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RESEARCH ARTICLE

Contraceptive Efficacy and Antioxidant Potential of *Prosopis juliflora* Pod Extract in Male Albino Rats

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Abstract

Objective: To investigate the contraceptive effects of *Prosopis juliflora* pod ethanolic extract in male albino rats. **Methods:** Group I is the control group. Group II this group rats were given TP intramuscular injection at a dose of 0.01mg/day for 30 days. Group III rats of this group were administered *Prosopis juliflora* pod extract at a dose level of 500 mg/kg body weight for 60 days. Group IV, a group of rats are treated with a combination of TP (0.01 mg/day intramuscular) and *Prosopis juliflora* pod extract (500 mg/kg body weight) for 30 days. The parameters viz. body and organ weight, serum biochemistry, testosterone level, hematology and antioxidants, lipid profile, sperm parameters, and histological changes, all these parameters observation was taken. One-way analysis of variance (ANOVA) was used to assess the data, after which Tukey's multiple comparison tests were conducted. Statistical data analysis was done using a graph pad prism. **Findings:** The group treated with pod extract of *Prosopis juliflora* showed a significant ($P \leq 0.001$) decline in sperm motility and density, serum testosterone level as compared to control, as well as the weight of testis and epididymis was significant ($P \leq 0.001$) reduced in comparison to control. From histological observation, it is revealed that spermatogenesis arrest, reduction in seminiferous tubule diameter, highly significant ($P \leq 0.001$) reduction in secondary spermatogonia, mature Leydig cell, and spermatid. With substantial elevation in LPO and decline in GSH. **Novelty:** This study was taken up because it is a weed plant to find if it has any novel properties, particularly regarding male fertility. A weed having such property will serve as a good source for controlling fertility. Less work has been conducted on this plant to reveal its therapeutic properties; this work helps to find its pod extracts' effect on fertility-controlling properties in male rats.

Keywords: Contraception; Prosopis juliflora; Antifertility; Plant Extract

1 Introduction

The increasing population is one of the major issues nowadays. India's population will reach 9.2 billion by 2050 because it is multiplying at an alarming rate and has reached 121 crores[1]. Therefore, this century needs to search for antifertility agents that can continuously tackle the problem of population explosion that may cause adverse impacts on the health and economic condition of families in particular and society in general. Population explosion also causes unemployment and poverty in developing and developed countries where the population growth is very high[2]. Nowadays, fertility control is considered a significant global and national public health issue. Most of the contraceptives available are only for females, for males are very few, and to date, no oral male contraceptives are available. Already available contraceptives are synthetic, having numerous adverse effects. Therefore, researchers are interested in safe, effective, readily available natural, active plant-based products to be used as contraceptives and abortifacients. Traditional plants and plant-based products have been essential parts of human society since the beginning of human civilization, which helps fight against several diseases[3]. The World Health Organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for their treatment of diseases due to various advantages such as ease of availability, affordability, and fewer side effects when compared to the allopathic system of medicines.

Prosopis juliflora plant belongs to (Mimosaceae) family. This plant shows various pharmacological properties such as antifungal, antibacterial, antioxidant, antimicrobial, and anticancer activity. The review of this plant suggests that secondary metabolites such as alkaloids, flavonoids, phenolic compounds, and other secondary metabolites are responsible for its medical activities; therefore, the plant plays a vital role in maintaining human health and well-being[4]. *Prosopis juliflora* pod extract was given to male and female rats along with daidzein and estradiol. Showing that the *Prosopis juliflora* pod has antifertility properties in females but not males[5]. The current study evaluated *Prosopis juliflora* pod ethanolic extract for contraceptive properties.

2 Methodology

2.1 Collection of plant material, identification and authentication

Prosopis juliflora pod was collected from Jai Narain Vyas University, New Campus, Jodhpur, Rajasthan, India. It was authenticated and verified by a botanical survey of India (BSI), Jodhpur, with authentication number BSI/AZRC/ I.12012/ Tech./ 23-24 (PI. ID.) 33 Dated: 27-4-23. For future reference, the sample was preserved in the BSI department.

