



# ANTIFERTILITY EFFICACY OF CITRUS LIMONUM SEED EXTRACT ON MALE ALBINO RATS.

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**Abstract:** Exploration of drugs that exhibit suppressive effect on fertilisation is the need of current time, and many time plant extracts have been investigated for there antifertility effect in female and male albino mice. *Citrus limonum* is native of north west region of India and is also cultivated throughout the Mediterranean. The ethanolic extract of the seed was administered orally to the rats for 30 days at a dose of 250mg/kg b.w and 500 mg/kg b.w. The results showed decrease in the level of protein when compared with control. At the same time the testosterone content also showed a considerable decrease. The concentration 500 mg/kg of b.w and dosing period 30 day of ethanolic extract showed more significant effect ( $p < 0.05$ ) on testosterone level in current study when compared compared with the concentration 250 mg/kg of b.w. Therefore in this present investigation it has been found that the extract of *Citrus limonum* shows signs of fertility suppression in males by decreasing the androgen level. Further detailed analysis of *Citrus limonum* seed and more research work in coming future will possibly make this a prospective antifertility agent for males.

**Keywords:** *Citrus limonum*, Antifertility, Testosterone.

## INTRODUCTION:

A good number of scientific papers have been already published related to the use of medicinal plants as antifertility. In the same direction many approaches pursued to identify antifertility agents from the Citrus limon seeds<sup>1,2</sup>. There is a need the search suitable safe products from indigenous medicinal plants<sup>3</sup>. *Citrus-limonum* commonly known as lemon, belongs to the citrus-Linn (Ruteaceae) family. Lemon peel is used as stomachic and carminative. Anti-pneumonia factor is said to be present in lemon juice and it is also used in the treatment of acute tropical dysentery and diarrhoea. Citrus fruits are rich in vitamin C and in mineral salts [4]. All parts of it are utilizable and have their own importance, like its juice is helpful in many ways including reducing fever [5] Its leaf that was treated as waste is also utilizable for extracting oil to serve as medicinal and nutritional property as well as have great importance as natural preserving agent [6]. Lemon is an important medicinal plant. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts[7]. Citrus limon seeds have shown significant antifertility activity in female albino mice[8,9]. The petroleum ether, alcoholic and aqueous extracts of *Citrus-limonum* (lemon) seeds were investigated for anti-fertility effect in female albino mice [10]. In the present study seeds of the species *Citrus-limonum* are investigated for their anti-fertility activity in male albino rats.

## MATERIALS AND METHODS:

### Collection of plant material

Fresh seeds were obtained from Birsa Agriculture College. The seeds were dried in shade for a period of 10-12 days, at an ambient temperature of 22°C. The dried seeds were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form. Powdered sample was extracted in soxhlet using ethanol (95%) as a solvent for (24) hr. then evaporated at 45° C by rotary evaporator to form a paste, and further transferred into sterile bottles and refrigerated at 4°C until used.

### Preparation of laboratory animals:

Adult male albino rats (150-200g) were used in the present study. The animals were kept in polypropylene cages for seven days and fed on rat chow and water ad-libitum and allowed to acclimatize to the laboratory condition for seven days. The animals were maintained under perfect veterinary supervision and in accordance to the guidelines of CPCSEA.

30 male albino rats were divided into three groups (n=10). Each group was kept in a separate cage. Group A served as control and treated with vehicle only. Group B and group C were treated with seed extract at the rate of 250mg/kg and 500mg/kg b.w. respectively for 30 days.

### Hormone assay

Blood sample was collected for serum separation to estimate testosterone by radioimmunoassay. Serum sample separated by standard procedures stored at -20°C for subsequent analysis. Serum levels of testosterone were assayed in duplicate using radioimmunoassay kit [11].

### Statistical Analysis:

All the values were statistically analyzed by using students t- test. The values were considered significant at  $p < 0.05$ .

