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ORAL ADMINISTRATION OF *PETROLEUM* ETHER EXTRACT OF *TECOMELLA UNDULATA* LEAVES AFFECTS SPERMATOGENESIS AND FERTILITY OF MALE RATS.

Pawan Kumar Soni and Pratap Chand Mali*

Reproductive Biomedicine and Natural Product Lab, Department of Zoology, Centre for Advanced Studies, University of Rajasthan. Jaipur -302004 (India).

*** Corresponding Author: Dr. Pratap Chand Mali**
Reproductive Biomedicine and Natural Product Lab, Department of Zoology, Centre for Advanced Studies, University of Rajasthan. Jaipur - 302004 India.

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ABSTRACT
The 50% petroleum ether extract of *Tecomella undulata* leaves was administered orally at the dose level of 50,100 and 200 mg/kg body wt/day to male rats for 60 days to search a reversible male contraceptive agent. The result reveals that do not caused any significant change in their body weights, however, the weights of reproductive organs i.e. testes, epididymides, seminal vesicles and ventral prostate were decreased significantly as compared to controls. Sperm motility and sperm density in cauda epididymides was significantly declined which resulted in reduction of fertility. Level of testosterone and LH hormones were significantly reduced after the treatment of *T.undulata* leaves extract. The level of protein, sialic acid, glycogen and cholesterol contents were significantly decreased in testis and other accessory sex organs with the *T.undulata* leaves extract treatment. There was no significant alteration was observed in SGOT, SGPT, acid phosphatases, alkaline phosphatases, cholesterol and LDH level in the serum of treated rats. Histoarchitecture of the testis exhibit degenerative changes in germinal epithelium and all spermatocytes stage of spermatogenesis support the decreased sperm density in treated rats. Intertubular space was increased in between seminiferous tubules as compared to controls. In conclusion, oral administration of *T.undulata* leaves extract for 60 days caused androgen deprivation affects spermatogenesis in treated rats without caused any side effects might be due to antiandrogenic nature of the drug.

KEYWORDS: *Tecomella undulata*, fertility, spermatogenesis, rats, hormones.

1. INTRODUCTION
Population explosion effects not only economy but also caused environmental degradation, depletion of natural resources and pollution around the world. However, several preventive care and effective methods of contraceptives are available for fertility control in both male and female but none of which is safe and reversible.[1,2] Plants are important sources of novel pharmacologically active compounds, with many blockbuster drugs being acquired directly or indirectly. It has been reported that the use of plants products as fertility regulating agent may cause minimal side effects as compared to currently available synthetic contraceptives.[3, 4] According to the World Health Organization, more than 80% of the world's population rely upon traditional medicinal plant based systems for primary health care. In India, approximately 95% of the prescriptions are plant based used in the traditional systems like Ayurveda, Homeopathic etc.[5] The plant *Tecomella undulata* (Family *Bignoniaceae*) is commonly known as Rohida. Phytochemical studies of *T.undulata* different parts have lead to identification of pharmacologically relevant compounds such as Iridoid, glucoside[6], phytosterols, flavonoid glycoside, naphthoquinone[7,8], flavonol[9], fatty alcohol[10] fatty acid[11] and triterpenoids.[12]

The percent extractive values for leaves are highest with petroleum ether.[13] A qualitative phytochemical test of petroleum ether extracts of leaves show some constituents such as steroids, tannins and saponins.[13] *T.undulata* leaves have various bioactive compounds like oleanolic acid, ursolic acid, betulinic acid, triacontanol, cirsimaritin, cirilineol, pentariacontanol and 4, 5-dihydroxy-3, 6, 8 trimethoxyflavones.[14, 15]

In modern pharmacopeia different parts of *Tecomella undulata* are extensively explored to use for the treatment of several human diseases like spleen internal tumours, diseases of abdomen, wound healing, conjunctivitis, hepatosplenomegaly, blood purifier, syphilis and gonorrhoea. Pharmacological studies have reported its anti-HIV[14], antibacterial activity, analgesic and anti inflammatory, antioxidants, hepatoprotective[16]

activities. Several traditional uses and chemical composition of *T.undulata* leaves prompted us to explore for possible effects on fertility. Therefore, the present study was planned to investigate the affects of the petroleum ether extract of *T. undulata* leaves to develop a safe and reversible male contraceptive agent.

