

Research Article

Fertility Enhancing Effect of Saponin Rich Butanol Extracts of *Tribulus terrestris* Fruits in Male Albino Rats

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ABSTRACT

The fertility ability of saponin rich butanol extracts of *Tribulus terrestris* fruits against 2,3,7,8 tetrachlorodibenzo-p dioxin (TCDD) induced infertility in male albino rats were examined. The rats were injected with 40µg TCDD/kg for one week. After ensuring the oligospermic condition the animals were divided into 5 groups, namely, Group I (Normal control) was administered only with the vehicle (2% CMC suspension). Group II served as negative control received TCDD alone. Groups III-IV was treated with SFTT at 200 mg/kg and 400mg/kg respectively. Group V served as standard and treated with clomiphene 10mg/kg. Drugs were administered daily using oral dosing needle continually up to 28 days. The administration of the SFTT for 28 days significantly ($P<0.01$) increased the relative weights of the total body weight as well as reproductive organs. The SFTT caused significant ($P<0.01$) increases in sperm motility, sperm count and sperm morphology. The SFTT treatment also reduced the levels of FSH and LH. It subsequently increased the level of testosterone values. The haematological and biochemical parameters were also investigated which revealed that there is a significant difference in the creatinine and serum cholesterol values. These results suggest that SFTT might be a potential agent offering protection against the development of TCDD-induced infertility.

Keywords: *Tribulus terrestris*, TCDD, Male rats

INTRODUCTION

In our society sterility is a taboo subject till today. It is primarily a male factor in 40% of these couples and in an additional 20% of these couples; it is a combination of male and female factors. Therefore, when a couple is having trouble in conceiving it makes sense not only to evaluate the woman but to evaluate the man as well.¹ In general, the exposure of human to environmental pollutants will adversely affect male reproductive organs.² The most important groups are the dioxins which is the polychlorinated aromatic hydrocarbons that produces the toxic responses and induce adverse effects in humans and animals, including cancer, reproductive and developmental effects, immunotoxicity, and cardiovascular disease.³ Recent studies have demonstrated that oxidative stress is an important constituent in the mechanism of TCDD toxicity.⁴ 2,3,7,8 tetrachlorodibenzo-p dioxin is a well known dioxin produced during the municipal garbage incineration process and also released as the by-product from gasoline and the pulp industries. Recently, it has received much attention worldwide as an endocrine disrupter.⁵ The increased production of reactive oxygen species, lipid peroxidation, and DNA and membrane damage are always associated with TCDD exposure.⁶ The lipophilicity of TCDD combined with its low rate of metabolism leads to its accumulation and persistence in adipose tissue and cause the reproductive disorders.⁷ The aryl hydrocarbon receptors are the dreadful mechanism

that leads to the toxicity of TCDD.⁸ The interaction of the transformed AhR complex with a specific dioxin responsive element (DRE) of the nuclear DNA elicits the activation of certain genes, including CYP1A1, in the cytochrome P450 superfamily, this gene is responsible for the development of subsequent toxicity.^{9,10,11} In particular, the induction of CYP1A1 leads to excessive generation of reactive oxygen species as a result of the depletion of cellular antioxidants.^{12,13} Male rats exposed to TCDD showed reduced testicular and epididymus weight. There was also a drastic change in the daily sperm production and cauda epididymal sperm counts. TCDD has been reported to produce the epididymis-specific decrease in cauda epididymal sperm counts. The high doses of TCDD lead to an inhibition of spermatogenesis and of 3β-hydroxysteroid dehydrogenase activity in Leydig cells.¹⁴ Herbal medicines have been extensively used in developed countries hence they are natural and relatively safe.¹⁵ Saponin-containing herbs possess a broad range of bioactivities and have been commonly used in folk medicine for their health-promoting properties. *Tribulus terrestris* L. is one such saponin-containing herb used from high antiquity to energize, vitalize, and improve sexual function and physical performance in men.¹⁶ *Tribulus terrestris* is a flowering plant in the family Zygophyllaceae, native to warm temperate and tropical regions of the Old World in southern Europe, southern

