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ORIGINAL ARTICLE

Antiandrogenic efficacy of *Tecomella undulata* bark extract in male albino rats with special reference to histology and sperm dynamics

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ABSTRACT
Fertility control is the major issue of national and worldwide public health concern because increasing growth rate of world's population showing negative impact on environment, economic growth and poverty reduction in developing countries. Male alternatives are less and relatively underused as compared with female contraceptives. Chemical based male synthetic contraceptives are showing unsatisfactory results due to side effects such as total spermatogenic arrest, sterile effects. The search for male antifertility drugs which are more effective, safe and reversible with least side effects remains thus a challenge. Plants have been always praised for their potential health benefits mainly in folk medicine. They are now highly investigated as safe natural phytochemical sources. Several plant extracts has been evaluated for their antifertility activities in male like reduction in sperm counts, alteration in sperm mobility, alteration in hormone levels. The present study was carried out to assess the contraceptive effect of T. undulata bark extract in male albino rats. A significant decrease (P≤0.001) in sperm count in testis and cauda epididymis and a significant increase (P≤0.001) in sperm mortality was observed in male albino rats treated by oral administration of the ethanolic bark extract of T. undulata at the dose of 500 mg/kg body weight and ethanolic bark extract of T. undulata with TP.
KEY WORDS: Antifertilty, spermatogenic, contraceptive, phytochemicals.

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INTRODUCTION

Day by day increasing population is triggering enormous problems and threats. Only in 20th century, the world population has increased from 1.65 billion to 6 billion and it is expected that it will rise to 9.4 billion by 2050.Society and environment are severely affected by population explosion and unintended pregnancies [1]. Modern or traditionally classified contraceptive methods are used to check growing population. It we compare male and female contraceptives, male contraceptive options are few and under used or either not encouraged. Periodic abstinence and withdrawal are traditional contraceptive methods for male on the other hand; modern methods which include vasectomy (which never affects sexual health) and condom (barrier method) are globally used as an alternative by only 8.9% population [2]. It is true that in comparison to female, no single new contraceptive drug is available, although experiments are being done.Lot of side effects have been registered about available contraceptive methods like failure rate or irreversibility resulting from total spermatogenic arrest [3].

Nowadays, scientists are striving hard to explore the efficacy and virtue of plant products to use them as potential male contraception without any side effects because plant products are accessible and devoid of harmful pathogen dwells [4]. Previous studies showed that several plants are sharing antifertility effects in both male & female [5]. Antifertility activities include reduction in sperm count, sperm mobility, testicular changes & altered hormonal levels, have been studies and recorded in plant extracts. The present study was carried out to evaluate the efficacy of anti-fertility effect of ethanolic bark extract of *Tecomella undulata* plant in male albino rats.

MATERIAL AND METHODS

Collection of plant material & extraction: Bark material of *Tecomella undulata* was collected from Jodhpur region which was then recognized by experts of Department of Botany, Jai Narain Vyas University, Jodhpur.The collected plant material will be shade and then passed to soxhlet extraction with 70% ethyl alcohol to obtain a crude extract under reduced pressure. Brownish colored crude extract was obtained after evaporation ethanol. Extract will be stored in sterile containers at- 4^0C.

Experimental animals: Healthy adult albino male rats having weight about 150-200gm were used for experimentation. Fertility of all animals was proved by examination of reproductive profile gathered from Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, India.

All the animals were provided proper care. Animals were placed in standard environmental all conditions, i.e., 26^0 C temperature, 12hr light and 12 hr dark cycle, standard rat pellet diet was provided and water ad libitum. All the animals were acclimatized to environment of lab before starting of experiments. The whole experiment was approved by Institution Ethics committee ((IAEC NO. 1646/GO/ERe/S/19/CPCSEA).

Drug and dose regime:-Exposure and treatment of ethanolic bark extract of *T.undulata* at the dose of 500mg/kg body weight with distilled water to the experimental rats for 60 days.0.01 mg/day intra muscular Testosterone propionate (TP) was given to rats for 30 days the dose of the extract was determined by LD_{50} 30test.

Experimental Design:

Twenty adult healthy rats were divided into four groups of five each.

