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Antifertility studies of the flower extract of *Tecomella undulata* in male albino rats with special reference to testicular cell population dynamics

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Abstract
Aim of present research work was to study the antifertility properties of *Tecomella undulata* ethanolic extracts of flower in male albino rats. Fertile male rats were divided in four treatment groups each having 5 animals. Prepared flower extract was orally administered at dose level of 500 mg/kg body weight for 60 days and TP was administered intramuscularly at dose level of 0.01 mg/day for 30 days. After completion of the dose schedule autopsy was done under mild anesthesia. Different fertility parameters were studies such as reproductive organ weight, sperm motility and density, testicular population count and histology. Statically significant ($P \leq 0.001$) decrease in weight of reproductive organs in treated groups in comparison to control was observed. Sperm Motility (Cauda) and Sperm density (Cauda and testes) was significantly reduced in all treated groups incomparison to control group. In comparison to control group, degenerative changes and arrest in spermatogenesis was observed in histology of testis of all treated group treated with extract. Reduction in spermatogonia, primary and secondary spermatocytes, spermatids, immature and mature leydig cells was noticed in treated groups (II and III) while regaining in these parameters in group IV was noticed as compared to control. From all research findings we come to conclusion that flower ethanolic extracts of *Tecomella undulata* with TP has reversible antifertility properties in male albino rats and can serve as oral contraceptive for male without any negative impact.

Keywords: Antifertility, Rohida, *Tecomella undulata*, Testicular population dynamics

INTRODUCTION

Now days, most of countries facing the biggest problem of increasing population, which affecting the whole factor of development such as employment, health care, education, sanitation, environment, and housing[1]. The world population is growing at alarming rate of 176 people per minute, with a current estimate of 6 billion people predicted to exceed 10.8 billion by the year 2050[2]. Over population is one of the considerable problems in the developing countries like India, by the year 2050 that would reach to 9.2 billion (united nation population division, (2007)[3]. Fertility regulation in today's scenario is major concern of people in developed and developing countries because over population cause harsh effect on environment, natural resources declination, increasing poverty and unemployment[4]. Various contraceptive agents that available now a day are synthetic, with various adverse effects like hypertension, hormonal imbalance, weight gain and cancer[5]. Male contraceptive is very few and not in much use, in comparison to female contraceptives. Modern contraceptive methods account only 8.9% global use and consist of mainly use of vasectomy (sterilization) and condom (barrier method) (united nation population division, (2009)[6]. Search of best contraceptive method with least side effect and which become safer and more effective was a challenge. In folk medicine, plant have been used because of their effective health benefits, from this point of view, various plant extracts are evaluated with contraceptive activity in both female and male, in male acted as spermicidal, reduction in sperm count and sperm motility[7]. Saponins, steroids, flavonoids, alkaloids, and phenolic acids are various metabolites of plant having contraceptive potential it was previously studied[8, 9].

Tecomella undulata plant belongs to family Bignoniaceae, mainly found in arid zone region. It is also known as desert teak and marwar teak. Medicinal properties of this plant also mentioned in ayurveda.

Rohitakarista, rohitakaloha, rohitakagharta, rohitakadyachoorna are various ayurvedic product prepared by bark of *Tecomella undulata*. This plant consists of various medicinal importances as it has properties like hepatoprotective, antibacterial, anti-inflammatory etc.[10]. The present research focused on ethanolic extract of *Tecomella undulata* flower to evaluate its contraceptive efficacy in male albino rats by testicular activities i.e, antispermatogenic and antianderogenic.

MATERIALS AND METHOD

Collection and identification of plant material: - Flowers of *Tecomella undulata* plant of family Bignoniaceae were collected from Jodhpur region and plant was identified by the expert of Department of Botany (JNVU), Jodhpur.

Extract preparation method: - Shade dried flowers of *Tecomella undulata*, were grinded into fine powder. 150 grams of powdered material of flower was taken and run in a soxhlet apparatus in 70% ethanolic solution for 20 hours. After running solution for 20 hours, with the help of muslin clothsolution was filtered. From obtained filtrate excess solvent was evaporated to obtain crude dark brown extract. Obtained extract were weighed and stored in clean air tight container at -4˚C.