2. MATERIALS AND METHODS

2.1 Identification of Plant and preparation extract

The plant *T. undulata* was identified by taxonomist at Department of Botany, University of Rajasthan, Jaipur (Rajasthan, India) and voucher herbarium number of this plant is RUBL-211334. The leaves of *T. undulata* were collected, shade dried, crushed and powdered. This powder was subjected to soxhlet with 50% petroleum ether for 24 hrs (8 hrs. X 3 days) according to the WHO protocol 1983.[17]

2.2 Animal model: Colony-bred, healthy adult fertile male albino rats (*Rattus norvegicus*) weighing between 150-200 gm were used for the present study. The animals were kept under controlled environment conditions and maintained on standard.

2.3 Treatment protocol: Rats of similar body weight, size age were grouped into 5 groups. The experiment was accomplished to observe antifertility effect, possible mode of action/effects nature of the extract and reversibility effects.The animals were divided into five treatment groups, each group consisting of 8 animals.

Group-A: Control vehicle treated rats (sterile distilled water) alone orally for 60 days.
Group-B: *T. undulata* leaves extract at a dose of 50 mg/kg.b.wt./day for 60 days.
Group-C: *T. undulata* leaves extract at a dose of 100 mg/kg.b.wt./day for 60 days.
Group-D: *T. undulata* leaves extract at a dose of 200 mg/kg.b.wt./day for 60 days.
Group-E: *T. undulata* leaves a dose of 100 mg/kg.b.wt./day for 60 days were kept for a recovery period of 30 days.

2.4 Fertility test and scarification schedule

Fertility test of individual rat was done prior to the experiment and after 55 days of treatment. To assess fertility of each male rat was cohabitated with proestrous females in 1: 2 ratio, vaginal smear was examined every morning for positive mating and number of litters delivered was noted. Twenty-four hours after their last dose, the rats were weighed and sacrificed under light ether anaesthesia.

2.5 Study Parameters

2.5.1 Body and organ weights

The initial and final body weights of the animals were recorded. The testes, epididymides, seminal vesicles, ventral prostate were dissected out, cleared off fat, blood vessels and connective tissue before weighing on single pan balance.

2.5.2 Sperm count and motility

For sperm motility and density, 50 mg of cauda epididymis was minced in 1 ml of physiological saline immediately within 5 min after scarification, 1 drop of evenly assorted sample was applied to a glass slide under a cover glass. The percent motility was calculated by counting both motile and immotile spermatozoa per unit area. After that Cauda epididymal sperm density was determined and express as millions/mm^3 suspension.[18]

2.5.3 Serum biochemistry

Serum was separated from blood and stored at -20^oC for the cholesterol[19], serum glutamic oxaloacetic transaminase (SGOT)[20], serum glutamic pyruvic transaminase (SGPT)[20], acid phosphatases[21], alkaline phosphatases[22] and LDH[23] determination.

2.5.4 Tissue biochemistry

The testis, cauda epididymis, seminal vesicles were freezed for the analysis protein[24], glycogen[25], cholesterol[26] and sialic acid[27] contents.

2.5.5 Hormone assay

FSH, LH and testosterone were measured in serum in duplicate by Chemiluminescence method.

2.5.6 Histopathological study

Contra lateral side of the testis was fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and free from adherent tissue and embedded in paraffin wax. 6 µ sections prepared and stain with Harris's haematoxylin and eosin to observe histopathological changes.

2.5.6 Statistical analysis

Data were expressed as mean ± S.E. and analysed for statistical significance by using student's "t" test. The data considered as significant and highly significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.[28]

2.5.7 Ethical aspects

The study will be carried out under the supervision of ethical committee of the Department of Zoology, University of Rajasthan, Jaipur and CPCSEA (2006) guideline was followed.[29]

3. RESULTS

Oral administration of *T.undulata* leaves extract at all the dose level did not cause any significant change in the body weight of rats. Result of serological parameters - SGOT, SGPT, Acid phosphates, alkaline phosphates, cholesterol and LDH showed no significant alteration in all the treatment groups (Data were not shown) as compared to control.