Asia, throughout Africa, and Australia. It can thrive even in desert climates and poor soil. Like many weedy species, this plant has many common names, including puncturevine, caltrop, cathead, yellow vine, goathead, burra gokharu and bindii. *Tribulus terrestris* contain substance like Dioscin, protodioscin and iosgenina compounds responsible for curing number of sexual disorders.¹⁷ It also increases levels of various hormones in the steroid family, including testosterone, DHEA and estrogen.¹⁸ In the present study we report the isolation of n-ButoH extract of *Tribulus Terrestris* and their enhancement in the physical characteristic of semen parameter

studies. The inbred animals were taken from animal house

Table 1: Effects of SFTT extract on Body and organ weights male rats

S.No.		1	2	3	4	5
Group		Normal	Control	Test I	Test II	Standard
Treatment and dose		2% CMC 5ml/kg	40µg TCDD/kg	SFTT 200mg/kg	SFTT 400mg/kg	Clomiphene 10mg/kg
Body weight (gm)	Initial	167± 10.21	176±10.18	182±11.61	179±12.44	185±10.52
	Final	236± 10.63*	204±9.86	244±10.12**	256±12.54**	234±10.66*
Testes (mg/100g B.W.)		845±4.76**	715± 4.03	905± 5.27**	928± 6.44**	862± 4.32*
Epididymides (mg/100g B.W.)		322±7.75*	295± 9.26	347± 11.16*	382± 10.12**	335± 9.60*
Seminal vesicle (mg/100g B.W.)		367.21±7.72**	288.86±6.15	394.22±9.44**	412.53±8.25**	377.81±7.12**
Ventral Prostate (mg/100g B.W.)		172±5.55*	145±4.12	202±5.92**	218±5.40**	204±4.07**
Vas deferens (mg/100g B.W.)		60± 0.40**	45± 1.47	78± 1.89**	85± 1.47**	81± 1.22**

Data are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$ significant level compared with the control group.

in Vel's College of pharmacy Chennai, India. The animals were maintained in well ventilated room temperature with natural 12 ± 1 h day–night cycle in the propylene cages. They were fed with balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai, India and tap water *ad libitum* was provided throughout the experimental period. The animals were sheltered for one week prior to the experiment so they get acclimatized to laboratory temperature. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline.

Acute toxicity: Acute oral toxicity study was conducted with SFTT extracts in Wister albino rats according to the guidelines of Organization for Economic Co-operation and Development (OECD, 425)²⁰. The Saponin fraction of *Tribulus terrestris* (SFTT) extract was suspended in 2% (v/v) CMC solution. Healthy rats of either sex, weighing between 120-250g were divided in groups of 6 (3male + 3female). The extract was administered by gavages at doses of 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0, g/kg body weight in single doses to both female and male rats. The animals were observed for general behavioral changes, signs of toxicity and mortality continuously for 1h after treatment, then intermittently for 4h, and there after over a period of 24 h. The rats were further observed for up to 14 days following treatment for behavioral changes and signs of toxicity /death and the latency of death. Mortality rate was recorded after 24 hours.

Subacute toxicity study: The sub-acute oral toxicity study was conducted according to the guidelines of the Organization for Economic Co-operation and Development (OECD, 407)²¹. Twenty four animals were randomly divided into four groups of six animals each. Group I served as control and received a suspension of 2ml/Kg CMC *p.o.* (2% v/v of CMC) Group II- Animals received 100mg/Kg body weight of SFTT in 2ml/Kg of 2% CMC *p.o.* daily for 28 days. Group III: Animals received 200mg/Kg body weight of SFTT in 2ml/Kg of 2% CMC *p.o.* daily for 28 days. Group -IV: Animals received 400mg/Kg body weight of SFTT in 2ml/Kg of 2% CMC

MATERIALS AND METHODS

Collection of plant material: The ripe fruits of *Tribulus terrestris* were collected from the Sairam siddha Medical College and research center India and were authenticated by Dr. Sankaranarayanan, Assistant Director, Dept of Research and Development, of the same college. The voucher specimen is also available in herbarium file of the same center.

