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Reversible antispermatogenic and antisteroidogenic activities of *Feronia limonia* fruit pulp in adult male rats

Ramaiyan Dhanapal[1*], J.Vijaya Ratna[2], I. Sarathchandran[3], Malaya Gupta[4]

S1Department of Pharmaceutics, Kakatiya Institute of Pharmaceutical Sciences (KIPS), Pembarthi (V), Hasanparthy (M), Warangal, Andhra Pradesh, India-506 371
2Pharmaceutical Technology Division, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India-530003
3Department of Pharmaceutics, Gokula Krishna College of Pharmacy, Sullurpet, Nellore, Andhra Pradesh, India-524 121
4Department of Pharmaceutical Technology, Division of Pharmacology, Jadavpur University, Kolkata, West Bengal, India-700032

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ABSTRACT

Objective: To explore the antispermatogenic and testicular antisteroidogenic activities of *Feronia limonia* fruit pulp southern India. **Methods:** Fourty Wistar male albino rats (*Rattus norvegicus*) were equally divided into four groups. Experimental groups were administered with the ethanolic extract of *Feronia limonia* (*F. limoni*) fruit pulp at doses of 250 and 500 mg/kg body weight once daily for 55 days. All treated rats had corresponding recovery groups. At the end of each treatment periods, various spermatological indices, tissue biochemicals and testicular enzymes levels were analysed. Blood profiles were also estimated. **Results:** Compared with the control, the *F. limonia* fruit pulp at both dose levels did not decrease body weight, which were associated with decline in epididymal sperm count, motility, viability and increased percent of abnormal sperm. Further, *F. limonia* fruit pulp at 500 mg/kg body weight markedly reduced the epididymal and testicular protein content by 24.58% and 29.86%, respectively, as well as the glucose-6-phosphate dehydrogenase and Δ^5-3 β -hydroxy steroid dehydrogenase) levels by 42.82% and 38.08%, respectively, while a significant elevation was observed in testicular cholesterol and ascorbic acid content. A gradual recovery of all parameters was observed after 55 days of treatment withdrawal. No significant alterations in haematological indices were observed. **Conclusions:** The present findings indicate that *F. limonia* fruit pulp may have reversible antispermatogenic and antisteroidogenic properties, and could partially support the traditional use as male contraceptive.

1. Introduction

Control of fertility constitutes a global health issue, as overpopulation have both major personal and societal impact and it is necessary to control it on the time. As we know the entire available contraceptive in the market are not safe, mostly they are steroid in nature and they have more or little hazardous side effect. Attention has now been focused on indigenous plants for possible contraceptive effect[1-3]. The traditional knowledge on the medicinal use of plants should be assessed under laboratory conditions using appropriate biological assays to disclose if the traditional claims are evidence-supported.

Feronia limonia (*F. limonia*) Linn, syn. *Feronia elephantum* Correa and *Limonia acidissima* Linn (Family: Rutaceae) is a small deciduous tree found throughout the plains of India[4,5]. All parts of this plant are prescribed in the indigenous system of medicine for treatment of various ailments. The fruits of this plant are used in diarrheoa and dysentery[6], tumors, asthma, wounds, cardiac debility and hepatitis[4]. Recently, the fruit pulp of this plant is studied to have anti-inflammatory, antipyretic and analgesic activities[7], antiulcer[8], hepatoprotective, wound healing and antioxidant activities[9,10]. The fruit shells were reported to contain antifungal compounds, namely, psoralene, xanthotoxin, 2,6-dimethoxybenzoquinone and sterol[11]. Our survey revealed that the fruit pulp of this plant is traditionally used for male contraception by the rural people of Thanjavur district, Tamilnadu, India (Personal communication). To the best of our knowledge, no scientific reports on the antispermatogenic and testicular antisteroidogenic effects of this plant were so far available.

*Corresponding author: Ramaiyan Dhanapal, Associate Professor, Department of Pharmaceutics, Kakatiya Institute of Pharmaceutical Sciences (KIPS), Pembarthi (V), Hasanparthy (M), Warangal, Andhra Pradesh, India-506 371.
Tel: +91-9652698238
E-mails: ramaiyandhanapal@gmail.com

Being part of a programme to find new compounds with antifertility activity, the fruit pulp of *F. limonia* was extracted with 70% (v/v) of ethanol and evaluated in male rats.

