



EVALUATE ANTIFERTILITY EFFECTS OF *TECOMELLA UNDULATA* TO DEVELOP AN ORAL MALE CONTRACEPTIVE.

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ABSTRACT **Objectives-** To evaluate antifertility activity of *Tecomella undulata* bark to develop a reversible male contraceptive. **Methods-** 50% petroleum ether extract of *Tecomella undulata* bark was administered orally at three different doses in male Wistar rats for 60 days. The clinical investigation of serum was performed, reproductive organs weight was recorded. Sperm density and motility were calculated. The tissues were analyzed for sialic acid, protein, fructose and cholesterol contents. The level of testosterone, FSH and LH were measured. The damage of sperms DNA was assessed by comet assay. Data were analyzed statistically using Student's t-test. **Key findings-** Data do not showed any alteration in general body weights; but weights of reproductive organs, sperm density and motility were declined significantly, followed to decrease fertility. Levels of testosterone, FSH and LH were significantly decreased. The levels of sialic acid, protein, glycogen and cholesterol contents were reduced significantly in reproductive organs. Testis histoarchitecture exhibits degenerative changes in germinal epithelium and reduced numbers in spermatocytes, spermatids. Comet assay expressed DNA damage in sperms of extract treated groups. **Conclusions-** It can be concluded that oral administration of *T.undulata* bark extract caused androgen deprivation affects spermatogenesis in treated rats might be due to antiandrogenic nature of treatment.

KEYWORDS : Antifertility, *Tecomella undulata*, histoarchitecture, androgen.

Introduction

At present, population explosion is one of the social problems since uncontrolled growth declined natural resources and cause threatens to sustainable development. Many methods of contraceptives have been designed for women, while need to paid satisfactorily attention for men in this issue (Soni et al., 2015) and secondary thing is the synthetic contraceptives use for fertility control create several harmful effects such as weight gain, hypertension, increased possibility of cancer and hormonal imbalance (Shafik, 1994) Thus demand to replace these synthetic agents by safe and effective agents based on plants. In Ayurveda medicine system, these plant based drugs are more use because of these low toxic natures and long standing experience of exposure (Chaudhary et al., 2011).

The bark of plant *Tecomella undulata* (Family *Bignoniaceae*) is rich source of biological active compounds such as tecomellosides, tecomine, tecosides lapachol, β -sitosterols, undulatosides, chromones glycosides, veratric acid, tannins and saponins (Rastogi & Mehrotra 2006, Ambasta, 2000, Nandkarni & Nandkarni 2006, Kumawat et al., 2012). The bark extract of *Tecomella undulata* possesses anticancer, analgesic, antibacterial, antifungal, antitermite, cardiotoxic, hepatoprotective, immunomodulatory, smooth muscle relaxant activity and anti-hyperglycaemic effect (Ravi et al., 2011, Khare, 2007, Chal et al., 2011, Das et al., 2015). Plant extract is use in treatment of gonorrhoea, syphilis, gout and also reported as blood purifier (Parveen et al., 2007). Antifertility activity of bark powder and leaves extract (Soni & Mali 2016) were reported earlier. Traditionally females of tribal communities take bark powder of *T.undulata* for abortion at Samahni valley in Pakistan (Muhammad & Khan 2008).

Sperm with damaged DNA may not perform fertilization and indicate it poor pregnancy so maintenance of sperm DNA integrity is necessary for healthy sperm to fertilize the egg cell (Morris et al., 2002). Therefore, this study was designed to evaluate the effects of different dosages of 50% petroleum ether extract of *T.undulata* bark treatment with emphasis on the sperm DNA, as well as the withdrawal thereof on the reproductive structure of Wistar male rats.

2. Materials and Methods

2.1 Identification of Plant and preparation of 50 % petroleum ether extract

The plant *T. undulata* (Family *Bignoniaceae*) was authenticated using specimen vouchers at the Department of Botany, University of Rajasthan, Jaipur (Rajasthan, India) and voucher herbarium number of this plant is RUBL-211334. Plant material was shade dried and

powdered. This powder was subjected to Soxhlet extraction with 50% petroleum ether for 24 hrs (8 hrs. X 3 days) according to the WHO protocol (WHO, 1983a). Seventeen gm of solid matter was obtained from 250 gm of *T. undulata* bark powder in the aqueous extract giving a yield of 6.8 %.