Preparation of Saponin rich Fraction of *Tribulus terrestris*: The saponins isolation was carried out according to the method of Kostova et al., 2002.¹⁹ The fruits of *Tribulus terrestris* (500 g) were shade-dried and pulverized to a coarse powder and was extracted with 70% ethanol (3 × 24 h) at room temperature. The combined ethanol solutions were concentrated to a small volume and extracted in succession with chloroform (3 × 24 h) and n-BuOH (3 × 24 h). The n-BuOH layer was concentrated to dryness to give crude saponin extract. The presences of saponins were confirmed by frothing test (0.5 ml filtrate + 5 ml distilled water). The saponins content was calculated as, a percentage of total saponins=weight of residue ×100/weight of sample taken. The yield of the saponins prepared by the above procedure was found to be 0.34 % (w/w). All the extracts obtained were stored at 4°C in air tight containers until assay.

Experimental animals: Adult albino male rats of Wister strain weighing 120 - 250 g were used for the toxicological

Table 2: Effect of SFTT extract on Testicular cell population dynamics in male rats.

S. No.	Group	1	2	3	4	5
Treatment and dose		Normal 2% CMC 5ml/kg	Control 40µg TCDD/kg	Test I SFTT 200mg/kg	Test II SFTT 400mg/kg	Standard Clomiphene 10mg/kg
Germinal cell types	Spermatogonia primary	14.65±1.62**	08.14± 1.89	27.28± 1.76**	32.44± 2.00**	25.91± 0.98**
	Spermatocyte secondary	9.88±1.04*	6.51± 0.45	15.52± 0.70**	21.66± 0.82**	17.52± 0.78**
	Spermatocyte Spermatids	13.54±1.16	11.21± 3.11	48.32± 3.35**	56.94± 4.17**	44.16± 2.85**
	Fibroblast	34.52±1.20	27.18±0.88	77.10±0.62**	102.10±5.72**	97.18±4.99**
	Mature Leydig cell	30.02±1.11	25.15±0.98	46.32±1.22*	62.90±1.46**	55.12±1.94**
Interstitial cell type	Immature Leydig cell	32.12±2.11**	18.05±1.52	41.25±1.34**	52.10±1.22**	54.55±2.02**
	Degenerating cell	41.20±1.01**	22.42±0.92	62.13±1.40**	74.26±1.32**	68.45±1.56**
		58.23±4.54**	35.12 ± 1.33	24.48 ± 1.46*	16.36 ± 0.98**	18.11 ± 1.72**

Data are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$ significant level compared with the control group.

Table 3: Effect of SFTT extract on Semen parameters

S. No.	Group	Treatment and dose	Sperm Count (10 ⁶ /ml)	Sperm Motility (%)	Morphology (% Normal)
1.	Normal	2% CMC 5ml/kg	14.92±0.66**	71.26±0.57**	72.26 ± 2.31**
2.	Control	40µg TCDD/kg	8.71±0.52	55.32±0.36	36.03 ± 1.42
3.	Test I	SFTT 200mg/kg	21.10±0.87**	74.98±0.68**	74.08 ± 2.48**
4.	Test II	SFTT 400mg/kg	25.96±0.62**	80.26±0.70**	82.16 ± 2.62**
5.	Standard	Clomiphene 10mg/kg	19.84±0.44**	72.45±0.61**	78.30 ± 2.76**

Data are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$ significant level compared with the control group

p.o daily for 28 days. Food and water intake was recorded daily; body weight was recorded once in a week throughout the study period.

Induction of oligospermia by TCDD in rats: Group of rats were injected with 40µg TCDD/kg i.p. After one week TCDD exposure, a rat from each group was selected and tested for induction of oligospermia and after ensuring the oligospermic conditions animal grouping was done for further treatment.

Animal grouping and Treatment pattern: Thirty adult male rats were randomly divided into five groups of six animals each.

Group I (Normal control) was administered only with the vehicle (2% CMC suspension)

Group II served as negative control received TCDD alone
Groups III-IV was treated with SFTT at 200 mg/kg and 400mg/kg respectively.