2. Materials and methods

2.1. Plant materials and extraction

The fruit pulp of *F. limonia* was collected during the months of December from Thanjavur district of Tamilnadu, India, in the year of 2006. The plant specimen was identified and authenticated by Dr.P.Jayaraman, M.Sc., Ph.D, Plant Anatomy Research Centre (PARC), Chennai Tamil Nadu, India. A voucher specimen (PARC/2006/382) has been deposited in the herbarium of the same department. The fruits pulp were carefully removed and separately dried in shade, pulverized by a mechanical grinder and passed through 40-mesh sieve. The powder was subjected to extraction in Soxhlet extractor, was defatted with petroleum ether (40-60℃) and later extracted successively with 70% v/v ethanol at 68℃. The extracts were collected in 5 liter individual conical flasks, filtered, and the solvent was evaporated to dryness under reduced pressure in an Eyela Rotary Evaporator (Japan) at 40-45℃ and were stored in a vacuum desiccators. The yield (% w/w) of the prepared extract was found to be 19.4%, with regard to dried powder. The extracts were dissolved individually in 1% Tween-80 solution, and were used for experimental purpose.

2.2. Animals

Adult Wistar strain male and female albino rats, *Rattus norvegicus* (90 days old), weighing 150-200 g, procured from animal house, were housed in groups of five per cage made of polypropylene (8″ × 12″× 8″) with metal grill tops. The animals were maintained with standard pellet feed (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. All experimental procedures described (CPCSEA) were reviewed and approved by the University Animal Ethical Committee.

2.3. Acute oral toxicity study

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development, revised draft guidelines 423 B ("Up and down" method)[12] received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)[13], Ministry of Social Justice and Empowerment, Government of India. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 h. The substance is tested using a stepwise procedure, each step using three animals of a single sex (females). Since there was no information on the substance to be tested (*i.e.* extracts), starting dose was 2 000 mg/kg body weight up to 5 000 mg/kg body weight. Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 h. In all cases death was observed within first 24h. Attention was also given to observations of tremors and convulsions.

2.4. Design of experiment

Fourty healthy male albino rats were selected and divided into four groups containing ten rats each and treated as follows: Group-1 received distilled water (10 mL/kg body weight) as normal control and Group-2 received 1% Tween 80 dissolved in distilled water (10 mL/kg body weight) as vehicle control. Groups-3 and 4 received the ethanolic extract of *F. limonia* fruit pulp at the doses of 250 and 500 mg/kg body weight, respectively. The vehicles and the plant drug were administered intragastric (*i.g.*) route on consecutive days for 55 days. At the end of the experimental period, five animals from both control and experimental groups were given anesthesia under mild sodium pentobarbital 24 h after the last dose and 18 h after fasting. Blood was also obtained by cardiac puncture from these animals for the analysis of hematological profiles. The testis, cauda epididymal ducts and seminal vesicles were dissected out, trimmed off from adherent fats and weighed and recorded to the nearest milligram on a digital balance. Sperm from cauda epididymal ducts were released in phosphate buffer solution (pH-9.0) media and used for spermatological studies[14]. The testes were used for biochemical estimation. The remaining five rats from groups 3 and 4 were left for recovery studies over a period of next 55 days (from 56th day to 110th day). All spermatological parameters were repeated in order to ascertain the nature of action of extract, *i.e.* reversible or irreversible.

2.5. Gravimetric analysis of body and reproductive organ

The body weights of the animals were recorded, prior to and after treatment and recovery. Testis, epididymis, seminal vesicles and ventral prostate gland were weighted to the nearest milligrams.

2.6. Spermatological studies

The cauda epididymal duct on one side was exposed and incised. The connective tissue capsule around the cauda epididymidis was teased out and the epididymal duct was uncoiled. The semen that oozed into the cavity block was quickly sucked into a capillary tube upto 0.05 μL mark and transferred to an Eppendorf tube. It was diluted 200 (0.05 μL of sperm with 99.95 μL of PBS) times in physiological saline. After thorough mixing, the sperm suspension was used for analysis of motility[15].