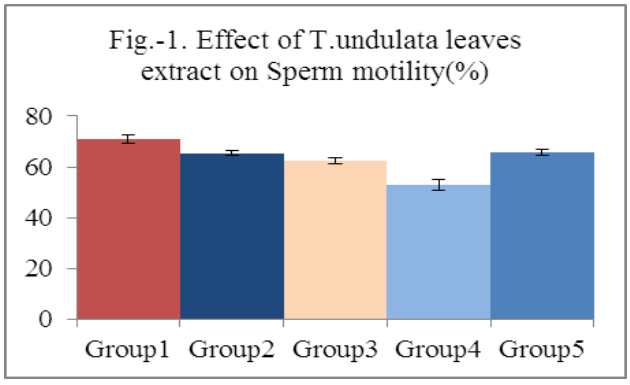
T.undulata extract treatment decreased the weight of testes, epididymides, seminal vesicles and ventral prostate in dose dependent manner (Table-1). The fertility rate of treated rats lessens up to 70% after the treatment of *T.undulata*.

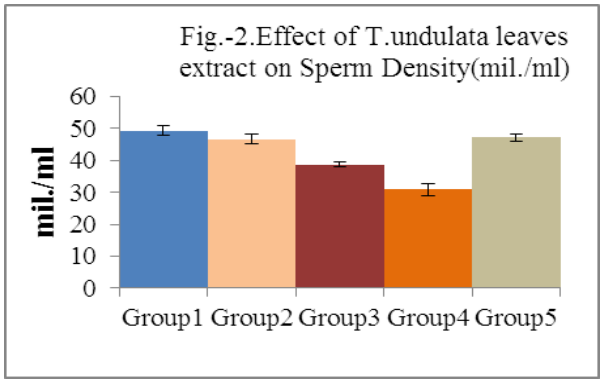
Table-1 Changes in body, organs weight and Fertility of male rats treated with 50% pet. ether extract of *T.undulata* leaves for 60 days.

Treatment	Body Weight (gm)		Organs Weight mg/100gm.b.wt.				Fertility Test (%)
	Initial	Final	Testes	Epididymides	Seminal Vesicle	Ventral Prostate	
Group-I	149.62 ± 4.22	168.5 ± 3.12	808.88 ± 5.29	522.58 ± 2.31	440.95 ± 2.76	176.51 ± 1.86	93.75%
Group-II	151.87 ± 5.42^{ns}	168.12 ± 4.21^{ns}	793.78 ± 5.83^{ns}	514.15 ± 3.69^{ns}	432.28 ± 4.79^{ns}	169.41 ± 3.41^{ns}	25% (-)
Group-III	151.87 ± 3.77^{ns}	166.25 ± 4.6^{ns}	788.36 ± 6.71*	511.98 ± 3.75*	427.48 ± 3.92*	167.03 ± 2.36*	37.5% (-)
Group-IV	150.0 ± 3.27^{ns}	165.62 ± 3.59^{ns}	786.08 ± 4.66*	510.80 ± 3.81*	425.26 ± 3.79*	165.88 ± 2.85*	62.5% (-)
Group-V	149.37 ± 3.19^{ns}	166.25 ± 2.95^{ns}	798.95 ± 3.05^{ns}	515.51 ± 4.16^{ns}	433.42 ± 4.30^{ns}	171.45 ± 3.12^{ns}	93.7%

(Mean ± SEM of 8 Animals) Treated Groups II, III, IV and V Compared with Control Group I.
***= highly significant (p≤ 0.001), ** = significant (p≤ 0.01), * = significant (p≤ 0.05), ns = non-significant.

Administration of *T.undulata* leaves extract to male rats caused significant to highly significant diminish litters, sperm motility (p≤ 0.001) and concentration of cauda epididymal sperms (p≤ 0.001) at the all dose level (Fig-1,2).





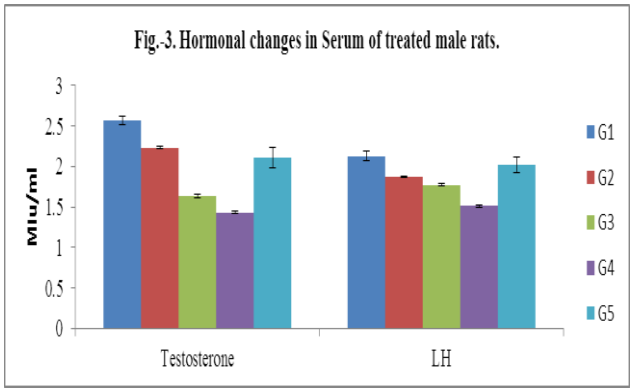
The level of protein was significantly decreased in testis (p≤ 0.01), epididymis (p≤ 0.05) and seminal vesicle (p≤ 0.05) after administration of *T.undulata* leaves extract. In the treated rats sialic acid level were significant decrease in testis (p≤ 0.05), epididymis (p≤ 0.01) and seminal vesicle (p≤ 0.05) (Table-2). In the testis, level of glycogen (p≤ 0.05) and cholesterol (p≤ 0.05) were significant decreased (Table-2).