Group V served as standard and treated with clomiphene 10mg/kg *p.o*.

Two rats from each treatment group were randomly sacrificed after 14 days of SFTT administration while the remaining animals were treated continually up to 28 days. Drugs were administered daily using oral dosing needle and twenty four hours after the last dose, blood samples from all the groups was collected and sacrificed. All procedures regarding handling of the test animals were in accordance with the existing CPCSEA and IAEC guidelines.

Blood sample and organ collection: After the last dosing of SFTT, all the animals were sacrificed by employing euthenesia procedure and the testes, epididymides, vas

deferens, seminal vesicles and ventral prostates were identified, dissected out, blotted free of blood and cleared of connective tissue or fat. The organs were weighed immediately using an electronic digital balance. Blood samples were collected by retro-orbital puncture into anticoagulant pre-coated and also in plain sterile eppendorff tubes and allowed to clot at room temperature. Serum samples was separated by centrifugation at 3000 rpm for 10 min and stored at -20°C for hormone assay. Anticoagulant added blood samples were used for the studying haematological parameters.

Sperm collection and Measurement of sperm parameters: To prepare sperm suspension, the connective tissue capsule around the cauda epididymidis was teased out and the epididymal duct was uncoiled and spermatozoa expressed out by cutting the distal end of the cauda epididymal tubule approximately into 1.0mm³ pieces and several incisions were made on it to allow sperm swim out using a sharp razor blade and the spermatozoa with epididymal fluid diluted with pre-warmed beaker containing 2 ml of physiological saline (maintained at 37°C) was placed on a thin glass slide. Sperm characteristics were evaluated for sperm count, motility, viability and morphology.

The caudal epididymal spermatozoa count: The sperm count was determined with an improved Neubauer haemocytometer and viability was assessed by eosin-nigrosin dye exclusion test under the light microscope at 100x magnification. Aliquots of the sperm suspension were placed on both sides of the new improved Neubauer's hemocytometer and allowed to settle by keeping in a humid chamber for 1 hour. The number of spermatozoa per

Table 4: Effect of SFTT extract on Hormone levels

S. No.	Group	Treatment and dose	FSH (mu/ml)	LH (mu/ml)	Testosterone (ng/ml)
1.	Normal	2% CMC 5ml/kg	1.94 ± 0.08**	0.72 ± 0.05**	3.72 ± 0.27*
2.	Control	40µg TCDD/kg i.p.	2.78 ± 0.04	1.06 ± 0.05	4.60 ± 0.24
3.	Test I	SFTT 200mg/kg	0.56 ± 0.06**	0.48 ± 0.03**	5.15±0.30*
4.	Test II	SFTT 400mg/kg	0.47 ± 0.04**	0.36 ± 0.03**	6.06±0.41**
5.	Standard	Clomiphene 10mg/kg	0.61 ± 0.05**	0.52 ± 0.04**	6.10±0.62**

Data are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$ significant level compared with the control group.

mililitre in the appropriate squares of the haemocytometer was counted under the phase contrast light microscope at 100X magnification. Six counts were taken provided the difference between the two counts did not exceed 10% and the average of all the six counts taken as one observation. The count is expressed as million/ml of suspension. Epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility.

Sperm morphology assessment: Caudal sperm were taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin. Sperm morphology was done using the Wall and Ewas stain and determined by examining stained smears in wet preparations using phase contrast light microscope at 400X magnification and their percentages were calculated.

The sperm cells were categorized as follows:

- Normal head and tail
- Isolated heads (whether the head was misshapen or not)
- Head- only defects (i.e., misshapen head with normal tail)
- Tail defects (i.e., normal head with abnormal tail or misshapen head with abnormal tail)
- Fused sperm and was expressed as a percentage of morphologically normal sperm.

Haematological and biochemical parameters: Blood samples were used to investigate Hemoglobin (gm %), RBCs count (million/cu.mm), WBCs count (thousands/cu.mm), Blood sugar (mg/dL), Blood urea (mg/dL), Serum cholesterol (mg/dL), Creatinine (mg/dL) and Serum protein (mg/dL) using the standard method.

