protein may leads to infertility in male. Thus, it was decided to study the effects of aqueous leaf extract of *Aegle marmelos* on seminal proteins in relation to antifertility.

MATERIALS AND METHODS

60 adult Swiss albino male mice (*Mus musculus*) were included in the investigation. These mice were procured from the animal stalk of University department of Zoology, T.M. Bhagalpur University, Bhagalpur and were maintained at uniform animal husbandry conditions.

Fresh and mature leaves of *Aegle marmelos* were taken from the gardens of Bhagalpur district. The leaves of *Aegle marmelos* were washed under tap water and dried at room temperature. Dried leaves were grinded with electric blender. 100 gm of *Aegle marmelos* leaf powder were kept in a jar and added 1000 ml distilled water and left it to overnight. The mixture was filtered and centrifuged at 1000 rpm for 15 minutes. After centrifugation, the supernatant was taken out from centrifuge tube for administration.

Total mice were divided into six (6) groups and each group containing six mice. One group was considered as control group while other was considered as experimental group. Treated groups of mice were fed 0.1 ml (350 mg/kg body wt. /day) aqueous extract of *Aegle marmelos* leaves for 10, 20, 30, 40 and 50 days. The control group of mice were fed 0.1 ml of distilled water for same days of exposure (Sathyaraj *et al*, 2010).

After completion of days of exposure, all mice were sacrificed. The cauda epididymis were operated out from the testis and tinged with 2 ml of normal saline (1 epididymis/ml of normal saline). Each of the cauda were crushed and seminal content were sieved by metallic filter. The seminal fluid was centrifuged for electrophoretic study. Electrophoresis was done by using polyacrylamide disc gel after the method of Smith (1976).

RELATIVE MOBILITY:

Relative mobility refers to movement of a polypeptide bands through the gel relative to the movement of marker in the gel. Relative mobility of different proteins were calculated in the relation with movement of the marker Bromophenol blue (BPB). The bands moving towards the cathode are called cathodic proteins and the bands moving towards the anode are called anodic proteins. Concentration of different protein bands were calculated against the known concentration of BSA (Bovine Serum Albumin). The results were expressed in the Mean value, ± standard error of the mean. Student **t- test** was applied to compare the data and calculation of level of significance. The values of data were significant at 0.01 level of significance and highly significant at 0.001 level of significance.

RESULT

Quantitative studies of different electrophoretic protein bands (Table-1, Fig; 1) show the marked difference in concentration of anodic proteins, cathodic proteins and total electrophoretic proteins in the *Aegle marmelos* leaf extract treated group of mice than the control group of mice.

Anodic proteins

Due to treatment of aqueous extract of *Aegle marmelos* (Bael) leaf at a dose of 0.1ml/day/mice, concentration of anodic protein in the semen of treated group of mice

increased significantly at P<0.1 and P<0.01 after 10 to 20 days *Aegle marmelos* leaf extract treatment, while after 30 days of treatment, there is highly significant increase (P<0.001) in anodic protein concentration in the semen of treated group of mice than the control. In 40 to 50 days of treated group of mice, the concentration of anodic protein increased highly significantly (P<0.001) than the control group of mice. The result shows that the concentration of anodic protein in semen of *Aegle marmelos* leaf extract treated group of mice increases significantly when duration of exposure ages (Table- 1, Fig; 1).

Cathodic proteins:

Treatment of *Aegle marmelos* leaf extract at a dose of 0.1 ml/mice/day to the treated group of mice after 10, 20, 30, 40 and 50 days of treatment, concentration of cathodic protein increases insignificantly than the control. The table-1 shows that the cathodic protein concentration in the treated group of mice goes up to maximum concentration after 50 days of treatment.