Experimental animals: - Healthy male albino rats were used for current studies which were procured from Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, India. Animal were acclimatized and maintained under normal temperature i.e, 26˚C and 12-hour light/dark cycle in animal house, and normal basal diet was given and water Ad libitum was present. Institutional Animal Ethical Committee (IAEC No. 1646/GO/Ere/S/19/CPCSEA) approved the protocol used for all experimental work. Veterinary adviser regularly supervises the all animal with some time interval.

Physiological dose identification: - For identification of physiological test, first LD_{50} was calculated by using the fixed dose procedure given by Walum[11]. 70% ethanolic flower extract of *Tecomella undulata* was given to albino male rats orally at 500mg/kg body weight per day for about 60 days and 0.01mg/day T.P was injected intra muscularly for 30 days.

Experimental group: - For this experiment 4 groups were designed having 5 animals in duplicatein each group.

1) **Group:1** Intact control: vehicle treated control group rats fed with normal rat feed for 60 days.
2) **Group:2** Intact + *Tecomella undulata* ethanolic flower extract: - (500 mg/kg body weight) orally for 60 days
3) **Group:3** Intact +Testosterone propionate (TP: - 0.01 mg/day i.m.) for 30 days
4) **Group:4** Intact + Testosterone propionate (TP) + *Tecomella undulata* ethanolic flower extract: - (0.01 mg/day i.m. + 500 mg/kg body weight orally) for 30 days.

Autopsy schedule: -Overnight fasted animal after completion of the experiment of 30 and 60 days, and were autopsied under mild anesthesia. From left ventricle puncture blood sample was taken and kept in both EDTA coated test tube and normal tubes for hematological and serum studies. Remaining Collected blood was centrifuged for 15 minutes at 3000rpm to obtain serum. Reproductive organ (testes, cauda, caput, ventral prostate, vas deference and seminal vesicle) and vital organ like heart, kidney, and liver was removed and fixed in 10% formalin and further processed for histological examination.

Sperm motility and density: - For this study epididymis and testis were taken. From epididymis cauda was separated for sperm motility and for calculation of sperm density both cauda and testis were used[12].

Hematological parameters: -Hematological parameters like PLT (platelet thrombocyte count), HCT (Hematocrit), HB (hemoglobin), WBC (white blood corpuscles), etc. were studied in blood using standard method.

Biochemical profile of serum: - Using standard commercial kit procedure different biochemical parameters like SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase), total cholesterol, urea, triglyceride, uric acid, creatinine, were estimated.

Histology: - Vital organ like liver, kidney, and heart, and reproductive organs like cauda, caput, testis, vas deferens, ventral prostate, seminal vesicle, were dehydrated in alcohol series and then kept in xylene. After that tissue was embedded in molten paraffin wax and block were prepared. Using microtome, 5µm thick sections were cutted and then stained with haematoxylin and eosin stain. Using light microscopeslides of sections were observed, for study of histopathological changes. Using ImageJ software and protocol given by Abercrombie[13], Dixon and Massey[14], Histometery of histological slides and testicular cell population counting was done.

RESULTS AND DISCUSSION

Worldwide population increase is a major problem, and many strategies are being employed, particularly in emerging nations, to lower the overall fertility rates for both men and women[15]. Speaking about drastic measures, the World Health Organization launched a population control programme that incorporates studies connected to conventional medical practices[16].

An imperative exists for male oral contraceptive for controlling fertility in safer way. Current topic was up taken to find medicinal alternative to control fertility in male.

Effect on body and organs weight: Statically insignificant changes were noticed in the initial and final body weight of animal dosed with Flower extract of *Tecomella undulata* and TP for 60 and 30 days respectively. The findings indicated that the control group and the intact group treated with TP showed no significant alterations in initial and final body weights. This is in support with earlier research that has reported no significant alterations in body weight with TP administration[17]. Similarly, the intact groups treated with flower extract of *Tecomella undulata* furthermore did not exhibit noteworthy changes in body weights. These findings give an impression that the interventions used in the study didn't possess a major impact on overall body weight.