A drop of dilute semen was transferred to an Eppendorf tube containing one drop of 10% nigrosine and one drop of 1% eosin then the sperm viability test was done by the method as described in the WHO Laboratory Manual[16].

Sperm morphology was observed adopting Papanicolaou staining. The staining solutions were prepared according to the method of Raphael[17]. Sperm counts were made according to the method described by Gopalakrishnan[18].

2.7. Biochemical estimations

2.7.1. Estimation of cholesterol content

Testis tissues about 3 mg weight, were carefully homogenized in Potter Elvehjem homogenizer using chloroform: ethanol mixture (2:1) and non-polar part was extracted out and total cholesterol content was estimated according to the method of Sperry and Webb[19]. The optical density was determined in spectrophotometer at 620 nm against blank (chloroform).

2.7.2. Estimation of ascorbic acid content

About 5 mg of testis tissue was homogenized in Potter Elvehjem homogenizer using 45 μL ice cold 5% meta-phosphoric acid and centrifuged for 20 min at 3 500× *g*. Then, 30 μL supernatant, 15 μL acetate buffer, and 15 μL of 2,6-dichlorophenol-indophenol sodium (0.1 mg/mL) were mixed and optical density was measured against blank (distilled water) at 540 nm. Standard curve was drawn against known concentrations of ascorbic acid content[20].

2.7.3. Estimation of Δ^5-3 β -hydroxysteroid dehydrogenase (Δ^5-3 β -HSD)

Testis were homogenized in 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10 000× *g* for 10 min at 0℃. Nicotinamide adenine dinucleotide (NAD; 0.2 mL) and 0.1 mL of dihydroxyepiandrosterone were added to supernatant and mixed well. This solution was kept in shaking incubator at 35 ℃ for 90 min, acidified with 0.1 mL 3M acetate buffer (pH 5.0) and extracted with 10 mL of ethyl acetate and evaporated. Residue was dissolved in 2 mL of ethanol and optical density was measured at 240 nm against blank (ethanol). The specific activity was expressed per mg of protein[21].

2.8.4. Estimation of glucose-6-phosphate dehydrogenase (G-6-PDH)

Testis were homogenized and centrifuged at 1 000× *g* (5 min) and 10 000 × *g* (10 min) at 0 ℃. Tris-HCl buffer (0.025 mL; pH 8.3; 0.5 M), 0.01 mL of 20 mM of nicotinamide adenine dinucleotide phosphate, 0.02 mL of supernatant, and 0.025 mL of glass distilled water was added and mixed well and optical density was measured at 340 nm against blank (distilled water). The activity of G-6-PDH was estimated by the method of Lohr and Waller [22].

Protein was estimated with Folin's phenol reagent and the activities of enzymes were expressed in unit per mg of protein[23]. Fructose content in seminal vesicle was measured as described in WHO Laboratory Manual[16].

2.8. Estimation of hematological profiles

The whole blood sample was analyzed for RBCs and WBCs count, hemoglobin[24], blood sugar[25], urea[26], and serum was analyzed to estimate phospholipids[27] and cholesterol[28].

2.9. Statistical analysis

Results are expressed as mean±SEM. Data obtained were statistically analysed by using Graphpad Prism, version 4.03 for Windows (Graph Pad Software, San Diego, California, USA). Results were compared using one-factor analysis of variance (ANOVA) with Dunnett's *post-hoc* test. Values were considered significant at $P<0.05$ or less. In many cases results were calculated as percentage of relevant control values to make understanding of the results easier.

3. Results

3.1. Acute oral toxicity

The LD_{50} Cut off value was found to be 2 500 mg/kg body weight for the ethanolic extract of *F. limonia* fruit pulp.

3.2. Body weight and the weight of reproductive organs

The data revealed that the body weights of rats were not much altered after the treatment of *F. limonia* fruit pulp at both doses, whereas, a significant ($P<0.01$) decrease in the reproductive organ weights was observed in relation to the control (Table 1). These changes were more marked at higher-dose. However, the organ weights of rats in group-3 and group-4 recovered gradually to control levels by 55 days after cessation of treatment (data not shown).