2.2 Extract Preparation

Plant material, i.e., a pod of *Prosopis juliflora*, was dried in the shade to reduce the moisture level. The dried pod was finely ground to powder using the grinder, and 200 gm of powdered pod material was mixed with 70% ethanol. For 32 hours, this extract was subjected to the Soxhlet extraction apparatus. Intermittent shaking was done repeatedly after some intervals for an hour to avoid sticking the material in the flask bottom. After 32 hours, the solution was filtered using a muslin cloth, and the filtrate was kept for evaporation to obtain a semi-solid paste of extracts under reduced pressure. Obtained extract materials were weighed and stored in clean, sterile, and air-tight containers at -277K temperature.

2.3 Maintenance of experimental animal

For the present study, 200 gm body weight female and male albino rats were used. Females are used for fertility tests, and males are used for experimental work. All experimental animals were kept at 23±2 °C standard temperature with a 12-hour cycle of light and dark. Before starting the experiment, all animals are acclimated for seven days. Animals are fed with standard pellets and have easy access to water as a base diet. All the experimental work performed for this research was approved by the Institutional Ethical Committee (IAEC) S. No. UDZ/IAEC/2022/16 Dated- 20/09/2022, and for animal handling guidelines from CPCSEA (committee for control and supervision of experiments on animals), Government of India. The veterinary advisor regularly supervised the animal.

2.4 Determination of Physiological dose

The LD50 was determined using the fixed-dose procedure described by Walum (1998)[6]. For 60 days, animals were dosed with 500mg/kg body weight. Ethanolic pod extract of *Prosopis juliflora* was given by an oral route every morning for experimental duration.

2.5 Fertility teste of male albino rats

Before starting dosing rats, rats were subjected to fertility tests by pairing up healthy females and males in 3:1 in individual cages for 5-6 days, i.e., one adult male and three adult females. For the vaginal smear test, every female underwent for 5 to 6 days to check the sperm presence. Fertile males identified by this process were used for the treatment.

2.6 Treatment Protocol

Healthy and fertile male rats weighing 150-200 gm were used as experimental model animals. In this experiment, four groups were made, each having five animals in duplicate.

Group. 1 (Control): This group of male rats was fed a regular diet and 2ml distilled water/day for 30 days.

Group II (TP): With Testosterone propionate at 0.01 mg/day for 30 days, this group of rats was injected intramuscularly.

Group III (Pod Extract): Rats of this group were orally dosed with *Prosopis juliflora* ethanolic pod extract at a dose level of 500 mg/kg body weight dissolved in 2ml distilled water for 60 days.

Group IV (Pod+TP): Rats of this group were orally dosed with *Prosopis juliflora* ethanolic pod extract at a dose level of 500 mg/kg body weight dissolved in 2ml distilled water and intra-muscular injections of TP at a dose level of 0.01 mg/day for 30 days in combination. Throughout the experiment period, all treated groups were fed with a standard diet.

2.7 Scheduling autopsy

Under mild anesthesia, overnight fasted animals were autopsied after completion of the experimental period of 30 and 60 days. Using a sterile and clean syringe via puncturing the left ventricle, blood samples were collected in EDTA-coated and regular test tubes for the serum biochemistry, lipid profile, hematological, and antioxidant studies. At 3000 rpm, blood was centrifugated for 15 minutes to obtain serum. All the vital organs (liver, kidney, heart) and reproductive organs (testis, caput, cauda, ventral prostate, seminal vesicle, and vas deference) were removed for histological studies, with normal saline dissected tissues were cleaned and fixed in 10% formalin. These tissues were further processed for histological slide preparations.

2.8 Body and reproductive organ weight determination

All experimental group animals' initial and final body weights were recorded. After dissecting and removing extra tissues adhered to all vital and reproductive organs, they were weighed and recorded.

2.9 Sperm motility and density

Testes and epididymides were used to study sperm motility and density. Epididymides were immediately removed after collecting the blood, and cauda was taken for sperm motility and density calculation.

2.10 Serum biochemistry

Albumin, globulin, bilirubin, Serum total protein, uric acid, urea, creatinine and SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase), and ALP (alkaline phosphatase) liver marker enzymes were estimated by using standard kits.

2.11 Hematological parameters

Using a Standards kit, various Hematological parameters of blood were determined.