While significant decline in reproductive organ weight i.e., seminal vesicle, cauda epididymis, testis, ventral prostate in male albino rats of treated group with flower extract of *Tecomella undulata* and TP. When Male rat treated with combination of flower extract of *Tecomella undulata* and TP showed the significant elevation in the reproductive organ weight (Table 1). Administration of TP and flower extract of *Tecomella undulata* brought to a notable decrease in testes weight in comparison to the control group. This finding is relation with earlier research that has reported testicular atrophy as a result of exogenous testosterone administration[18]. Moreover, Abd El Tawab *et al*. 2014[19] demonstrated that certain herbal extracts can also resulting in a decrease in testicular weight. These studies support the current findings and suggest that both TP and herbal extracts can exert suppressive effects on testicular weight.

The present investigation revealed a noteworthy decrease in epididymis weight following administration of TP and flower extract of *Tecomella undulata*. This is agreeing with earlier studies that that has reported decreases in epididymal weight with testosterone treatment[20]. Administration of flower extract of *Tecomella undulata* resulted in a significant reduction in seminal vesicle weight, while TP treatment also significantly affects this organ in negative way. Based on findings these results are not in a queue from prior research, which have documented increased seminal vesicle weight with testosterone administration[21]. However, it is valuable to note that the current analysis focused on herbal extracts in combination with TP, which may have contributed to these divergent results. The study discloses a significant reduction in ventral prostate weight with TP and flower extract of *Tecomella undulata* administration. This corresponds to previous research showing that testosterone treatment can be connected to a decrease in prostate weight[22].

Effect on sperm dynamics: Animals treated with flower extract of *Tecomella undulata* and TP, showed significant decline in number of motile sperm in the cauda epididymis and sperm density in the testis and cauda epididymis also significantly decreased in comparision to control group**.** While when animal is treated with combination of flower extract of *Tecomella undulata* and TP together showed significant elevation in the number of motile sperm in cauda epididymis and sperm density in testis and cauda epididymis as compared to flower extract and TP treated group alone (Fig.1, Fig.2, and Fig.3). The observed reductions in sperm motility in several treated groups indicate a potential negative impact on sperm function. Decreased motility can hinder sperm's ability to reach and fertilize the egg, leading to infertility. Preceding studies have denoted that various factors, such as exposure to toxins, hormonal imbalances, and the effects of oxidative stress on sperm motility impairment[23]. The administration of TP and flower extract of *Tecomella undulata* may have impacted these elements, resulting in the observed reductions in motility.

The significant declines in sperm density across the treated groups suggest a potential disruption in spermatogenesis or sperm production. Decreased sperm density is related with lower fertility potential and can devote to male infertility. Prior research has published that a number of aspects, including exposure to environmental pollutants, heat stress, and certain medications, can reduce sperm density[24]. The dosing of TP and flower extract of *Tecomella undulata* might have impacted these variables, resulting in the observed reductions in sperm density.

Dynamics of testicular cell population: As compared to control group significant decline in mature leydig cell, numbers of spermatid and spermatocytes, germ cell were noticed, whereas there was elevation in number of degenerative cell in male rats treated with flower extract of *Tecomella undulata* and TP alone, as compared to control group. While male animal treated with flower extract of *Tecomella undulata* and TP together showed the elevation in number of mature and immature germ cell, spermatid, spermatocytes and reduction in number of degenerative cell as compared to flower extract and TP alone treated group (Table 2). Additionally, a significant reduction in fibroblast cells, immature and mature Leydig cells and an increase in degenerating cells were observed after administration of flower extract of *Tecomella undulata*, indicating disrupted leydig cell function and increased cell death. Similar observations of TP-induced testicular damage have been reported in previous studies[25].

This finding is consistent with previous studies that have reported alterations in testicular morphology following the administration of plant extracts[26].