1) **Groups:1** Intact (control): This group will receive vehicle only.
2) **Groups:2** Intact + *Tecomella undulata* ethanolic bark extract:- (500 mg/kg Body weight) orally for 60 days
3) **Groups:3** Intact + Testosterone propionate (TP:- 0.01 mg/day i.m.) for 30 days
4) **Groups:4** Intact + Testosterone propionate (TP) + *Tecomella undulata* ethanolic bark extract :- (0.01 mg/day i.m. + 500 mg/kg Body weight orally) for 30days.

After completion of experiments, overnight fasted animals were then exposed to mild ether anesthesia and after that their autopsy was done. Using cardiac puncture method, blood of each animal was collected and then stored at -20^0 C until assayed for lipid profiles and haematological analysis and other parameters. Kidney, heart, liver and reproductive organs such as testis,epididymis,ventral prostate and seminal vessicle were separated weighed and fixed in 10% formalin for further histrological study.

- **Haematology:**
  Haemoglobin, RBC, WBC counting was done by using Lynch *et al*., (1969) method [6].
- **Serum Biochemistry:** Total cholesterol, Triglyceride, Urea, Creatinine, SGOT, SGPT all above parameters were done by standard kit method.
- **Histology:** All reproductive organs and vital organ such as Testis, Epididymides, Vas deference, Seminal Vesicle, Liver, Heart, Kidney, Ventral Prostate will be taken for histology to see the effects of plant extract.

Sperm dynamics analysis:

By using standard methods, sperm dynamics Such as sperm mortality in cauda, sperm density in cauda and testis were done by Prasad *et al*., (1972)[7].

RESULTS

Effect on body and organs weight: It was observed that there were no any significant changes in body weight of all treated groups as compared with control group. The weight of testis in bark extract plus TP treated group shows a slightly decrease ($P \leq 0.05$), while other treated groups shows non-significant changes in weight of testis, epididymis, ventral prostate and seminal vesicle when compared with control group (Table1).

Effect on sperm Dynamics: Sperm denties in cauda epididymis and testis and total number of motile sperms in cauda epididymis were analysed (Fig.1 & Fig.2).

Rats administered with ethanolic bark extract of *T.undulata* for 60 days and TP treatment for 30 days lead to highly significant decrease ($P \leq 0.001$) in sperm dynamic parameters such as total sperm counts in testis and cauda went down in compared with control group. Slightly changes ($P \leq 0.05$) were observed in TP + bark extract treated group for 30 days.

On the other hand mean while sperm density in cauda and testis was declined and in group of animals who received dose of ethanolic bark extract, TP and TP+Bark extract increase in sperm mortality was observed as compared to control group.

Serum Biochemistry: Serum biochemistry results are shown in (table 2) as in comparison to control group, serum total concentration of cholesterol, triglyceride, urea and creatinine was not substituted all these parameters are in normal range ,while on the other hand serum SGOT activities were noticed rising in all the treatment groups that is significant changes (P≤0.001) were observed in Bark extract treated group, TP and TP+Bark extract treated groups.

Hematological Study: As compared to control groups no signification changes were observed in haematological parameters.

TABLE-1: Effect On Body And Organ Weight In Ethanolic Extract Of *T. Undulata* Bark Treated Albino Male Rats.

TREATMENT GROUP	Body weight		Testis	Epididymis	Seminal vesicle	Ventral prostate	Heart	Kidney	Liver g/100g BW
	Initial	Final	mg/100 g BW						
Control (Gr.I)	202± 22.80	222± 22.80	1111.5± 29.49	547± 35.19	420.25± 27.40	305.75± 21.02	455.7± 18.36	566.75± 24.10	4.24± 0.77
Bark extract (Gr.II) 60 day	180± 14.14	202± 17.88	634.2± 30.93^{c}	342.29± 18.14^{b}	245.45± 20.71^{a}	159.6± 28.05^{d}	354.05± 30.28^{d}	722± 27.67^{d}	3.32± 0.45^{d}
TP (Gr.III) 30 day	150±0	173± 2.88	820.5± 26.27^{c}	269.3± 18.82^{c}	263.3± 22.47^{c}	138± 14.10^{b}	469.3± 25.02^{d}	659.5± 33.02^{d}	3.26± 0.33^{d}
Bark+TP (Gr. IV 30 day	167± 10.60	155± 7.07	1025± 35.35^{a,g}	440.8± 32.36^{d,g}	358.5± 21.92^{d,g}	242± 33.94^{d,f}	373.5± 28.56^{a,h}	670±2 8.28^{a,h}	4.26± 0.33^{d,e}

Group I compared with Group II, III ,IV — Gr.III compared with Gr. IV

P≤ 0.05 = a — P≤ 0.01 = b — P ≤ 0.05 = e — P ≤ 0.01 = f

P≤ 0.001 = c — Non-significant = d — P ≤ 0.001= g — Non- significant = h

TABLE-2: Effect On Lipid Profil In Ethanolic Extract Of *T. Undulata* Bark Treated Albino Male Rats.