3.3. Effect on sperm morphology and viability

In the vehicle control (Group 2) rat, 90.6% of spermatozoa possess normal morphology (Table 2). On the other hand, in the rats fed with ethanolic extracts of *F. limonia* fruit pulp at the doses of 250 and 500 mg mg/kg body weight the lower percent of spermatozoa possess normal morphology (68.5% and 37.3%, respectively).

The abnormalities of the epididymal sperm morphology observed were flexed head, detached head and sticking or fusion of spermatozoa. The degree of morphological abnormality expressed as percentage was significant ($P<0.01$). The morphology of rats treated with *F. limonia* fruit pulp extract at a dose of 500 mg/kg body weight exhibited higher percentage of abnormality (71.4%) than those treated with 250 mg/kg (42.5%). However, the percentage of the normal sperm gradually recovered to control levels after cessation of treatment for 55 days (Table 2).

The sperm viability reduced significantly ($P<0.01$) in rats that were treated with ethanolic extract of *F. limonia* fruit pulp at both doses. Thus, in comparison with the vehicle control group (88.2%), the groups treated with extract at the doses of 250 and 500 mg/kg body weight showed about 57.5% and 38.6%, respectively (Table 2).

Table 1
Effect of ethanolic extracts of *F. limonia* fruit pulp on the body weight and weight of reproductive organs of male rats after 55 days of treatment.

Parameters	Treatment design			
	Group-1 (Normal 10 mL/kg body weight)	Group-2 (Vehicle 10 mL/kg body weight)	Group-3 (EEF 250 mg/kg body weight)	Group-4 (EEF 500 mg/kg body weight)
Initial body weight (g)	181.16±8.53	180.43±3.27	182.26±4.16	184.24±2.65
Body weight after treatment (g)	236.43±6.64	234.47±8.53	235.53±5.34	236.61±4.76
% increase in body weight	30.50	29.95	29.22 ns	28.42 ns
Testis (mg)	2.81±0.08	2.82±0.07	2.34±0.05** (−17.02)	1.97±0.06** (−30.14)
Caput epidimidis (mg)	348.86±5.74	347.65±8.76	314.66±2.58** (−9.48)	285.12±2.43** (−17.98)
Cauda epidimidis (mg)	237.46±7.76	235.63±8.46	212.17±6.42** (−9.95)	185.84±3.23** (−21.13)
Seminal vesicles (mg)	286.52±15.66	287.46±13.57	255.67±3.18** (−14.53)	235.36±4.76** (−21.60)
Ventral prostate (mg)	135.14±3.16	134.11±4.23	115.38±1.74** (−13.96)	104.33±4.36** (−22.20)

**P<0.01 significantly different from vehicle control; ns=Non- significantly different from vehicle control. Figures in parenthesis are % increase (+) or decrease (−) over vehicle control; EEFL= ethanolic extracts of *F. limonia* fruit pulp.

Table 2
Effect of ethanolic extracts of *F. limonia* fruit pulp on spermatological parameters after 55 days of treatment and 56-110 days after withdrawal of the treatment.

Parameters		Treatment design			
		Group-1 (Normal 10 mL/kg body weight)	Group-2 (Vehicle 10 mL/kg body weight)	Group-3 (EEF 250 mg/kg body weight)	Group-4 (EEF 500 mg/kg body weight)
Normal sperm (%)	After treatment	91.3±2.7	90.6±3.2	68.5±2.6**	37.3±2.6**
	Recovery	91.4±2.3	92.3±1.2	89.6±1.8 ns	87.4±2.7 ns
Abnormal sperm (%)	After treatment	8.7±1.6	9.3±1.4	42.5±3.4**	71.4±2.3**
	Recovery	8.2±1.7	7.6±2.4	9.5±1.2 ns	10.1±2.3 ns
Sperm viability (%)	After treatment	89.4±1.6	88.2±1.4	57.5±2.3**	38.6±1.3**
	Recovery	90.2±1.3	89.3±1.5	83.9±1.6 ns	82.4±2.4 ns
Sperm count × 10^6 sperm/mL	After treatment	65.4±2.3	64.8±3.6	47.6±1.8**	33.4±2.7**
	Recovery	66.3±1.2	66.7±2.4	62.8±2.6 ns	61.5±2.2 ns
Motility duration (mins)	After treatment	105.0±2.0	103.0±3.0	47.0±2.0**	38.0±4.0**
	Recovery	104.0±3.0	103.0±5.0	98.0±2.0 ns	98.0±5.0 ns
Types of motility	After treatment	Rapid progressive	Rapid progressive	Sluggish	Sluggish
	Recovery	Rapid progressive	Rapid progressive	Progressive	Mild progressive