Table: 2 Biochemical changes in Tissue of male rats treated with 50% pet. ether extract of *T.undulata* leaves for 60 days.

	Protein (mg / gm)			Sialic Acid (mg / gm)			Cholesterol (mg / gm)	Glycogen (mg / gm)
	Testis	Epididymis	Seminal Vesicle	Testis	Epididymis	Seminal Vesicle	Testis	Testis
Group-I	257.83 ± 2.10	245.31 ± 3.74	213.61 ± 3.32	5.84 ± 0.27	6.64 ± 0.12	5.13 ± 0.138	5.97 ± 0.18	3.29 ± 0.13
Group-II	245.12 ± 4.01*	232.29 ± 2.86*	205.71 ± 2.58^{ns}	4.71 ± 0.33*	5.84 ± 0.39^{ns}	4.70 ± 0.31^{ns}	5.01 ± 0.33*	2.83 ± 0.34^{ns}
Group-III	242.35 ± 4.21*	231.25 ± 2.78*	203.60 ± 4.02^{ns}	4.59 ± 0.32*	5.83 ± 0.35^{ns}	4.33 ± 0.27*	4.42 ± 0.42*	2.63 ± 0.26*
Group-IV	239.17 ± 3.19**	230.84 ± 2.61*	201.37 ± 2.89*	4.46 ± 0.32*	5.58 ± 0.27**	4.31 ± 0.26*	4.35 ± 0.45*	2.61 ± 0.26*
Group-V	248.23 ± 4.68^{ns}	238.20 ± 2.08^{ns}	209.61 ± 2.51^{ns}	5.14 ± 0.43^{ns}	5.95 ± 0.27^{ns}	4.70 ± 0.25^{ns}	5.21 ± 0.38^{ns}	3.16 ± 0.25^{ns}

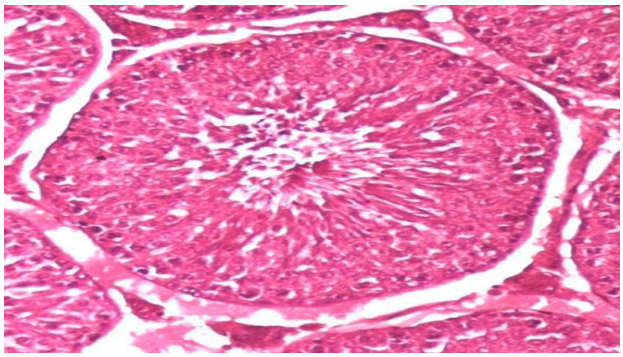
(Mean ± SEM of 8 Animals) Treated Groups II, III, IV and V Compared with Control Group I.
***= highly significant (p≤ 0.001), ** = significant (p≤ 0.01), * = significant (p≤ 0.05), ns = non-significant.

Serum FSH level was not affected significantly, but the level of serum testosterone ($p \leq 0.001$) and LH hormone ($p \leq 0.001$) were declined in all of the treated groups in comparison to control group. (Fig.-3)