2.2 Animal model

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

2.3 Treatment protocol

The experiment was accomplished to examine antifertility effects, possible mode of action / effects nature of the extract and reversibility. Five treatment groups of the animals were required for the study, each group exist 8 animals. Animals of group I was served as control vehicle treated rats (sterile water) alone orally and group II, III and IV were treated with different doses of extract as 50mg, 100mg and 200mg/kg b. wt for 60 days respectively. Animals of group V served as recovery group and treated with 100 mg dose for 60 days and kept of recovery period of 30 days.

2.4 Fertility test

Fertility test of individual rat was accessed 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 reported every morning for positive mating and number of litters delivered was noted.

2.5.1 Body and Organs weight

The weights of the animals were noted prior of experiment and before scarification. Twenty-four hours after their last dose, the rats were sacrificed under light ether anaesthesia. The testes, seminal vesicles, epididymides and ventral prostate were dissected out, cleared of adhering fat and extraneous tissue before weighing on single pan balance.

2.5.2 Sperm count and motility

For measurement of sperm motility and density 50 mg of cauda epididymis was used and minced in 1 ml of physiological saline immediately within 5 min after scarification; one drop of evenly assorted sample was applied to a glass slide under a cover glass. Both motile and immotile spermatozoa were counted per unit area for the calculation of percent motility. After that Cauda epididymal sperm density was determined in millions/mm³ (WHO 1983 b).

2.5.3 Serum biochemistry

Serum was separated and stored at -20°C for determination of the cholesterol (Zlatkis et al., 1953), serum glutamic pyruvic transaminase (SGPT) (Reitman and Frankel, 1957), serum glutamic oxaloacetic transaminase (SGOT) (Reitman and Frankel, 1957), acid phosphatases (Gutman & Gutman, 1940), alkaline phosphatases (Fiske & Subbarow, 1925), and LDH (Cabaud and Wroolewski, 1958)

2.5.4 Tissue biochemistry

The testis, cauda epididymis, seminal vesicles were freezeed for the analysis protein (Lowry et al, 1951), glycogen (Montgomery, 1957), cholesterol (Mann, 1964), sialic acid (Warren, 1959) and fructose (Foreman et al, 1973) contents.

2.5.5 Hormone assay

The serum samples were stored in a -20 °C freezer and FSH, LH and testosterone hormone were measured in blood plasma samples using Chemiluminescence immunoassay method.

2.5.6 Histopathological study

The Contra lateral side of the testes from both control and treated groups were dissected out and fixed in Bouin's fluid. For histological examination the tissues were processed and paraffin wax (Melting point 55o-62oC) sections were made at 6 μ and stained with Harris's hematoxylin and eosin to observe histopathological changes under light microscope.

2.5.6 Comet assay

The comet assay is use to detect DNA damage in various cell types (Moller, 2005). 1.0 % Normal melting agarose (NMA) and 0.5% low melting point agarose (LMPA) were prepared in PBS. For primary layer, 110 μL of NMA were added to ice cold microscope slides. For second layer, 75 μL of LMPA containing sperm cells were added. Finally, 75 μL LMPA was added for third layer preparation. For lysis of sperm cells, Slides were dipped in lysis solution for 1h at 4°C, and after that slides were kept in alkaline buffer for 15 minutes to DNA unwinding. Electrophoresis was processed with the slides at 25 V and 300 mA for 30 minutes, after this, slides were washed with neutralizing buffer and stained with ethidium bromide (2 mg/10 ml distilled water). The slides were analysed under fluorescent microscope at 400X magnification (Singh et al., 1998).

2.5.7 Statistical analysis

Data were demonstrating as mean ± S.E. and student's "t" test was used for statistical significance. The data considered as significant and highly significant at p≤0.01 and p≤0.001, respectively (Gupta, 1978).

2.5.8 Ethical aspects

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

3. Results

After treatment of *T. undulata* bark extract, no significant change in the body weight was found. Result of serological parameters - SGPT, SGOT, Acid phosphates, alkaline phosphates, cholesterol and LDH showed no significant alteration in all treated groups (Data were not shown) as compared to control.

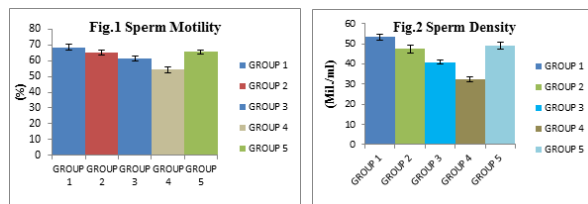
The oral administration of *T.undulata* extract, the weight of reproductive tissues as testes, epididymides, seminal vesicles and ventral prostate were decreased according to dose level (Table-1).