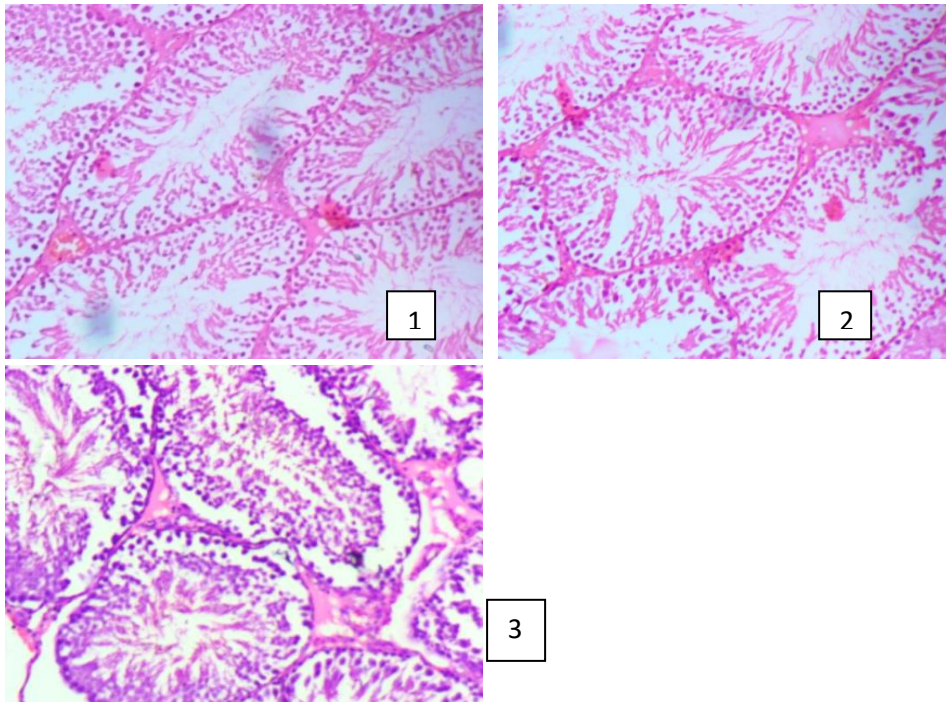
## RESULTS:

Table1: Effect of *citrus limonum* seed extract on testosterone, protein and glycogen.

Parameters	A Control	B L.D(250mg)	C H.D(500mg)
Testosterone (ng/ml)	2.05±0.30	1.97±0.19	1.82±0.50
Protein(mg/g)	3.55±1.74	3.51±1.85	3.46±1.76
Glycogen(mg/g)	2.51±0.36	2.36±0.39	2.21±0.32

Values are expressed as mean± SD, where n=10 \* p<0.05

The treatment brought about significant depletion in the level of testosterone ( $p < 0.05$ ). Protein and glycogen content of the testis was also decreased significantly (Table 1). Testosterone reduction was dose dependent i.e. high dose 500mg/kg.b.w treated group drastically reduced followed by less in low dose 250mg/kg.b.w group.

**Histopathological observation of testis**

Photomicrograph-1, Group-A (X 100 H.E.) testis of control rats showing a normal histoarchitecture, well developed germinal epithelium.

Photomicrograph-2, Group-B (X 100 H.E.) testes of rat treated at 250 mg/kg b.w. showing degenerated epithelium.

Photomicrograph-3, Group-C (X 100 H.E.) testes of rat treated at 500 mg/kg b.w. showing decreased spermatocytes.

**DISCUSSION**

Maintenance of structure and functional integrity of accessory reproductive organs requires continuous supply of androgen [12, 13]. Decreased weights of accessory sex glands indicates diminished secretory ability which reflects the decreased level of testosterone as these organs are androgen dependent [14]. The low levels of testicular protein are usually indicative of inhibition of spermatogenesis [15,16,17]. A decrease in glycogen content of the testis reduces the energy source for spermatogenic activity which could affect protein synthesis [18, 19].

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