Hematological study: Male albino rats dosed with flower extract of *Tecomella undulata* and TP alone and with combination of flower extract and TP showed insignificant changes in all hematological parameters as compared to control group. The results specify that hematological parameters remained within the normal range in both the control and experimental groups. These criteria are necessary to assess the general wellbeing and functionality of the blood cells and are commonly used in clinical practice to assess various blood disorders. It is crucial to remember that the lack of significant changes in these hematological parameters recommends that the treatment of TP and flower extract of *Tecomella undulata* did not have a noticeable impact on the blood cell composition or related parameters. This implies that the extract did not cause any adverse impacts on the hematological system.

Serum Biochemistry: Male albino rats treated with flower extract of *Tecomella undulata* and TP alone showed significant elevation in serum total cholesterol and serum SGOT in comparison to control group. Whereas significant decline was observed in the level of serum total cholesterol and serum SGOT in treated group with flower extract and TP together as compare to TP alone treated group. serum SGPT and serum triglycerides level observed significantly elevated in animal of treated group with flower extract and TP as compare to control group, while animals treated with flower extract and TP in combination showed significant decline in level of serum SGPT and serum triglycerides as compare to flower extract and TP alone treated group. The observed significant increase in SGOT levels in several groups under treatment in comparison to the control group suggests a potential impact on

liver function. SGOT is an enzyme found predominantly in the liver and heart, and its elevation in the blood can indicate liver dysfunction. Previous research has reported that certain herbal extracts and compounds can affect liver enzymes and potentially alter liver function[27]. The administration of TP and flower extract of *Tecomella undulata* and compounds may have influenced liver metabolism or caused mild liver injury, resulting in the observed increase in SGOT levels.

SGPT is an enzyme found primarily in liver cells, and its elevation in the blood can indicate liver dysfunction. Conversely, a decrease in SGPT levels can indicate improved liver health. The oral dose of flower extract of *Tecomella undulata* may have influenced liver metabolism, leading to the observed changes in SGPT levels. Further Research is required to understand the underlying mechanisms.

Histopathological observation: In untreated group observed normally arranged connective tissue, developing interstitial cell and spermatogenic cell. Primary and secondary spermatocytes, Sertoli cell, and spermatogonia are observed at the basement membrane, all this observation indicates normal active spermatogenesis process. In inter-tubular space of interstitial tissue fully developed leydig cell observed. Mature sperms are filled in lumen (Fig. 4(a)). In comparison to control/untreated group the male albino rat treated with flower extract and TP alone showed the significant decline in nuclear diameter of leydig cell, diameter of seminiferous tubule and arrest in spermatogenesis (Fig. 4(b & c)). As compared to TP treated group, male albino rat treated with combination of flower extract and TP showed restoration of active spermatogenesis process and in diameter of nuclei of leydig cell and seminiferous tubule (Fig. 4(d)). In flower extract of *Tecomella undulata* and TP alone treated male albino rats observed decreased epithelial cell lining and reduction in sperm count in lumen of cauda epididymis as compared to control group exhibiting normal histology of cauda epididymis, in the lumen number of mature spermatozoa observed, cauda tubule was lined with pseudostratified epithelial cell. Group dosed in combination with flower extract and TP showed significant reduction in epithelial cell height and significant increase in number of mature sperm in comparison to group treated with TP alone. Caput epididymis was lined by tall columnar epithelial cell and lumen filled with mature spermatozoa, as compared to cauda the less inter-tubular stroma with connective tissue was present in caput of male albino rat of control group showing normal histology. Treated group with flower extract and TP alone showed significant decline in number of spermatozoa and height of epithelial cell, while in comparison to control group there was no change in inter tubular stroma and stereocilia of caput epididymis. Male albino rat treated with combination of flower extract and TP showed restored Histoarchitecture of caput epididymis with restore of epithelial cell height and number of spermatozoa in lumen as compare to group treated with TP and flower extract alone. The histological observations of the testes in this study provide a valuable insight into the effects of various extracts and compounds on spermatogenesis and testicular architecture. The control group (Gr. 1) exhibited normal histoarchitecture, with well-organized seminiferous tubules containing all stages of spermatogenesis, indicating active and normal spermatogenesis. This finding is consistent with previous studies that have described the histological characteristics of normal rat testes[28].