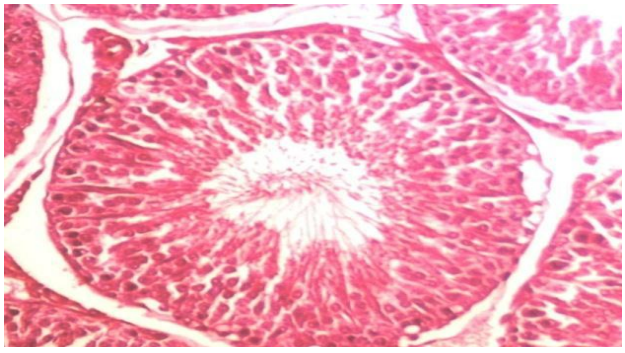


3.1 Histological observation of testis

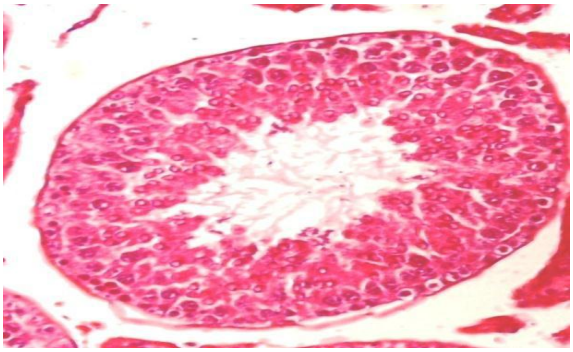
Histological observation of control rat (photomicrograph-1) testis exhibits normal structure of seminiferous tubule, which contains all successive stages of spermatogenesis, lumen filled with mature sperm cells. Oral administration of *T.undulata* leaves extract caused degenerative germinal elements of testis and reduced primary, secondary spermatocytes, spermatids and sperm in lumen of Seminiferous tubules (Photomicrographs 2 to 4). Interstitial space was comparatively loose. Lumen with less sperms and disrupted Leydig cells and cell debris are also visible. Histoarchitecture of testis of recovery group rats (photomicrograph-5) exhibited normal structure as similar to control group.



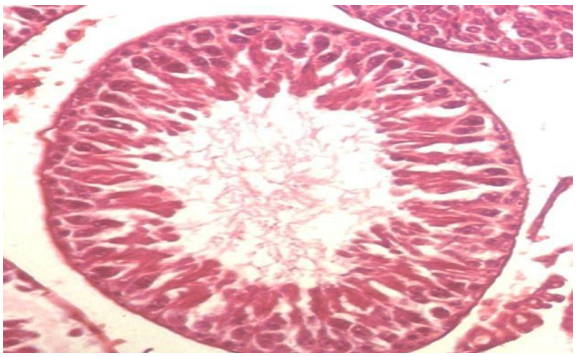
(Photomicrograph-1) [Group I]



(Photomicrograph-2) [Group II]



(Photomicrograph- 3) [Group III]



(Photomicrograph-4) [Group IV]

4. DISCUSSION

The male reproductive system is a complex that consists of the testes, epididymides, seminal vesicle, vas deferens and ventral prostate. These structures work together to maintain the fertility, and male secondary sexual characteristics. Reduction in the weights of testes and other accessory sex organs might be due to insufficient level of androgen supply.[30] Rat prostate glands contain some androgen receptors which are the direct target of androgen action[31] and mainly dependent on testicular androgens.[32] Spermatogenesis is a continuous process in which spermatogonial stem cells mature in a step-wise manner into specific germ cells before terminally differentiating to form spermatozoa. This dynamic process depends upon Sertoli cells that provide nourishment and structural support to germ cells throughout their development and Leydig cells that synthesize steroidal hormones necessary for germ cell differentiation. Testosterone is produced by Leydig cells in the testes and therefore degenerated Leydig cells in the treated rats are responsible for decreased of testosterone production[33], which might have affected the fertility of treated rats as compared to control.

It is known that the structure and function of the epididymis are dependent on androgens.[34] In the present study, a dose related suppression of cauda epididymal sperm motility in all treatment groups suggest an undersupply of testosterone to epididymis and therefore, an impaired epididymal function.[35,36,37] The reduction in sperm motility in cauda epididymis is of importance with regard to fertilization.[38]

In present study, protein content in testes and other sex organs significantly decreased following *T.undulata* leaves extract administration probably due to the absence of the spermatogenic stages in the testes.[39] Low level of sialic acid in testes, epididymides and seminal vesicles in treated rats may be correlated with loss of androgen.[40,41] Sialic acid acts as a lubricant to facilitate the downward movement of sperm and to reduce friction among spermatozoa.[41] The reduced sialic acid content might change the structural integrity of acrosomal membrane, ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa.[41-42] The low glycogen content in the testis after *T.undulata* leaves extract administration is possibly due to the inhibition of phosphorylase activation or the depletion of certain other enzyme which could block androgen synthesis.[43] Change in level of cholesterol after the treatment caused degenerative changes in treated rats.[2]

All these factors thus brought about functional sterility in the extract treated rats. However, the induced infertility was completely reversed after withdrawal of treatment of another period of 60 days. Also no apparent abnormality was observed in the litters delivered by the females mated with the males of recovery group. Non-toxicity of 50% petroleum ether extract of *T.undulata* is further supported by the data obtained after examination of serological parameters, which remain unaltered.

5. CONCLUSION

From present study it can be concluded that *T.undulata* is capable to suppress male fertility without altering general metabolism. Hence the possible male contraceptive efficacy of *T.undulata* leaves extract cannot be ignored paving way to the smooth development for the clinician interests in clinical trials towards emergence of a potent herbal male contraceptive.

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