TREATMENT GROUP	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	SGOT (U/L)	SGPT (U/L)
Control (Gr.I)	55.96±5.06	46.22±2.34	25.95±3.63	0.57±0.19	34.61±1.32	32.61±3.85
Bark extract (Gr.II) 60 day	53.78±9.04^{d}	92.69±4.40^{c}	28.04±4.66^{d}	0.46±0.24^{d}	157.26±75.19^{a}	40.17±9.55^{d}
TP (Gr.III) 30 day	56.4±1.07^{d}	61±3.56^{c}	28.5±1.83^{d}	0.28±0.02^{a}	66.17±2.18^{c}	34.21±1.44^{d}
Bark+TP (Gr. IV) 30 day	62.15±3.86^{a,e}	53±2.12^{b,e}	30.5±0.77^{a,e}	0.21±0.04^{a,e}	104.8±0.19^{c,g}	41.86±1.22^{c,g}

Group I compared with Group II, III,IV — Gr.III compared with Gr. IV

P≤ 0.05 = a — P≤ 0.01 = b — P ≤ 0.05 = e — P ≤ 0.01 = f

P≤ 0.001 = c — Non-significant = d — P ≤ 0.001= g — Non- significant = h

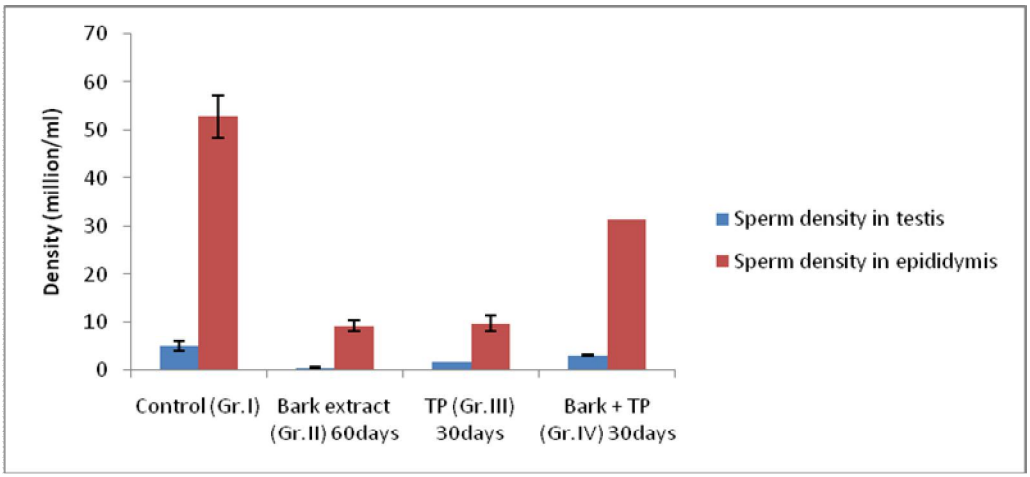

Fig.1-Effect of ethanolic bark extract 60 days,TP 30days,Bark+TP 30 days on sperm density of treated rats.

