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Evaluation of the effect of *Citrus limonum* Seeds on Testosterone and Sperm Morphology

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Abstract

There's a rapid increase inpopulation expansion across the world and the current status is alarming particularly in countries that are developing or those that are under developed. The threatening effect can be observed on resources that supports life on earth, which may lead to certain adverse effect like rise in poverty, depletion of natural resources, infectious diseases, epidemics, health and nutrition problemsetc. To combat this fertility regulation in both males and femalesmakes an important issue of concern for better reproductive health. Contraceptive methodfor controlled conception is preferred to avoid the risk. In this study the seed extract of Citrus limonum is investigated for its antifertility potential.The extract administered at a higher dose level for 60 days showed considerable reduction in the level of circulating testosterone. The aim of this study is to highlight the effect of Citrus limonum seed potential acting as a source of male fertility regulation agent.

Keywords: *Contraception, fertility, Citrus limonum, sperm, testosterone.*

Introduction

Fertility regulation an important aspect comprising contraception that not only manages conditions of infertility conditions, but also constitutes an important part of reproductive health[1].The term contraception is usually meant to prevent conception with the help of certain contraceptive devices, surgical procedures, or implementing certain sexual practices, or use of chemicals, or drugs, Thus, any type of device which inhibits a female from conceiving or from getting pregnant is regarded as contraceptive. Contraception is required for protection against unwanted pregnancy as well as for protection against sexually transmitted diseases [2]. There are various studies that have confirmed both men and women's demand for novel male contraceptives. Research for male contraceptiveis going on for more than 60 years. Hormone based male contraception are such that they interrupt natural hormonal feedback regulation comprising of Hypothalamic-Pituitary-Gonadal (HPG) axis, to inhibit the process of spermatogenesis [3].*Certain compounds found in plants could prevent fertilization without the side effects of hormone-based contraceptives [4] as is evident from previous researches.* In female albino mice the seeds of *Citrus limonum*have shown prominent antifertility effect [5]. The seeds are having phytoestrogenic activities and the alcoholic extract of the seeds reveals the most promising antifertility effect in female albino mice. It has been found that the treatment with lemon seed extract leads to anti-implantation activity in female albino mice [6].

Materials and Methods

Study design and parameters investigated

The experimental study was performed in the premises of Ranchi University. In the present study male Albino rats (175-200g) 3 months old were used. These rats were kept in polypropylene cages in the animal house in university department of zoology. They were kept at a room temperature of about 25±2°C and 50±15% relative humidity with a 24-hour day night pattern. The research experiments were performed after the approval from institutional animal ethics committee (IAEC) of Ranchi University, Jharkhand. Ref no z/1172/2029 [5]. Therats were kept under appropriate veterinary supervision according to the regulations of CPCSEA [7].

Parameters studied were the hormone testosterone level and sperm morphology of white albino rats.

Sample collection

The *Citrus limonum*seeds were procured from Birsa Agriculture University of Ranchi and identified in Botany department of Ranchi University[5].

These fresh seeds were dried in shade under 28±3°C for around 7 days. Then they were crushed into fine powdery substance and was subjected to ethanolic soxhlet extraction.

Experimental design

Treatment phase: Treatment phase was divided into two doses with time varying groups which are mentioned.

a) Group I-control

b) Group II -200 mg/kg for30 days and 60 days(group IIa and IIb, respectively).

c) Group III-400 mg/kg for 30 days and 60 days (group IIIa and IIIb, respectively).

Recovery phase: In recovery phase the animals were withdrawn from extract administration and was observed for 45 days.

Statistical Analysis

The data was analysed using t-test. ANOVA was performed for comparative study. Statistical Software (SPSS) was used to determine, $p<0.05$ and $p<0.01^{**}$ was considered to be statistically significant[8].

Results and Discussion

For analysis of sperm morphology cauda epididymis was minced and suspended in 0.5ml PBS placed in a watch glass maintained at 37°C. The tissue fraction was removed, and the sperm suspension was used for sperm analysis.

Sperm suspension was made and a drop on a glass slide was positioned, on which drop of equal volume of PAP stain (1:1) was placed and mixed thoroughly at 37°C then a drop of the mixture was placed on other glass slide and was observed under microscope.

Vaginal smears of the mated females were examined early morning after mating for observing the sperm morphology. Sperm abnormality represented in figure.1.1, figure.1.2 and figure.1.3.In group-I normal morphology of the sperm is clearly depicted. In the initial stage of dose administration, the destruction was minor but with the advancement of dose level in groups(group III) conspicuous destruction in the sperm head and tail has been observed.

Fig: 1.1sperm morphology group I

Fig: 1.2 Sperm morphology group II

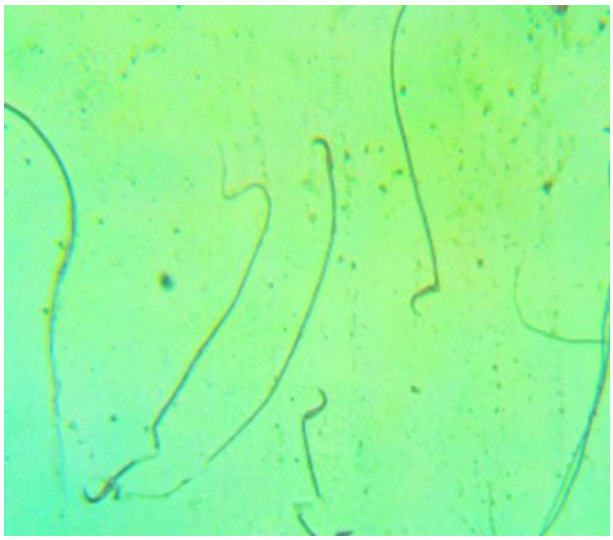

Fig: 1.3 Sperm morphology group III

For the determination of testosterone sample of circulatory blood was taken. the level of testosterone in group-II animals was insignificantly different when compared with control animals. In group III, with the advancement of dose and time the concentration of testosterone observed was reduced significantly. However, during recovery phase, the depleted level of testosterone was resumed back to its normal level and was comparable to the control group. There are numerous chemical agents present in extract of plants that leads to hormonal imbalance. Alkaloids present in the seeds may contribute to the lowering of testosterone level. According to some studies [9]it has been reported that alkaloids and flavonoids have the ability to reduce plasma concentrations of some fertility hormones.

Table 1: Estimation of testosterone level (in ng/ml) in the blood of rat following the administration of different doses of C. limonum seed extract

	Treatment phase		Recovery Phase	
Number of Groups	**30 days**	**60 days**	**75 days**	**105 days**
Group I	1.75±0.05	1.79±0.03	1.80±0.02	1.83±0.04
Group- IIa	1.74±0.02		1.79±0.02	
Group -IIb		1.77±0.02		1.84±0.02
Group -IIIa	1.70±0.06**		1.74±0.08	
Group -IIIb		1.68±0.13**		1.78±0.09

Conclusion

The findings of the investigation states that *Citrus limonum* seeds have the potential to be a natural spermicidal agent with the optimum dose of 400mg/kg body weight. The seeds showed destructive property and completely hampered the sperm, making its function impaired. The reduction in the level of testosterone indicates that it can be considered as a suitable agent for fertility regulation and upgrades its acceptance value regarding its antifertility potential in males. The seeds of *Citrus limonum* have various medicinal components therefore, to explore its properties as antifertility agent more investigation needs to be assayed and authenticated for future use.

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