**P<0.01 significantly different from vehicle control; ns=Non- significantly different from vehicle control. Figures in parenthesis are % increase (+) or decrease (−) over vehicle control; EEFL= ethanolic extracts of *F. limonia* fruit pulp.

Table 3
Effect of ethanolic extracts of *F. limonia* fruit pulp on the contents of epididymal protein, seminal vesicular fructose and the testicular cholesterol, ascorbic acid and protein contents in the rats after 55 days of treatment and 56-110 days after withdrawal of the treatment.

Parameters		Treatment design			
		Group-1 (Normal 10 mL/kg body weight)	Group-2 (Vehicle 10 mL/kg body weight)	Group-3 (EEF 250 mg/kg body weight)	Group-4 (EEF 500 mg/kg body weight)
Seminal vesicular fructose (mg/g)	After treatment	4.80±0.23	4.90±0.22	3.90±0.13** (−20.40)	3.00±0.12** (−38.77)
	Recovery	4.80±0.02	4.80±0.04	4.80±0.02 ns	4.70±0.12 ns
Epididymal protein (mg/g)	After treatment	218.53±4.67	215.38±5.86	186.16±2.24** (−13.36)	162.42±2.24** (−24.58)
	Recovery	216.43±2.12	214.14±3.35	210.42±1.66ns	210.72±3.44ns
Testicular protein (mg/g of testis)	After treatment	186.30±2.40	188.50±1.70	145.60±1.30** (−22.75)	132.20±1.40** (−29.86)
	Recovery	191.50±1.40	190.60±1.80	188.40±0.40ns	187.90±1.20ns
Testicular cholesterol (μg/mg of tissue)	After treatment	87.26±2.34	88.43±2.42	123.37±2.54** (+39.51)	135.83±2.31** (+53.60)
	Recovery	88. 60±1.26	89.12±1.38	87.92±1.63ns	87.48±1.16ns
Testicular ascorbic acid (μg/mg of tissue)	After treatment	138.61±2.17	137.83±1.36	181.76±3.15** (+31.87)	197.62±2.46** (+43.37)
	Recovery	139.42±1.24	138.34±1.53	136.87±1.27 ns	136.68±0.52 ns

**P<0.01 significantly different from vehicle control; ns=Non- significantly different from vehicle control. Figures in parenthesis are % increase (+) or decrease (−) over vehicle control; EEFL= ethanolic extracts of *F. limonia* fruit pulp.

3.4. Effect on epididymal sperm count and motility

The cauda epididymal sperm count was significantly reduced ($P < 0.01$) in that were treated with ethanolic extract of *F. limonia* fruit pulp at both doses (Table 2). Thus, in comparison with the vehicle control group [(64.8±3.6)×10^6 sperm/mL], the groups treated with extract at the doses of

Table 4
Effect of ethanolic extracts of *F. limonia* fruit pulp on the activities of Δ5-3β-HSD and G-6-PDH in testis of rats after 55 days of treatment and 56-110 days after withdrawal of the treatment.

Treatment design	Specific activity of Δ5-3β-HSD (U/mg of protein)		Specific activity of G-6-PDH (U/mg of protein)	
	After treatment	Recovery	After treatment	Recovery
Group-1 (Normal 10 mL/kg body weight)	8.52±0.06	7.68±0.26	23.22±0.34	23.47±0.16
Group-2 (Vehicle 10 mL/kg body weight)	8.27±0.12	7.52±0.15	23.14±0.12	23.82±0.31
Group-3 (EEF250 mg/kg body weight)	6.36±0.12** (-23.09)	7.23±0.42 ns	18.42±0.13** (-20.39)	23.30±0.03 ns
Group-4 (EEF500 mg/kg body weight)	5.12±0.17** (-38.08)	7.35±0.12 ns	13.23±0.21** (-42.82)	23.19±0.21 ns

**$P<0.01$ significantly different from vehicle control; ns=Non- significantly different from vehicle control. Figures in parenthesis are % increase (+) or decrease (-) over vehicle control; EEFL= ethanolic extracts of *F. limonia* fruit pulp.