In contrast, administration of TP (Gr. 3) resulted in a significant decrease in germinal cells, including secondary spermatocytes and spermatids. These findings suggest an arrest in spermatogenesis, which is in line with previous research demonstrating the adverse effects of TP on testicular histology and spermatogenesis[29].

Furthermore, the reversion of TP-induced effects was observed when TP was administered along with the flower extract of *Tecomella undulata.* The number of germinal cells, including primary and secondary spermatocytes, spermatids, immature and mature Leydig cells, and degenerating cells, started to recover. This reversal effect suggests the potential of the flower extract of *Tecomella undulata* in mitigating TP-induced testicular damage. Similar protective effects of plant extracts against TP-induced testicular toxicity have been reported in previous studies[30].

Similar reversal effect of fertility parameters in male albino rats was reported after administration of combination of TP and bark petroleum ether extract of *Leptadenia reticulata*[31].

Table-1: Effects on body weight and organ of treatment with ethanolic extract *T. undulate* in albino rats. (Mean of 5 animals ± SEM)

TREATMENT GROUP	Body weight		Testis	Epididymis	Seminal vesicle	Ventral prostate	Heart	kidney	Liver
	Initial	Final	mg/100 g BW						g/100 g BW
Control (Gr.I)	201 ±22.80	221 ±19.49	1110.5 ±29.49	546 ±35.19	421.25 ±27.40	304.75 ±21.02	454.7 ±18.36	565.75 ±24.10	3.50 ±0.77
Flower extract (Gr. II) 60 day	182 ±11.90	208.3 ±12.5	994 ±30.04^{c}	327 ±6.35^{b}	241 ±12.5^{c}	134 ±11.96^{c}	404 ±13.27^{d}	654 ±81^{d}	3.68 ±1.74^{d}
TP (Gr.III) 30 day	156 ±6.45	172 ±2.88	821.5 ±26.27^{c}	268.3 ±18.82^{c}	262.3 ±22.47^{c}	137 ±14.10^{c}	466.3 ±25.02^{d}	658.5 ±33.02^{d}	3.28 ±0.33^{d}
Flower+TP(Gr.IV) 30 day	157 ±2.88	175 ±5.77	1319 ±18.21^{g}	555 ±33.87^{g}	435 ±17.85^{g}	262 ±16.86^{g}	414 ±37.86^{h}	681 ±40.14^{h}	3.73 ±0.28^{h}

Group I compared with group II, III
P≤ 0.05= a P≤ 0.01 = b
P≤ 0.001= c Non-significant = d

Group III compared with group IV
P ≤ 0.05= e P ≤ 0.01= f
P ≤ 0.001= g Non- significant = h

Table-2: Testicular cell population dynamics of *T.undulata* ethanolic extract treated intact rats. (Mean of 5 animals ±SEM)

TREATMENT GROUP	Germinal cell type				Interstitial cell types			
	Spermatogonia	Spermatocytes Primary	Spermatocytes Secondary	Spermatids	Fibroblast	Immature Leydig cell	Mature Leydig cell	Degeneration
Control (Gr.I)	26.12 ±1.01	20.72 ±0.67	70.51 ±3.01	159.12 ±6.21	62.12 ±2.01	52.01 ±3.76	73.76 ±2.76	13.17 ±0.97
Flower (Gr. II) 60 day	19.12 ±1.76^{b}	16.16 ±0.76^{a}	18.16 ±1.16^{c}	16.12 ±1.12^{c}	59.13 ±3.13^{d}	42.12 ±1.23^{a}	35.16 ±3.01^{c}	62.76 ±4.01^{c}
TP (Gr. III) 30 day	21.12 ±1.01^{a}	13.23 ±0.33^{b}	10.12 ±1.76^{c}	8.01 ±0.01^{c}	60.12 ±.01^{d}	30.12 ±0.20^{c}	34.12 ±1.01^{c}	76.13 ±3.76^{c}
Flower+TP (Gr. IV) 30 day	21.36 ±2.81^{h}	18.12 ±1.92^{f}	32.12 ±0.13^{g}	41.01 ±3.01^{g}	57.67 ±1.87^{h}	62.12 ±3.01^{g}	56.12 ±3.2^{g}	24.12 ±1.63^{g}