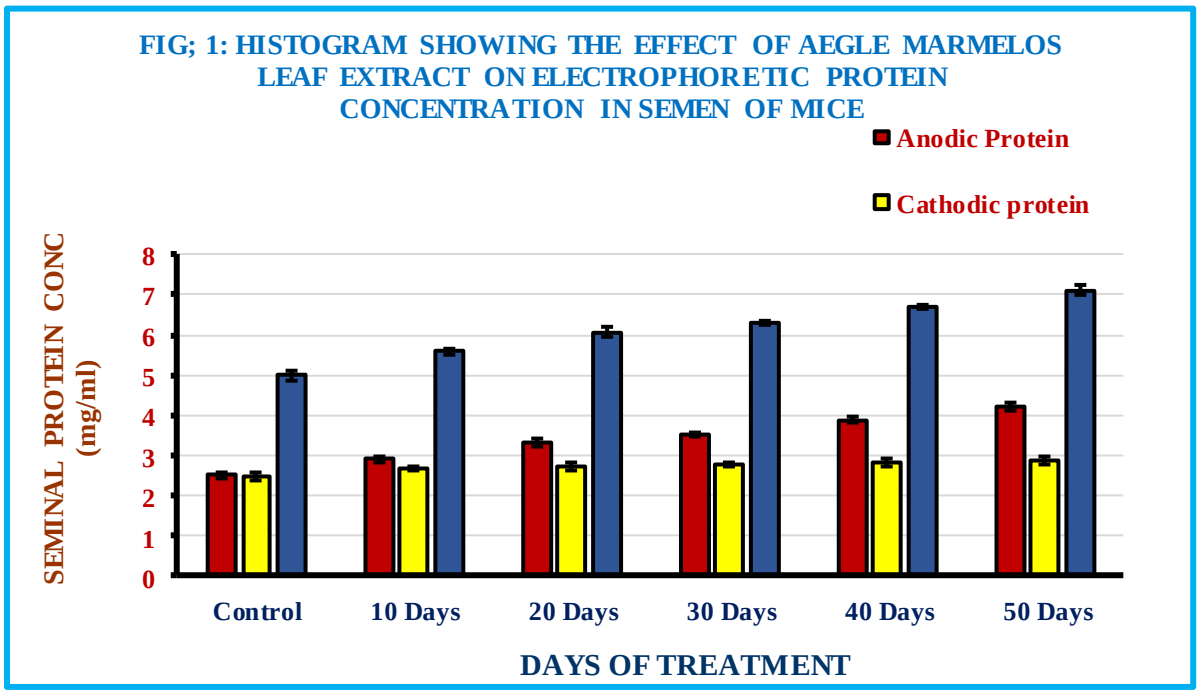
Total electrophoretic proteins

As table-1 shows, the total electrophoretic protein concentration in semen of treated group of mice after 10 to 20 days of treatment shows significant increase at P<0.1 and P<0.01 level of significance. After 30, 40 and 50 days of treatment, the total electrophoretic protein concentration shows highly significant increase (P<0.001) than the control group of mice. The gradual and significant increase in total electrophoretic protein of *Aegle marmelos* leaf extract treated group of mice reaches at maximum level after 50 days of treatment.

TABLE-1: Effect of aqueous leaf extract of Aegle marmelos on Seminal Electrophoretic Protein Concentration of mice in comparison with control

Groups	Anodic Protein Conc. (mg/ml)	Cathodic Protein Conc. (mg/ml)	Total Electrophoretic Protein Conc. (mg/ml)
Control (30)	2.51± 0.06	2.48± 0.08	4.99 0.11
10 Days of Treatment (6)	2.91± 0.09*	2.69± 0.06	5.60± 0.08*
20 Days of Treatment (6)	3.33± 0.11**	2.74± 0.10	6.07± 0.12**
30 Days of Treatment (6)	3.52± 0.07***	2.78± 0.04	6.30± 0.06***
40 Days of Treatment (6)	3.89± 0.12***	2.82± 0.09	6.71± 0.05***
50 Days of Treatment (6)	4.23± 0.13***	2.88± 0.11	7.11± 0.13***

*Data presented as Mean± SEM *, **, *** shows significant at 0.1, 0.01 and 0.001 levels with the value in control. Numbers within parenthesis denote number of samples.*



DISCUSSION

Seminal proteins are relevant for sperm function and related to interaction of sperm with the oocyte. Proteins of the seminal plasma responsible for establishing fertility (Rodriguez-Martinez *et al*, 2011). During ejaculation, the spermatozoa are transmitted by the seminal plasma. The interaction of the seminal plasma with spermatozoa induces binding of seminal proteins to the sperm surface membrane helps in sperm transport, survival and fertilizing ability in the female genital tract (Druart *et al*, 2018).

Proteins are one of the major seminal plasma components that modulate sperm function. Seminal plasma proteins have been found to influence sperm capacitation and sperm oocyte interaction (Caballero *et al*, 2012). The result of this study as shown in the table-1 (fig; 1 & 2) represent that the administration of *Aegle marmelos* leaf extract modulates the seminal plasma proteins. In the treated group of mice the concentration of total electrophoretic proteins also increased significantly ($P<0.001$) than the control. The increase in number of total electrophoretic protein concentration are may be due to significant increase in anodic proteins concentration.

After 50 days of treatment of *Aegle marmelos* leaf extract to the male albino mice, the total number of anodic protein band and their concentrations in seminal plasma increased highly significantly ($P<0.001$) than the control group of mice and reached maximum level. However, the total number of cathodic protein bands and their concentration increased insignificantly as compared to control group. Hence it is clear that the aqueous extract of *Aegle marmelos* leaf selectively influences the anodic proteins, due to which the number of anodic proteins increased as a result total electrophoretic proteins increased highly significantly.

Significant increase in anodic protein concentration in semen of *Aegle marmelos* treated mice may add more negative charges on sperm surface membrane which affect capacitation and fertilizing ability of spermatozoa that may cause infertility among treated group of mice than control (Bedford, 1963; Singh and Singh, 1988).

In a study, Vengaiah *et al* (2015) also showed that, administration of *Piper betel* leaf stalk at the dose of 50mg/kg body wt./day till 15 days to the male albino rats, total proteins increased significantly in all reproductive tissues, testis, epididymis, seminal vesicle and prostate gland. Verma *et al* (2016) reported that administration of *Piper betel* leaf stalk extract at the dose of 50mg/kg to the male albino mice up to 50 days, the total electrophoretic protein concentration and anodic protein concentration increase significantly ($P<0.001$) in the semen of treated mice. However, cathodic protein concentration do not show significant change.

CONCLUSION

From the above study it can be concluded that the aqueous leaf extract of *Aegle marmelos* directly affect the seminal proteins. After the 50 days of treatment of extract cause highly significant increase in concentration of anodic proteins in treated group of mice. Significant increase in anodic protein concentration in semen of *Aegle marmelos* treated mice may add more negative charges on sperm surface membrane which affect capacitation and fertility ability of spermatozoa that may cause infertility among treated group of mice than control. Hence, *Aegle marmelos* leaf extract could be a good and safe contraceptive agent for male.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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