Table 5
Effect of ethanolic extracts of *F. limonia* fruit pulp on hematological parameters in male rats after 55 days of treatment.

Parameters	Treatment design			
	Group-1 (Normal 10 mL/kg body weight)	Group-2 (Vehicle 10 mL/kg body weight)	Group-3 (EEF 250 mg/kg body weight)	Group-4 (EEF 500 mg/kg body weight)
Hemoglobin(g %)	12.72±0.46	12.44±0.32	12.94± 0.13 (+4.01)	13.83±0.22 (+3.13)
RBCs count (million/cu.mm)	5.51±0.12	5.57±0.04	5.72±0.03 (+2.69)	5.76±0.03 (+3.41)
WBCs count (thousands/cu.mm)	4.26±0.42	4.34±0.56	4.47± 0.36(+2.99)	4.53±0.82 (+4.37)
Blood sugar (mg/dL)	82.02±1.60	78.03±1.61	65.34±2.72(-16.26)	63.33±5.81 (-18.8)
Blood urea (mg/dL)	34. 30±1.30	31.72±2.52	31.03± 3.15 (+0.03)	33.04±3.03 (+4.16)
Serum cholesterol (mg/dL)	84.20±1.70	83.02±0.54	69.07±1.04(-16.80)	63.03±4.31 (-24.07)
Serum phospholipids (mg/L)	82.30±0.24	79.04±0.63	74.08±0.43(-6.27)	72.02±0.53 (-8.88)
Serum protein (mg/dL)	7.82±0.15	7.86±0.16	8.21± 0.64(+4.45)	8.43±0.09 (+7.25)

Figures in parenthesis are % increase (+) or decrease (-) over control.

250 and 500 mg/kg body weight showed about (47.6±1.8)×10^6 and (33.4±2.7)×10^6 sperm/mL, respectively and gradually restored following 55 days withdrawal of treatment.

In the rats from vehicle control group, cauda epididymal sperm exhibited rapid progressive motility and it was lasted for about 1 h 45 min. But, in the rats treated with *F. limonia* fruit pulp at the doses of 250 and 500 mg/kg body weight the sperm were showing sluggish motility for 47±2 and 38 ±4, respectively. Following withdrawal of the treatment for 55 days, the sperms were actively motile showing forward progression and recovered to normal level (Table 2).

3.5. Effect on seminal vesicular fructose, testicular and epididymal protein contents

Our results showed that, treatment of rats with *F. limonia* fruit pulp at the lower and higher-dose levels for 55 days, significantly ($P<0.01$) reduced the seminal vesicular fructose content in all treated groups, the effect was more marked at higher-dose (Table 3).

A significant reduction in the protein contents of testis (22.75% and 29.86%), and cauda epididymis (13.36% and 24.58%) was observed following the treatment of rats with *F. limonia* fruit pulp at the lower and higher-dose levels, respectively, for 55 days. The above changed parameters were brought to the normal level in testis and cauda epididymis after the withdrawal of the drug (Table 3). However, levels of seminal vesicular fructose, testicular and epididymal protein contents were recovered gradually to control levels after cessation of treatment for 55 days (Table 3).

3.6. Effect on cholesterol and ascorbic acid content

Significant ($P<0.01$) elevation was observed in testicular cholesterol and ascorbic acid content following the treatment of rats with *F. limonia* fruit pulp at the lower and higher-dose levels (Table 3). This effect was more marked at higher-dose. Thus, *F. limonia* fruit pulp extract at the doses of 250 and 500 mg/kg body weight showed the percentage of elevation of testicular cholesterol and ascorbic acid content about 39.51% & 53.60%, and 31.87 & 43.37%, respectively (Table 3) and gradually restored following 55 days withdrawal of treatment.