Group I compared with group II,III
compared with group IV
P≤0.05=a P≤0.01=b
P≤0.01=f

Group III
P≤0.05=e

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

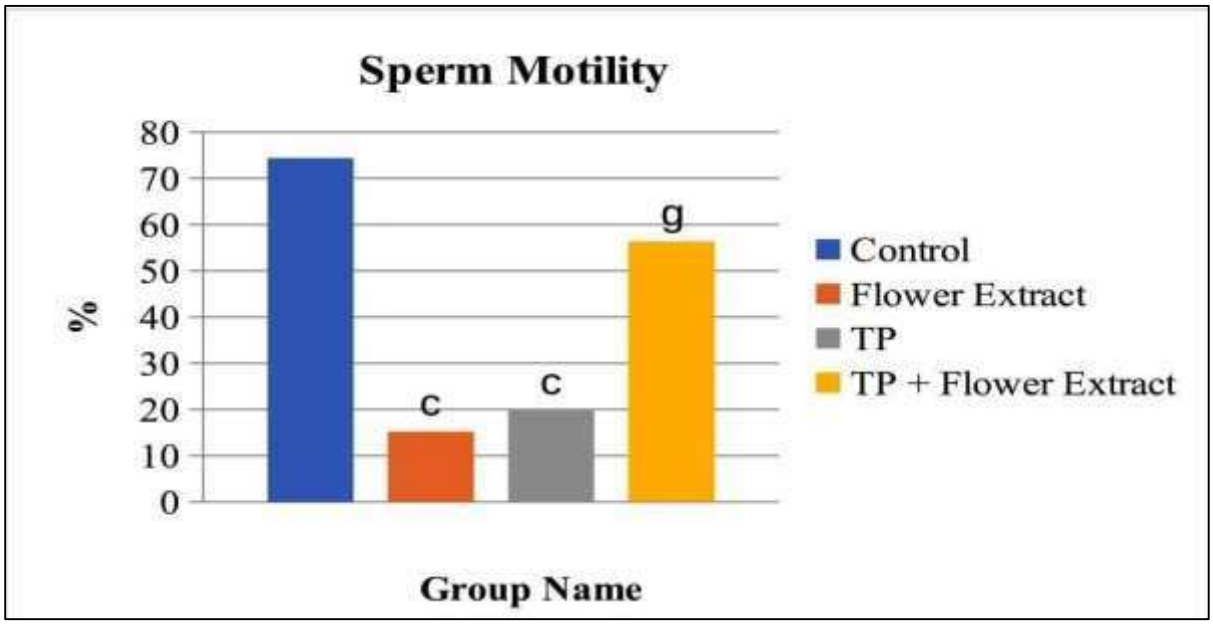

Fig 1: Effect of ethanolic flower extracts of *T.undulata* on sperm motility of male albino rats. Data was expressed in terms of Mean ± SD. Column Bars are with error bars representing SD of Mean. Superscripts denote significance level.