Table-1 Changes in body, organs weight and Fertility of male rats treated with 50% pet. ether extract of *T.undulata* bark 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	144.37 ± 3.46	170.62 ± 3.19	821.92 ± 4.74	533.39 ± 2.37	441.20 ± 3.66	173.95 ± 2.87	87.5%
Group-II	146.87 ± 3.65ns	171.25 ± 2.63ns	810.01 ± 8.48ns	526.12 ± 4.75ns	437.32 ± 2.78ns	165.17 ± 4.88ns	25% (-)
Group-III	149.37 ± 4.27ns	168.12 ± 3.77ns	789.53 ± 6.01***	521.96 ± 3.87*	424.51 ± 4.31*	162.99 ± 5.75ns	31.25% (-)
Group-IV	143.75 ± 3.98ns	161.25 ± 3.75ns	777.71 ± 5.34***	517.46 ± 4.31**	421.56 ± 4.84**	157.99 ± 4.17**	62.5% (-)
Group-V	151.87 ± 3.89ns	170 ± 2.84ns	810.22 ± 4.35ns	525.56 ± 2.79ns	436.29 ± 5.63ns	169.18 ± 3.13ns	81.25%

(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* bark extract to male rats caused highly significant sperm motility (p≤ 0.001) and sperms concentration of cauda epididymal (p≤ 0.001) at the all treatment groups (Fig-1,2). The fertility rate of treated rats lessens up to 70% after the treatment of *T. undulata*.



The protein level in testis (p≤ 0.001), epididymis (p≤ 0.001), vas deferens (p≤ 0.001), and seminal vesicle (p≤ 0.01) was significantly declined after administration of *T. undulata* bark extract. In the treated rats sialic acid level were significant decrease in testis (p≤ 0.01), epididymis (p≤ 0.001) and seminal vesicle (p≤ 0.01) (Table-2). In the testis, level of glycogen (p≤ 0.01) and cholesterol (p≤ 0.001) were significant decreased (Table-2). In the seminal vesicle, level of fructose (p≤ 0.001) was decreased.

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

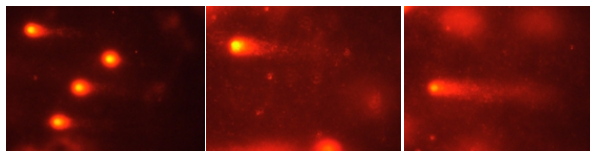
	Protein (mg / gm)					Sialic Acid (mg / gm)			Cholesterol (mg / gm)	Glycogen (mg / gm)	Fructos (mg / gm)
	Testis	Epididymis	Seminal Vesicle	Vas deferens	Ventral Prostate	Testis	Epididymis	Seminal Vesicle	Testis	Testis	Seminal Vesicle
Group-I	256.32 ± 5.28	251.98 ± 2.13	214.25 ± 4.62	243.42 ± 3.52	208.12 ± 3.67	5.97 ± 0.37	6.61 ± 0.28	5.35 ± 0.33	5.90 ± 0.32	3.79 ± 0.41	5.32 ± 0.30
Group-II	241.05 ± 2.80*	234.15 ± 3.82**	204.72 ± 2.24ns	229.22 ± 3.72*	199.19 ± 2.15ns	5.62 ± 0.44ns	5.21 ± 0.38*	4.39 ± 0.31ns	5.49 ± 0.26ns	2.74 ± 0.27ns	4.37 ± 0.30*
Group-III	235.77 ± 5.30*	228.72 ± 5.09***	201.73 ± 4.65ns	223.64 ± 5.01**	195.05 ± 4.68*	4.14 ± 0.24**	4.89 ± 0.41**	4.17 ± 0.27*	4.14 ± 0.45**	2.54 ± 0.30*	3.73 ± 0.42**
Group-IV	229.24 ± 2.92***	223.49 ± 3.52***	193.55 ± 3.58**	219.63 ± 3.41***	193.11 ± 3.57*	4.07 ± 0.29**	4.19 ± 0.34***	3.94 ± 0.18**	3.97 ± 0.39***	2.36 ± 0.18**	3.42 ± 0.25***

Group-V	249.23 ± 4.07ns	242.91 ± 3.81ns	206.13 ± 2.11ns	234.02 ± 2.81ns	202.13 ± 2.03ns	5.13 ± 0.35ns	5.65 ± 0.37ns	4.58 ± 0.28ns	5.15 ± 0.50ns	3.71 ± 0.40ns	4.66 ± 0.25ns
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(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.