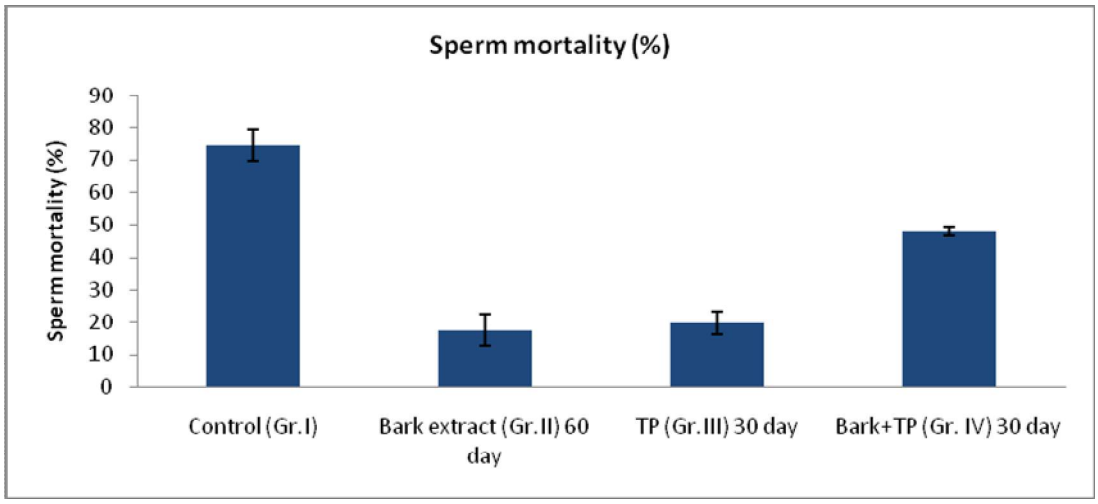

Fig.2-Effect of ethanolic bark extract 60 days,TP 30days,Bark+TP 30 days on sperm mortality of treated rats.

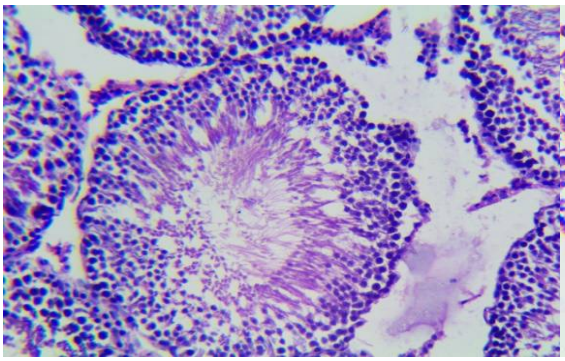

Fig.3 (a)

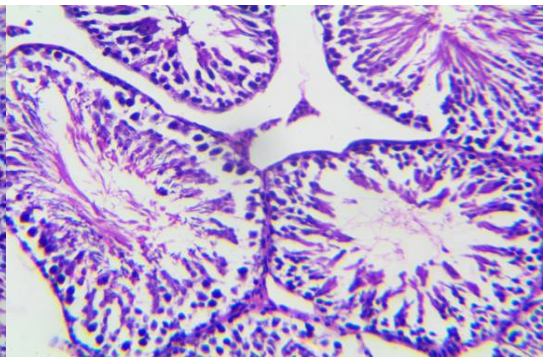

Fig.3(b)

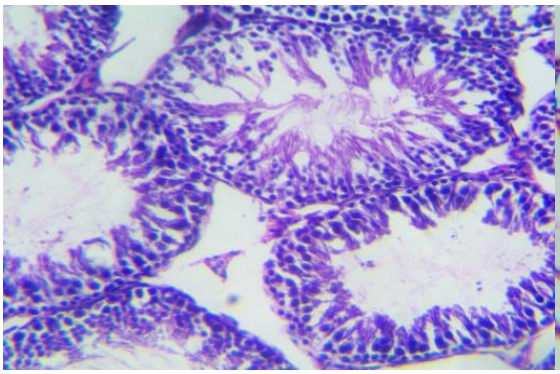

Fig.3 (c)

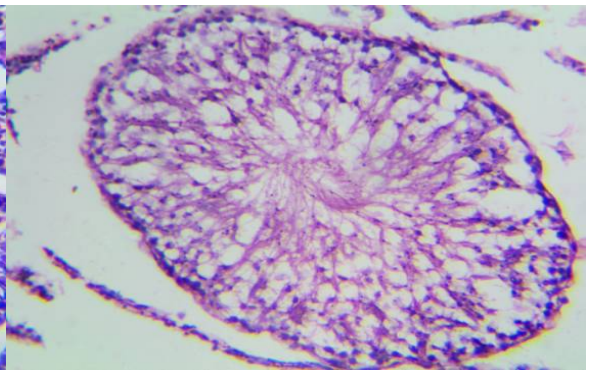

Fig.3 (d)

Figure 3: (a) Testis of vehicle control rats (200xHE). (b) Testis of 60 days *T. undulata* ethanolic bark extract treated rats (200xHE). (c) Testis of 30 days TP treated rats (200xHE).(d) Testis of 30 days *T. undulat* ethanolic bark extract plus TP treated rats (200xHE).