Hormone assays

FSH, LH and Testosterone hormones: Blood samples were spun at 2500rpm for 10 minutes in a table top centrifuge. The serum samples obtained were analyzed to determine the concentration of FSH, LH and Testosterone hormones were measured with immunoradiometric assay. The concentration of LH, FSH and Testosterone hormones in blood sample was measured by special LH and FSH hormones kits using ELISA (Enzyme-Linked Immunosorbant Assay) method. Also, the concentration of Testosterone hormone was measured by testosterone kit using ELISA method.

Organ Harvesting and Tissue processing for histological analysis: Both testes were carefully dissected out, trimmed of all fat and blotted dry to remove any blood. The raw testicular weights were noted and volume displaced measured using a 10ml measuring cylinder. In addition, the sizes (length and width) were recorded by use of a sliding gauge ($d = 0.1$) before fixation in a freshly prepared 10% formalin solution. Tissues were transferred and fixed into graded alcohol and then processed for 17.5 hours in an

automated Shandon processor after which they passed through a mixture of equal concentration of xylene. Following clearance in xylene the sections were then infiltrated and embedded in molten paraffin wax. However, prior to embedding, the mounted sections were orientated perpendicular to the long axes of the testes before ensure cutting with the aid of the rotary microtome. These sections were designated as "vertical sections". Serial sections of 5 µ m thickness were cut, floated onto clean slides coated with Mayer's egg albumin for proper cementing of the sections to the slides and were then stained with Haematoxylin and Eosin stains.

Statistical Analysis: The weight data were expressed in Mean ± S.E.M (Standard Error of Mean). Statistical analyses were done by using the student t-test and ANOVA using INSTAT V3 Version 3.0 for Windows-a computer software programme. Results were considered significant at 5% level of probability ($P < 0.05$).

RESULTS

Effect of SFTT extract on body weight and the reproductive organs: In the present study, administration of the SFTT for 28 days significantly ($P < 0.01$) increased the relative weights of the total body weight as well as reproductive organs. There were linear increases in the body weights of both the normal and treatment groups. In SFTT treated animals it was found that sperm numbers in testis were significantly higher than in the normal controls. The final body, testis and epididymis weights of all groups were statistically different to those of the controls. Administration of SFTT caused remarkable changes in the weight of the seminal vesicle and prostate gland. However, testicular weight was increased significantly ($P < 0.01$) after SFTT treatment in a dose and duration dependent manner when compared with the control.

It has been reported that male rats exposed to TCDD show adverse effects such as weight loss of the testicles, seminal vesicles and prostate glands, and changes in the form of the testes. In the present study, investigation was made for the ameliorative effect of SFTT on the toxicity of TCDD in rats. TCDD (40 µg/kg) caused a significant decrease the weights of testes and epididymis. On the other hands, testicular weight in SFTT treated rats was greater than in non-treated controls and significant increased sperm production. These results suggest that SFTT promotes testicular development, and thus, enhances sperm production

Effect of SFTT extract on testicular cell population: The two types of testicular cell population like germinal cell type and interstitial cell type showed high significant level when compared with the control. The SFTT treatment with 200mg/kg showed a atpar values when compared with the