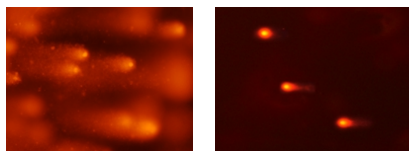
DNA damage in control rat sperm cells were analysed by comet assay with minimal DNA migration from nuclei. In contrast, DNA damage was reported to according dose in treatment group. The group IV (200 mg/kg.b.wt./day) sperm cells show significantly DNA migration from the nuclei with highly damaged DNA.



Photograph 1-Comet assay of sperm for control or vehicle treated. Shows the DNA migration patterns from typical sperm cells of control animal. The symbols + and - represent cathode and anode, respectively, during electrophoresis of negatively charged DNA. Magnification: X400. Dye: EtBr.

Photograph 2- Comet assay of sperm of rat treated with *T.undulata* at 50 mg / kg.b.wt/rat. DNA migration patterns of sperm cells with damaged DNA. Magnification: X400. Dye: EtBr.

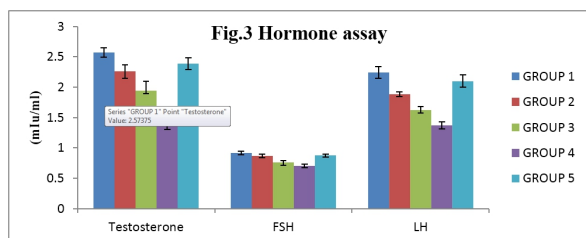
Photograph 3- Comet assay of sperm of rat treated with *T.undulata* at 100 mg / kg.b.wt/rat. DNA migration patterns of sperm cells with highly damaged DNA. Magnification: X400. Dye: EtBr.



Photograph 4- Comet assay of sperm of rat treated with *T.undulata* at 200 mg / kg.b.wt/rat. DNA migration patterns of sperm cells with highly damaged DNA. Magnification: X400. Dye: EtBr.

Photograph 5- Comet assay of sperm of rat kept for recovery. Magnification: X400. Dye: EtBr.

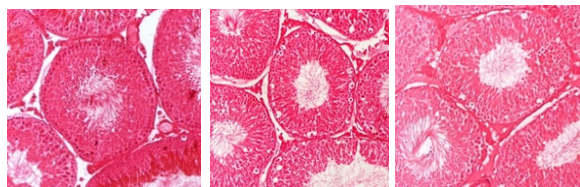
The level of serum testosterone (p≤ 0.001), serum FSH (p≤ 0.001) and LH hormone (p≤ 0.001) were decreased in treated groups in comparison to control group. (Fig.-3)



3.1 Histological Observation of Testes

In control rat (photomicrograph-1) testis exhibits normal structure of seminiferous tubule contains all successive stages of spermatogenesis with occurring maturation of spermatozoa near the lumen and the interstitium contained Leydig cells. The seminiferous epithelium was compactly arranged. Sertoli cells appeared granular and well defined cytoplasm exhibited typical, irregular nuclei. The oval shape spermatogonia were closely arranged with the basal lamina. Spermatocytes contain condensed nuclei and were closely arranged

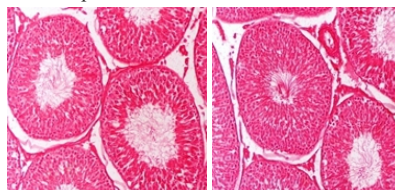
with Sertoli cell cytoplasm. Oral administration of *T. undulata* bark extract caused degenerative germinal elements of testis and reduced primary, secondary spermatocytes, spermatids and sperm in lumen of Seminiferous tubules(Photomicrographs 2 to 4). Intertubular space was enlarged in between seminiferous tubules as compared to controls. Lumen with less sperms and cell debris and disrupted Leydig cells were also visible. Histological observation of testis in recovery group rats (photomicrograph-5) exhibited normal structure as similar to control group.



Photomicrograph 1 Histoarchitecture of the testis of a control animal. The seminiferous tubules contain Sertoli cells and germ cells of various stages, covering the entire process of spermatogenesis. The lumen contains mature spermatozoa.

Photomicrograph 2 Histoarchitecture of testis of rat treated with *T.undulata* at 50 mg / kg.b.wt/rat. Shows structural disorganization and degenerative germinal elements of testis and reduced sperm in lumen.

Photomicrograph 3 Histoarchitecture of testis of rat treated with *T.undulata* at 100 mg / kg.b.wt/rat. Shows more reduced sperm in lumen and cell debris are also visible.