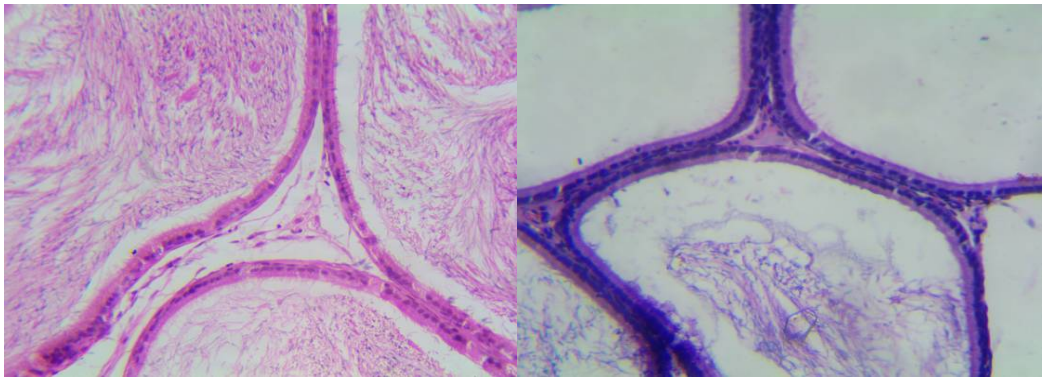

Fig. 4 (a) Fig. 4 (b)

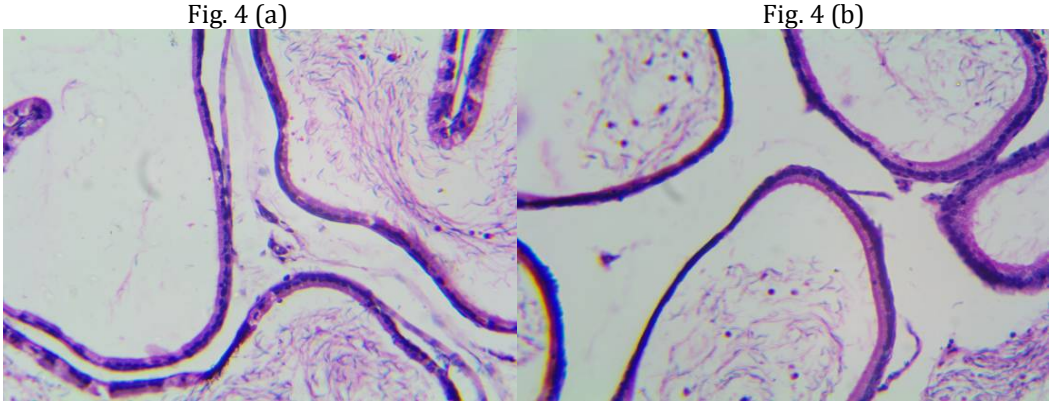

Fig. 4 (c) Fig. 4 (d)

Figure 4: (a) Cauda epididymis of vehicle control rats (200xHE). (b) Cauda epididymis of 60 days *T. undulata* ethanolic bark extract treated rats (200xHE). (c) Cauda epididymis of 30 days TP treated rats (200xHE). (d) Cauda epididymis of 30 days *T. undulata* ethanolic bark extract plus TP treated rats (200xHE).

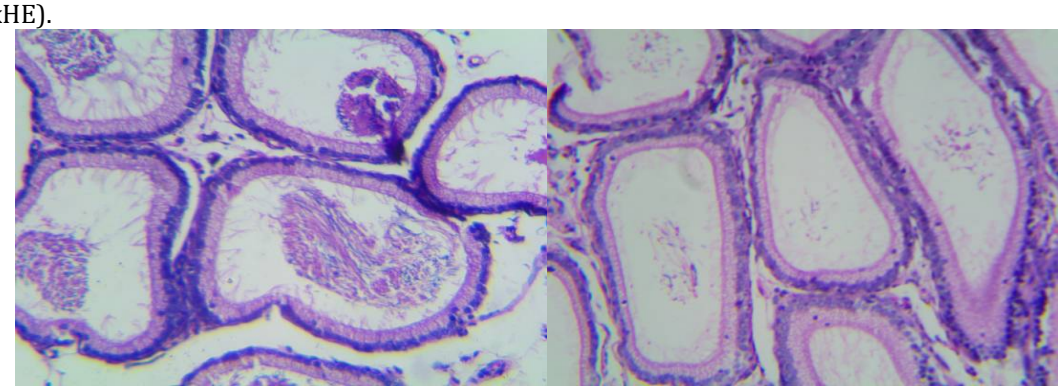

Fig. 5(a) Fig. 5(b)