Table 5: Effect of SFTT extract on Hematological and biochemical parameters

Parameter	Normal CMC 5ml/kg	2% Control 40µg TCDD/kg	Test I FFTT 200mg/kg	Test II FFTT 400mg/kg	Standard Clomiphene 10mg/kg
Red blood cell (mm ³)	7.80±0.52	7.85±0.40	7.54±0.64	7.88±0.42	7.80±0.52
HB (%)	52.60±1.10	55.33±0.87	58.08±0.80*	61.17±0.52*	52.60±1.10
Leukocyte (x10 ⁶ /mL)	10142±202.44*	8485±140	14735±242**	16120±319**	12146±128.48**
Blood glucose(mg/dl)	100.12±3.42	98.10±4.12	89.52±5.48	92.84±6.25	100.12±3.42
Urea(mg/dL)	60.48±2.72	62.50±4.18	70.12±8.12*	71.14±7.22*	60.48±2.72
Creatinine (mg/dL)	1.62±0.12**	1.80±0.21	1.56±0.19**	1.48±0.22**	1.62±0.12**
T. cholestrol(mg/dL)	38.45±4.00	40.42±5.82	36.21±4.42	34.02±3.60	39.45±4.00
Total Protein(g/dl)	8.10±0.40	8.12±0.38	8.10±0.42	8.18±0.32	8.10±0.40
Albumin(g/dl)	3.20±0.22	3.14±0.20	3.60±0.22	3.42±0.24	3.20±0.22
Globulin(g/dl)	4.12±0.60	4.16±0.54	4.20±0.43	4.81±0.38	4.16±0.42

Data are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$ significant level compared with the control group.

standard group. The SFTT treatment with 400mg/kg revealed the high significant levels $p < 0.01$ when compared with the standard groups. So the treatment of SFTT on male rats has given better results in the production of testicular cell population.

Effect of SFTT extract on Sperm morphology and spermatogenesis: 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 TCDD exhibited higher percentage of abnormality than those treated with SFTT. However, the percentage of the normal sperm gradually reverted to control levels after cessation of treatment for 30 days.

It is known that a major function of the epididymis is sperm maturation which leads to the acquisition of fertilizing ability and viability of spermatozoa. Therefore, improvement in the activities of the epididymis could have led to an increase in progressive motility of sperm in the experimental rats. The increased sperm count and motility thereby shows that treatment with SFTT improves and enhances the fertilizing capacity of the Semen. These qualities were often used as a measure of sperm production, testicular function and or male fertility.

Effect of SFTT extract on epididymal sperm count and motility: The SFTT caused significant ($P < 0.01$) increases in sperm motility and sperm count. Furthermore, the percentages of abnormal sperm cells in treatment groups were significantly different from the control. Caudal epididymal sperm count and motility were significantly increased ($P < 0.01$) after SFTT treatment compared with the control. The datas are confirmed by our observation on the increased sperm counts, motility. In the treated groups, there was a significant excess in the number of primary and secondary spermatocytes and round spermatids, similarly, the number of the sertoli cells and spermatogonia was observed more comparatively.

The significant increase in the absolute weight of the testis and epididymis could therefore be due to increased androgen biosynthesis as evidenced by a significant

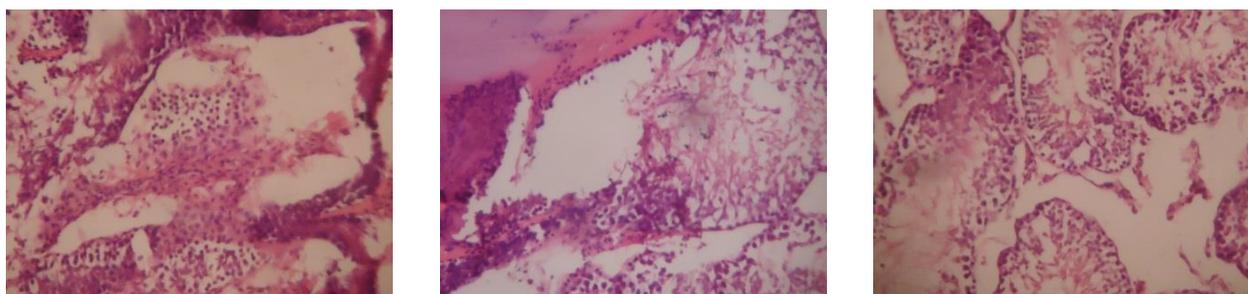
increase in serum testosterone levels in the experimental rats. Androgens have been shown to be necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands and studies have shown that the level is positively correlated with the weight of testis, epididymis, seminal vesicle and prostate glands.