2.12 Lipid profile

For estimation of total serum cholesterol, HDL (high-density lipoprotein), VLDL (Very-low-density lipoprotein), LDL (Low-density lipoprotein) and Triglyceride Standard commercial kits are used.

2.13 Estimation of hormonal level

A standard commercial kit was used through the Enzymes Immuno Assay method (EIA), and serum testosterone levels were determined.

2.14 Organ Histopathology

Reproductive organs (cauda, caput, testis, ventral prostate, vas deference, and seminal vesicle) and vital organs (kidney, liver, heart) were dehydrated in successive grades of alcohol. Then, they rinsed with xylene after fixing it with 10% formalin. Molten paraffin wax was used to embed tissues. For cutting the section with a thickness of 5 um, microtome was used, and for the staining section, Eosin and Hematoxylin were used. For studying the histopathological changes, slides were observed at 200 and 400 X magnification in the light microscope, prominently in the reproductive organs. For testicular cell population counting or histometery of histological slides, ImageJ software was used by using the protocol given by Abercrombie (1946)[(7)] and Dixon and Massey (1957)[(8)].

2.15 Estimation of Antioxidant

- LPO (Lipid peroxidation): LPO was done using the procedure given by[(9)].
- FRAP (Ferric Reducing Antioxidant Power Assay or The Ferric Reducing Ability of Plasma): - FRAP was done using the method given by[(10)].
- GSH (Glutathione): GSH levels in serum were estimated based on the procedure given by Beutler[(11)].

2.16 Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the data, after which Tukey's multiple comparison tests were conducted. Statistical data analysis was done using a graph pad prism.

3 Result and Discussion

3.1 Extraction

Extraction was done using the Soxhlet apparatus in 70% Ethanol as a solvent, and the percentage extraction yield for Pod was 38.61%. Bark powder of *Leptadenia reticulata* was used for the Soxhlet extraction procedure with ethanol and petroleum ether as a solvent, and the percentage extraction yield obtained was 2.33% ethanolic, and 2.02% petroleum ether as a solvent[(12)].

3.2 Effect on body and Reproductive organs weight

After 60 days of administration of *Prosopis juliflora* pod extract and 30 days of intramuscular injection of TP and a combination of Pod extract + TP to male rats, a statically insignificant difference in initial and final body weight was observed. A similar observation was observed when male rats were dosed with aqueous extracts of *Prosopis cineraria* leaf[(13)]. However, in the reproductive organ weight (testes, epididymides, seminal vesicle) of the treated group, a highly significant ($P \leq 0.001$) decline in testes and a significantly reduced epididymides weight in comparison to the control group (Table 1). The present findings and results are similar to previous research[(14)], which indicates that androgen is a crucial element in maintaining the functional and structural integrity of the reproductive organs in males. As a result, the animal's body should have enough androgen in circulation to sustain the reproductive organ's standard functionality and histoarchitecture. Similar results were obtained when male rats treated with aqueous neem leaf extract[(15)] showed a significant decline in accessory reproductive organs, showing the plant extract's antifertility and contraceptive capability. While the rats were treated with a combination of Pod ethanolic extract and TP, compared with TP (Group II), the treated group results showed an elevation in reproductive organ weight.

Table 1. Body and organ weight of Ethanolic pod extracts of *Prosopis juliflora* treated male rats

TREATMENT GROUPS	BODY WEIGHT (gm)		Reproductive Organ Weight (gm/100gm body weight)			
	Initial	**Final**	**TESTES**	**EPIDIDYMIDES**	**SEMINAL VESICLE**	**VENTRAL PROSTATE**
Group I (Intact Control)	196.50 ±14.94	210.50±13.53	1218.00±54.85	610.23 ±12.43	435.53±28.39	280.48±4.19

Continued on next page

Table 1 continued

Group II (TP-Testosterone propionate)	168.36 ±14.42	191.40±22.82	894.04±45.98^{c}	527.27 ±46.81^{c}	315.06 ±31.05^{b}	189.88±6.28^{c}
Group-III (Pod Ethanolic)	156.25 ±15.03	173.75±14.53	967.30±64.87^{c,h}	487.51±29.16^{c,h}	325.24±25.85^{b,h}	192.94±2.16^{c,h}
Group-IV (Pod + TP)	194.46 ±4.23	202.48±4.60	1047.84 ±38.44^{a,e}	548.27±14.84^{b,h}	398.22 ±23.65^{a,e}	199.31±9.76^{c,h}