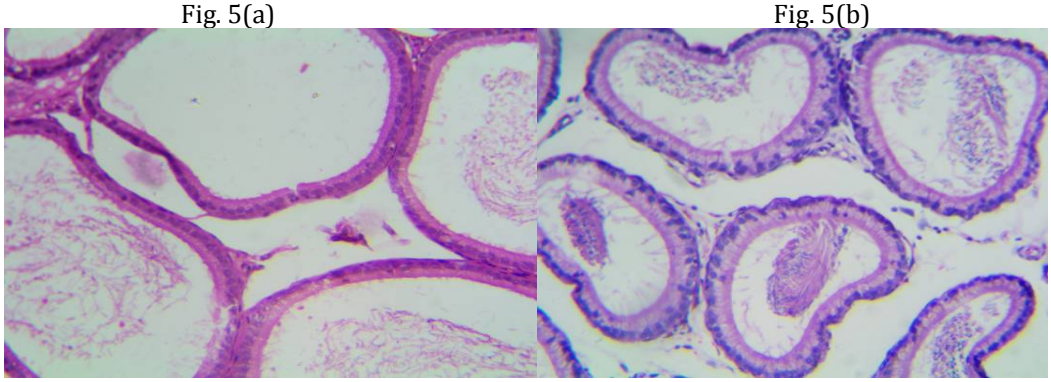

Fig. 5(c) Fig. 5(d)

Figure 5: (a) Caput epididymis of vehicle control rats (200xHE). (b) Caput epididymis of 60 days *T. undulata* ethanolic bark extract treated rats (200xHE). (c) Caput epididymis of 30 days TP treated rats (200xHE). (d) Caput epididymis of 30 days *T. undulata* ethanolic bark extract plus TP treated rats (200xHE).

DISCUSSION

Sudden increase in world population is todays concerning matter for developing as well as developed countries [8]. Few methods are available for male contraception due to highly sensitive feature of male reproductive system to various synthetic drugs so new inventions are required which are more efficient drugs and plant based with least side effects, safe and cost effective [9]. It is known to all that Indian traditional medicinal systems like Ayurveda and many herbal extracts have been used for decreasing rate of fertility since initiation of civilization. Decrease weight of testis and accessory structure depicted decreased level of androgen hormones as these organs are androgen dependent [10]. Motility plays crucial role in the success of fertilization and any decreasing rate of motility lead to debilitating and deleterious effect on fertilization [11]. A large number of plant such as *Lindenbergia indica* , *Elytraria acaulis, Adiantum lunulatum, Dactyloctenium aegyptium* are well known for their contraceptive effects in male rats through preventing spermatogenesis and reducing total sperm counts in testis and epididymis [12,13,14]. *T. undulata* also shows antifertility effects in male albino rats. It was previously reported that sperm motility and sperm density in epididymics were significantly decreased and the level of testosterone and LH hormone were also decreased when oral administration of petroleum ether extract of *T. undulata* leaves at the dose of 50, 100 and 200mg/kg body weight for 60 days in male rats [15]. Our results also shows a significant decrease in the level of sperm count, motility and a significant decrease in testis and epididymis weight were also observed in the ethanolic bark extract of *T. undulata* treated group when compared with control group. An aniandrogenic effect of the extracts is suggested by the reduced sperm motility in cauda epididymides mainly due to reduce androgen synthesis [16]. A decrease in the weight of accessory sex structure such as seminal vesicle and ventral prostate also support the view of decrease androgen level as this organs are androgen dependent [17]. The present study indicate that *T.undulata* extracts may have impaired androgen synthesis either by inhibited leydig cell function or hypothalamus pituitary axis resulting in fertility.It is concluded that ethanolic bark extract of *T. undulata* showing antifertility effect via reducing sperm total count and increasing sperm mortality in male rats.

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