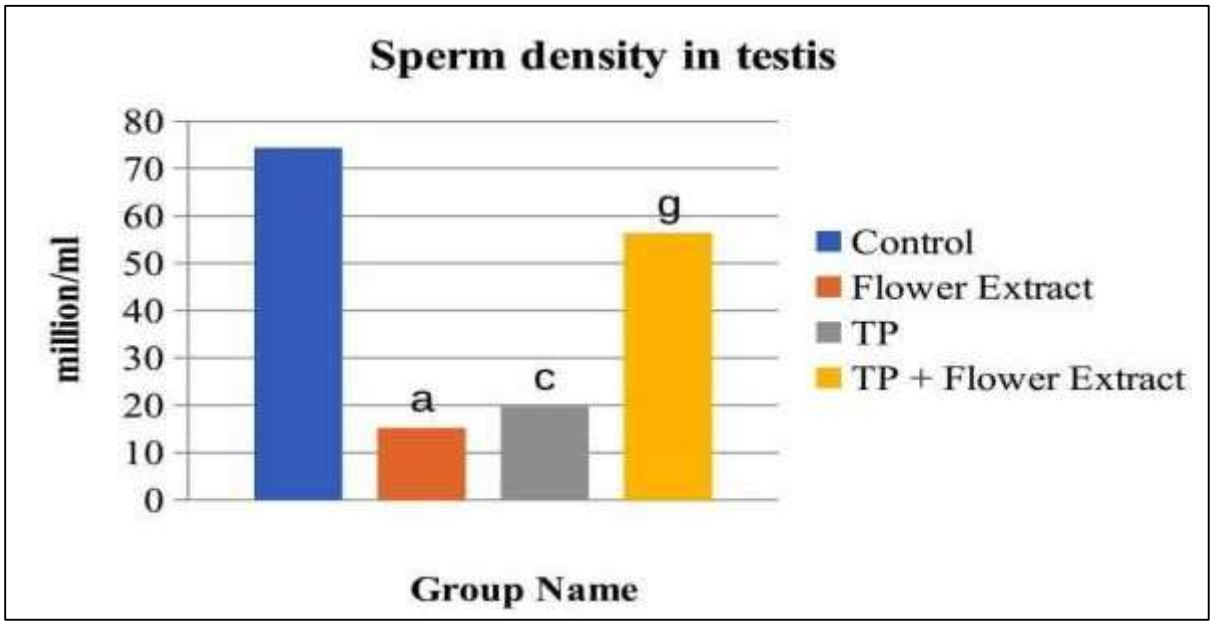

Fig 2: Effect of ethanolic flower extracts of *T.undulata* on sperm density (testis) of male albino rats. Data was expressed in terms of Mean ± SD. Column Bars are with error bars representing SD of Mean. Superscripts denote significance level.

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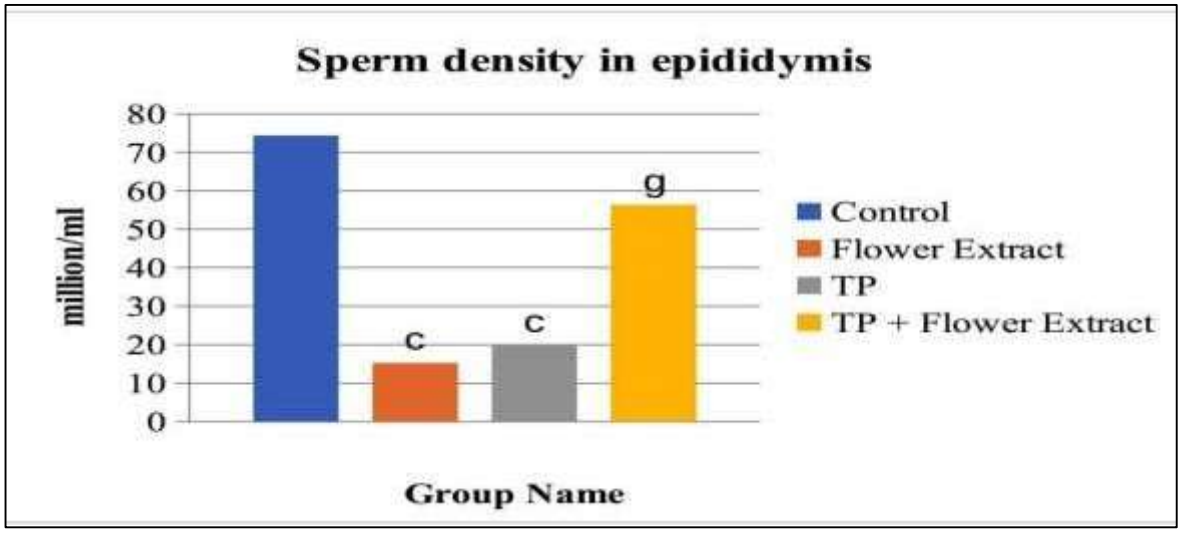

Fig 3: Effect of ethanolic flower extracts of *T.undulata* on sperm density (epididymis) of male albino rats. Data was expressed in terms of Mean ± SD. Column Bars are with error bars representing SD of Mean. Superscripts denote significance level.