Group II-IV compared with Group I
P≤ 0.05=a P≤0.01=b
P≤0.001=c Non-significant=d
Group III-IV compared with Group II
P ≤ 0.05=e P≤ 0.01=f
P ≤ 0.001=g Non-significant=h

3.3 Effect on sperm motility and density

Prosopis juliflora pod Extract dosed rats showed a reduction in sperm parameters (testes and epididymis). In comparison to the control group, a highly significant decrease (P ≤ 0.001) in sperm density and sperm motility was noticed in Groups II and III (Table 2). Sperm motility and viability are some of the most crucial parameters to determine the capability of mature sperms for regular fertilization[16]. In serum and plasma, testosterone levels correlate with sperm concentration and sperm motility. This alteration in sperm motility and viability could be attributed to the reduced levels of serum testosterone and enhanced production of reactive oxygen species, as previously suggested[17]. Similar findings were observed when male rats were administered *Mentha longifolia L*. methanolic leaf extract[18]. In Group IV, a significant elevation in sperm density and motility was observed compared to Group II (Table 2).

Table 2. Sperm dynamics,diameters and serum testosterone of Ethanolic pod extracts of *Prosopis juliflora* treated male rats

TREATMENT GROUPS	SPERM MOTILITY (%)	SPERM DENSITY (million/ml)		Seminiferous Tubule Diameter (μm)	Epithelial Cell Height (μm)		TESTOSTERONE(ng/dL)
		CAUDA	TESTES		Caput	Cauda	
Group I (Intact Control)	84.81±6.28	69.50 ±3.42	4.95±0.09	298.14 ±14.34	42.91 ±3.79	33.89 ±2.81	450.80 ±7.63
Group II (TP- Testosterone propionate)	22.68±1.61^{c}	14.52±1.99^{c}	1.96±0.81^{b}	178.31 ±11.50^{c}	30.79 ±2.52^{b}	24.69 ±2.04^{b}	135.53 ±4.80^{c}
Group-III (Pod Ethanolic)	33.93±3.9^{c,f}	21.43±2.00^{c,f}	1.81±0.34^{c,h}	201.89 ±19.41^{c,e}	33.16 ±3.26^{a,h}	27.97 ±1.98^{b,h}	267.5 ±8.89^{c,g}
Group-IV (Pod + TP)	67.54±2.59^{b,g}	30.37±1.03^{c,g}	4.02±0.50^{a,e}	254.13 ±12.04^{a,g}	39.03 ±2.59^{d,e}	29.58 ±1.75^{a,e}	303.79 ±5.88^{a,g}

Group II-IV compared with Group I
P≤ 0.05=a P≤ 0.01=b
P≤ 0.001=c non-significant=d
GroupIII-IV compared with Group II
P ≤ 0.05=e P ≤ 0.01=f
P ≤ 0.001=g non-significant=h

3.4 Effect on serum biochemistry and hematological parameters

Compared to the control group, insignificant alteration was observed in biochemical and hematological parameters of all treated groups with *Prosopis juliflora* pod extract. By average values of serum and blood parameters, low toxicity of the plant is demonstrated [19]. Similar results were noticed in the group when treated with *Leptadenia reticulata* leaf extract [20].

3.5 Effect on lipid profile

Compared to the control group, the lipid profile parameters like LDL, VLDL, HDL, and triglyceride had a slight elevation in the treated group, while this elevation was statistically insignificant. The total cholesterol level decreased in the treated group with *Prosopis juliflora* pod extract compared to the control group. This decrement of testosterone level may be because of the effects of saponin on serum cholesterol level, which is essential for testosterone synthesis by its action on the Leydig cells. Numerous studies reported that saponin lowers serum cholesterol levels and hence affects testosterone production & spermatogenesis [21] .