Photomicrograph 4 Histoarchitecture of testis of rat treated with *T.undulata* at 200 mg / kg.b.wt/rat. Shows Intertubular space was enlarged in between seminiferous tubules and reduced sperm number in lumen.

Photomicrograph 5- Histoarchitecture of testis of rat kept for recovery. Shows normal structure as similar to control group.

4. Discussion

The present investigation show that the 50% petroleum ether extract of *T. undulata* bark reduced serum concentration of testosterone, LH and FSH in 60 days treated animals. It is widely accepted that for production of testosterone, LH is responsible principally (Mali et al., 2002) and FSH stimulates spermatogenesis in the Sertoli cells. It may be possible that suppressed level of serum LH would responsible for dysfunctional Leydig cell, thereby resulting in suppression of testosterone secretion which indicate for decreased spermatogenesis and sperm counts (Chase, 1992, Shan & Hardy 1992). The supplies of androgens are necessary for structure and function of the epididymis (Cooper 1992). In the present study, sperm motility of cauda epididymis in all treated groups declined according to dose, suggest an undersupply of testosterone to epididymis. The improper epididymal function also correlates to reduce testis activity which disturbs motion of testicular fluid into the epididymal tissue (Lohiya et al., 1992, Ansari et al., 1998). It is also associated to decrease weight of epididymis. For the well functional accessory reproductive organ, require enough level of testosterone hormone. The decreased weights of ventral prostate and seminal vesicle also indicate to suppress of circulating testosterone concentration in serum (Lohiya & Ansari 1999). The fertility of treated males decreased up to 70%. The declined number of fertile males further support to reduce sperm concentration and motility.

For successful fertilization, Sperms must reach to the egg cell and penetrate to cervical mucosa because an appropriate number of sperms having normal function are required, any alteration in structure or function of sperm create infertility (Ghogar & Jiraungkoorskul 2017). This investigation exhibit that dose treatment with *T. undulata* bark for 60 days is reversible, safe and effective for sperm motility and density in rats.

The outcome of histopathological analysis of the testis shows gradual arrest of spermatogenic cycle in dose depend manner, as the number of cell types in the germinal epithelium reduce with higher doses. It might be either due to as a result of decreased level of fructose, cholesterol levels in reproductive tract/organs affects the germinal epithelium or might be indirect affect through its function on the serum hormone deprivation that influence normal spermatogenesis. In the 100 and 200 mg/kg/day treated animals (Group III, IV) show moderate and highly depletion in germinal epithelium.

Depletions in the sperm density, motility and histopathological examination of testis supported to interrupted microenvironment of testis and epididymis tissue due to extract treatment as exhibited by significant depletion in the reproductive tissues weight (Agrawal et al., 2012). The extract treated animals show significant increase number of vacuoles thereby depletion in the epithelial cell mass in the testes leading to the substantial reduce mass from the testes.

In present study, protein content in testes and other sex organs significantly decreased following *T. undulata* bark extract administration, it may be possible that stages of spermatogenesis are absence in the testes (Chinoy & Bhattacharya 1997). Change in level of cholesterol after treatment caused degenerative changes in treated rats (Mali et al. 2015). Low level of sialic acid in testes, epididymides and seminal vesicles in treated rats may be associated with decreases of androgen (Luhadia & Mali 2015). The loss of sialic acid content might changes in acrosomal membrane structure, finely effects on the metabolism motility and fertilizing efficiency of spermatozoa (Riar et al., 1973). Decrease level of Glycogen content in testis was correlated to decline number of post-meiotic germ cells (Gunaga et al., 1972).

DNA damage of spermatozoa in rat measured by microgel electrophoresis assay and correlated with infertility (Irvine et al., 2000). Both DNA damage in spermatozoa and decreased sperm motility show levels of reactive oxygen species in semen were increased (Aitken et al., 1998).

In the present investigation, the body weight of rats increased gradually and unaltered serological parameters in treated rats groups suggests that the bark extract of *T. undulata* does not induce any adverse side effects on general body mechanism of the male rats.

In conclusion, the oral administration of 50% petroleum ether extract of *T. undulata* bark inhibitory effects on sialic acid, fructose and protein contents in reproductive accessory organs in dose depend manner. The dose may affects inhibitory action on Sertoli and Leydig cells which may be responsible for suppress in testosterone production resulted in reduced spermatocytes number and changes in spermatozoa functions. These results are similar with the observations of *T. undulata* leaves extract treatment (Soni & Mali 2016).

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