3.7. Effect on △5-3β-HSD and G-6-PDH activity

The oral administration of *F. limonia* fruit pulp extract at the lower and higher-dose levels for 55 days treated resulted in a significant ($P<0.01$) reduction in the testicular $\triangle^5$-3β-HSD and G-6-PDH levels when compared to vehicle control group. This effect was more marked at higher-dose. Thus, *F. limonia* fruit pulp extract at the doses of 250 and 500 mg/kg body weight showed the percentage of reduction of testicular $\triangle^5$-3β-HSD and G-6-PDH levels about 23.09% & 38.08%, and 20.39% & 42.82%, respectively (Table 4). However, by 55 days of treatment withdrawal, the values recovered to control levels (Table 4).

3.8. Effect on Hematological parameters

No significant differences were found in the mean number of RBC and WBC, level of hemoglobin and in hematocrit value in *F. limonia* fruit pulp extract-treated rats compared

to controls (Table 5). But, a significant percentage of reductions were noted in the levels of blood sugar, serum cholesterol and serum phospholipids in the rats treated with *F. limonia* fruit pulp extract at the both dose levels when compared to vehicle control.

4. Discussion

The present study showed for the first time that the *F. limonia* fruit pulp extract impair reproductive activities in male rats possibly by inhibiting spermatogenesis and steroidogenesis. In our investigation, based on the results of acute oral toxicity study, 10% and 20% of the LD_{50} cut-off value were selected as doses and used for pharmacological screening.

In the present investigation, an insignificant change in the body weight of extracts treated rats when compared with control groups suggest that the tested *F. limonia* fruit pulp extract at both dose levels did not induce any over toxicity to the animals. The male accessory reproductive organs play an important role in the sperm maturation, motility and formation of semen[29]. Thus, in our study, a weight loss of the reproductive organs of the rats after treated with *F. limonia* fruit pulp extract could suggest a disturbance of the reproductive endocrine functions.

It is well established that, sperm count is one of the most sensitive tests for spermatogenesis and it is highly correlated with fertility[30]. A reduction in sperm count suggests alterations in sperm maturation and sperm production[3]. In our study, a decrease in the sperm count of cauda epididymis following treatment with *F. limonia* fruit pulp extract may be due to inhibition of the spermatogenesis.

The alterations in motility, viability and morphology of spermatozoa in treated rats are likely the result of adverse effect of the treatment on epididymal functions[31]. Inadequate concentration, sluggishly motile or immotile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova[32,33].

In our study, protein content in the testes and epididymis was significantly reduced with *F. limonia* fruit pulp extract, which might be a causative factor in reducing the weight of reproductive organs, sperm count and motility[2,34]. A marked reduction in the level of seminal vesicular fructose in the in *F. limonia* fruit pulp extract-treated rats may be another cause of reduction in sperm motility as motile sperm consume fructose after ejaculation[35], which provided energy for sperm motility.

Cholesterol and ascorbic acid, the principal precursor for the formation of androgens in biogenic pathway in the testis and involved in steroidogenesis in the testes[36-38]. In our study, an increased level of testicular cholesterol and ascorbic acid in rats treated with *F. limonia* fruit pulp extract resulting in impaired spermatogenesis[39]. Testicular inhibitory action was further strengthened by the inhibition of testicular $\triangle^5$-3β-HSD and G-6-PDH activities in rats after treated with *F. limonia* fruit pulp extract, as the $\triangle^5$-3β-HSD and G-6-PDH are the key enzymes involved in androgen biogenesis[34,40].

Nontoxicity of extract of *F. limonia* fruit pulp is further supported by the data obtained after examination of haematological parameters, which remained unaltered even at the higher dose. After withdrawal of the extract for a period of 55 days, the weight of reproductive organs, sperm count, motility, viability, morphology, testicular biochemicals and enzymes of the extract-treated male rats were similar to those of the vehicle-treated control group, which suggested that the impacts of *F. limonia* fruit pulp extract on male reproductive functions were reversible.

The present findings indicate that *F. limonia* fruit pulp may have reversible antispermatogenic and antisteroidogenic properties, and could then partially support the scientific rationale for the traditional use of this plant in inducing sterility in male.

Conflict of interest statement

We declare that we have no conflict of interest.

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