3.6 Effect on histopathology

After treating rats with *Prosopis juliflora* pod extract, an alteration in reproductive organ histology was observed. Degenerative changes like spermatogenesis arrest, highly significant ($P \leq 0.001$) decreases in seminiferous tubule diameter, and decline in the secretion of ventral prostate and seminal vesicle was observed (Table 2, Figure 1). A highly significant ($P \leq 0.001$) reduction in all these parameters was noticed in treated groups II and III as compared to the control (Figures 1 and 2). Androgen is the crucial component responsible for the proper maintenance of reproductive organs' structural and functional integrity in males. Hence, in the animal body, circulating androgen levels should be sufficient to maintain the reproductive organs' histoarchitecture and their standard functionality. A similar result was noticed when male albino rats were treated with bark extract of *Leptadenia reticulata* for 60 days [12]. Male rats dosed with a combination of Pod extract and TP (Group IV) showed a notable increase in all the above parameters concerning the TP (Group II) treated group.

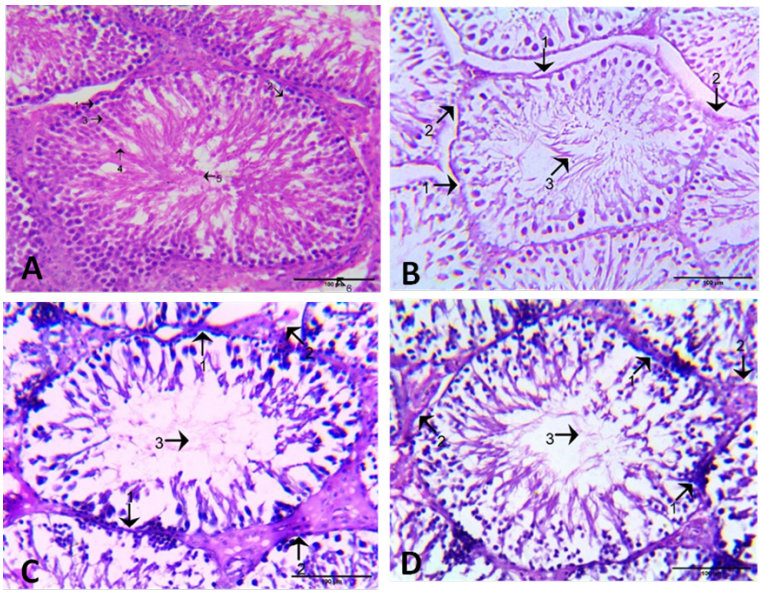

Fig 1. Microphotographs of different groups Testes; A. Control male albino rat Testes labelling showing various spermatogenesis stages used for histometery 1. Spermatogonia; 2. Primary Spermatocyte; 3. Secondary Spermatocyte; 4. Spermatid; 5. Lumen filled with spermatozoa; 6. Leydig Cells; HE, 200× B; TP- Testosterone propionate, and C. Pod Ethanolic treated male albino rat testes labelling showing: 1. Reduction in diameter of seminiferous tubule; 2. Reduction in Leydig cells; 3. Leumen with reduced spermatozoa; HE, 200×. D.TP + Pod Ethanolic Extract treated male albino rat Testes labelling showing 1. Recovering diameter of seminiferous tubule; 2. Regenerating Leydig cells; 3. Leumen with increased spermatozoa (HE, 200 x)