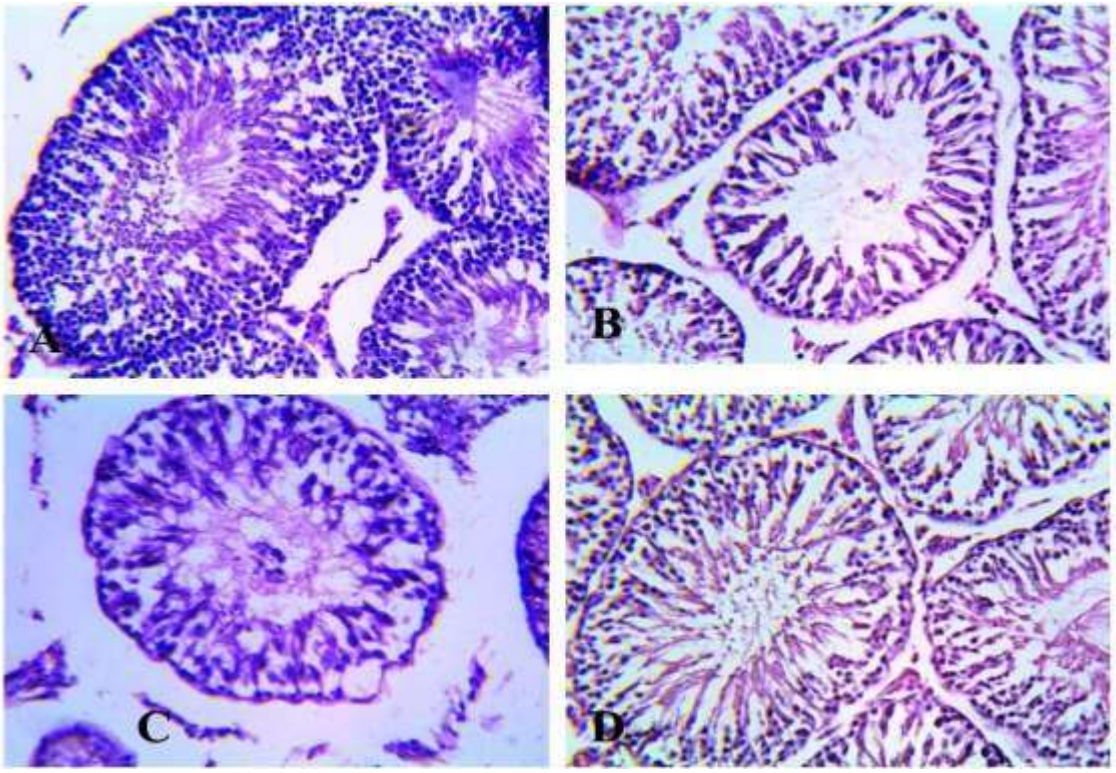

Fig: 4: Microphotomicrographs of testes of albino male rats. A. Group I (control) testes of albino male rat, B Group II (ethanolic flower extract of *T.undulata)*. C Group III (TP) & D Group IV (TP + ethanolic flower extract of *T.undulata*). HE, 200×

CONCLUSION

This can be inferred that ethanolic flower extract of *T. undulata* possess male antifertility potential without any side effect on normal body functioning. As treated groups showed reduced sperm motility, density, reduction in testicular cell population and spermatogenesis arrest. This can serve as potent male

oral contraceptive drug as it also has reversibility in its effect. As combinational treatment of flower and TP exhibited regaining in sperm motility, density, testicular cell population and spermatogenesis.

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CONFLICT OF INTEREST
No conflict

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