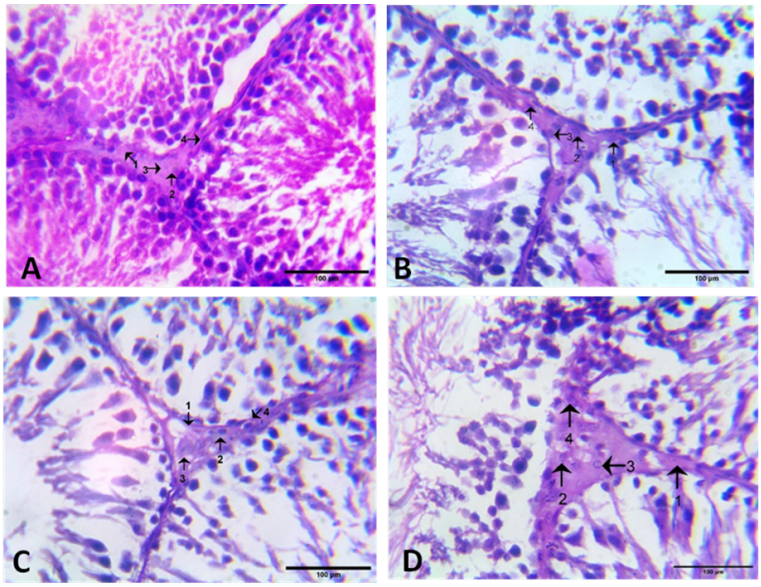

Fig 2. Microphotographs of interstitial cells of different groups: A. Control (Group I), B. TP-Testosterone propionate (Group II), C. Pod Ethanolic Extract (Group III), and D. TP + Pod Ethanolic Extract (Group IV) labelling showing: 1. Fibroblast Cells; 2. Immature Leydig Cells; 3. Mature Leydig Cells; 4. Degenerating Cells (HE, 400×)

3.7 Effect on testicular cell population

Significant ($P \leq 0.001$) decline in primary, secondary spermatogonia, spermatid, and mature Leydig cell, significant ($P \leq 0.001$) elevation in no. of degenerative cells, was noticed in the treated group dosed with *Prosopis juliflora* pod extract and TP in comparison to the control group (Table 3, Figures 1 and 2). The decline in the testicular and Leydig cells is associated with reduced hormone levels because, as we know, reduced testosterone and LH levels interfere with the growth phase of sperm production and spermatogenesis. Inhibition of steroidogenesis via the hypothalamic-hypophyseal gonadal axis, FSH, and LH secretion, and arrest in spermatogenesis reduced fertility, which is reflected by decreased sperm motility, density, and testicular population count [19]. Vacuolation, apoptosis, and degeneration in testicular cells such as primary and secondary spermatocytes, spermatogonia, and spermatids may result due to Sertoli cell micro milieu disruption, which in turn affects the differentiation of germinal cells by affecting protein synthesis machinery [22]. Similar findings were also noticed when male rats were treated with *Tecomella undulata* ethanolic root extract [14]. Male rats dosed with Pod extract and TP (Group IV) in combination showed elevation in primary, secondary spermatogonia, spermatid, and mature Leydig cell, relative to only TP (Group II) treated group (Table 3).

Table 3. Testicular cell population dynamics of Ethanolic Pod extract of *Prosopis juliflora* treated male rats

TREATMENT GROUPS	Germinal Cells				Interstitial Cells			
	Spermatogonia	Primary Spermatocytes	Secondary Spermatocytes	Spermatids	Fibroblast	Immature Leydig Cell	Mature Leydig Cell	Degenerating Cells
Group I (Intact Control)	63.62 ±2.41	56.82 ±3.64	97.21 ±5.31	163.61 ±6.37	81.85 ±2.57	62.09 ±2.33	79.15 ±2.31	19.65 ±1.21
Group II (TP-Testosterone propionate)	46.69 ±1.47^{c}	39.69 ±2.31^{c}	49.64 ±2.44^{c}	28.91 ±2.24^{c}	69.13 ±1.70^{c}	51.14 ±2.87^{b}	45.14 ±2.39^{c}	81.57 ±4.37^{c}

Continued on next page

Table 3 continued

Group-III (Pod Ethanolic)	58.78 $\pm 1.38^{a,g}$	38.89 $\pm 1.47^{c,h}$	25.13 $\pm 4.11^{c,g}$	39.47 $\pm 3.02^{c,f}$	71.42 $\pm 3.64^{b,h}$	54.28 $\pm 2.11^{b,h}$	46.37 $\pm 2.50^{c,h}$	48.51 $\pm 4.68^{c,g}$
Group-IV (Pod + TP)	61.35 $\pm 4.63^{d,g}$	44.24 $\pm 3.29^{b,h}$	39.22 $\pm .25^{c,h}$	53.24 $\pm 4.34^{c,g}$	71.28 $\pm 4.83^{a,h}$	58.32 $\pm 2.89^{a,h}$	51.12 $\pm 4.60^{c,e}$	59.99 $\pm 3.26^{c,g}$

Group II-IV compared withGroup I
P≤ 0.05=a P≤0.01=b
P≤0.001=c non-significant=d
Group III-IV compared with Group II
P ≤ 0.05=e P≤ 0.01=f
P ≤ 0.001=g non-significant =h

3.8 Effect on serum testosterone level:

In *Prosopis juliflora* pod extract (Group III) and TP (Group II), treated group animals, observed a significant ($P \leq 0.001$) decline in serum testosterone level in comparison to the control group (Group I) (Table 2). Testosterone has a pivotal role in the spermatogenesis process. Testosterone is the representative and primary androgen that controls the progression of spermatogenesis. Alteration in cell signaling, apoptosis, metabolism, DNA repair, meiosis & RNA processing may be observed due to a decline in testosterone level; this may be due to testosterone requirement in the development and maturation of male germ cells [23]. A similar finding was noticed when the animals were treated with *Momordica Dioica* Methanolic Root Extract [24]. Male rats dosed with Pod extract and TP (Group IV) in combination showed elevation in testosterone levels relative to only the TP (Group II) treated group (Table 2).

3.9 Effect on level of serum antioxidant:

Concerning the control group, there was a significant ($P \leq 0.001$) elevation in serum LPO, a slightly significant ($P \leq 0.05$) reduction in serum GSH levels in all treated groups, and the value of serum FRAP level remained unchanged (Table 4). Similar results were noticed when rats were treated with bark extract of *Leptadenia reticulata* [12]. It may be inferred that *Prosopis juliflora* Pod ethanolic extract has antifertility properties, most likely due to the degeneration of Leydig cells, which are responsible for testosterone levels in the blood. Increased serum LPO and decline in serum GSH levels support the degenerative changes in male albino rats.

Table 4. Serum antioxidant of various extracts of Ethanolic Pod extract of *Prosopis juliflora* treated male rats

TREATMENT GROUPS	LPO (μM MDA formed/mg Protein/hour)	GSH (μM GSH/mg Protein)	FRAP (μM FRAP in Ascorbic Acid Equivalent)
Group I (Control)	2.04 ± 0.24	118.21 ± 2.33	196.58 ± 18.11
Group II (TP-Testosterone propionate)	9.69 $\pm 0.77^{c}$	95.83 $\pm 7.54^{b}$	203.33 $\pm 5.66^{d}$
Group-III (Pod Ethanolic)	4.87 $\pm 0.19^{c,g}$	102.11 $\pm 9.11^{a,e}$	189.71 $\pm 12.77^{d,h}$
Group- IV (TP + Pod Ethanolic Ether)	2.56 $\pm 0.24^{d,g}$	106.21 $\pm 8.11^{a,h}$	192.61 $\pm 9.034^{d,h}$

It may be inferred that *Prosopis juliflora* pod ethanolic extract has an antifertility property because, as mentioned above, it causes a decline in no. of Leydig cells, which are responsible for testosterone level maintenance. Sertoli cells are affected because of low levels of testosterone in serum, which also causes spermatogenesis arrest; the extract may cause the alteration of the testosterone synthesis pathway. This study was taken into consideration since *Prosopis juliflora* is a weed plant to find if it has any novel properties, particularly regarding male contraception. A weed having such property will serve as a good source for controlling fertility. Less work has been conducted on the *Prosopis juliflora* plant to reveal its medical properties; this work helps to find its pod extracts' effect on contraceptive efficacy in male rats.

4 Conclusion

Antifertility parameters include sperm density, sperm motility, and testicular cell population. From all these parameters, we can conclude the antifertility ability of any herbal extract or drug. The present finding from various observations is a significant decline ($P \leq 0.001$) in sperm density and motility compared to the control group. Testicular cell population dynamics, prominently in secondary spermatogonia, spermatids, and mature Leydig cells, there was a significant reduction in no. as compared to the control group. The diameter of seminiferous tubules was also reduced. A highly significant ($P \leq 0.001$) decline in the testosterone level in the pod extract-treated group serum was observed. From all the observations mentioned above, we can conclude that *Prosopis juliflora* pod ethanolic extract has contraceptive efficacy and can be used as an effective reversible male oral contraceptive to regulate fertility without any side effects after extensive clinical trials.

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