However both increase in sperm motility and decrease in total abnormal sperm rate of rats in high dose group were statistically significant in comparison to the control group. Moreover, an effect is due to testosterone changes alone should have led to an increase in the weight of all accessory organs, it is therefore justifiable that the increased weight of the testis and epididymis reflects a dual effect of increased testosterone levels and sperm contained in these organs.

Effect of SFTT extract on Hormone levels: Testosterone is known to be critically involved in the development of sperm cells and derangement results widely in leydig cell dysfunction and testicular steroidogenic disorder. In males, testosterone is primarily synthesized in Leydig cells. The number of Leydig cells in turn is under the control of LH and FSH. Therefore, reductions in the levels of FSH and LH by the SFTT suggest beneficial effect on the number of Leydig cells, which in turn might have initiated the synthesis and release of testosterone. The observed increase in the sperm functions in the SFTT administered rat could be attributed to favourable and increased spermatogenic activities as results of high testosterone levels.

Effect of SFTT extract on Hematological and biochemical parameters: No significant differences were found in the mean number of RBC and Hb level in SFTT treated rats. A significant percentage of reduction was noted in the level of creatinine and serum cholesterol and serum protein levels were decreased but it was statistically not significant in the rats at the both dose levels when compared to control.

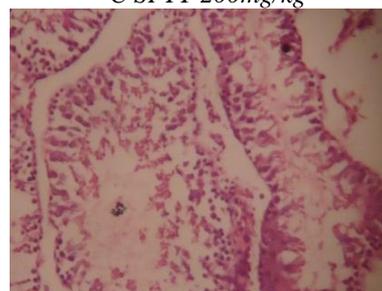
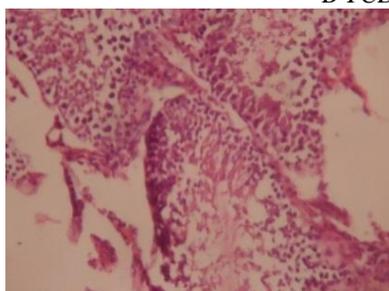
Effects of SFTT extract on Histopathology: After histological research of removed organs and evaluation it was stated that in the testicles of control group rats the



A Normal

B TCDD alone

C SFTT 200mg/kg



D SFTT 400mg/kg

E Standard

Figure 1(A-E): Photomicrograph showing effect of SFTT extract on testis in normal (A), TCDD alone (B), SFTT 200mg/kg (C), SFTT 400mg/kg (D) and Standard (E) administered rats for 28 days

incisions of the spermatic tubules were clearly seen. Between the tubules thin interstitial tissue with blood vessels and leydig cells are seen. The tubules are surrounded by connective tissue properia, from which a thin basic membrane stretches inside, on which spermatogenic epithelium is fixed. Spermatogonia are small round cells.

It is noticeable that the spermatic tubules are different in their structure depending on the phase of spermatogenesis, while penetrating inside the spermatic tubules, behind the spermatogonia in the initial stage of spermatogenesis primary spermatocytes can be well seen. These are biggest cells of spermatic tubules. The spermatozoids are accumulated in the radius of tubules with their prolonged heads sticking out from the cytoplasm of the Sertoli cells and their tails directed towards the radius of the tubules. In the histological preparations in treated groups shows a significant increased in the numbers of primary, secondary spermatocytes and spermatids could be distinguished in comparison with control group.

DISCUSSION

Androgen is essential for most of the stages of spermatogenesis, meiosis in particular. The three male sex steroids or androgens, namely are testosterone, dihydrotestosterone and estradiol. From a quantitative perspective, the most important androgen is testosterone. More than 95% of this hormone is produced by Leydig cells, with the rest produced by the adrenal glands. To supplement testosterone, the testes also produces a small amount of potent form of androgen called dihydrotestosterone (DHT), as well as weaker forms such as dehydroepiandrosterone (DHEA) and androstenedione. These cells also secrete a small quantity of estradiol, estrone, pregnenolone, and progesterone, and 17-alpha-hydroxyprogesterone. Sperm production cannot proceed

optimally to completion, without a continuous androgen supply. Interference of testosterone production leads to atrophy of the organ and impairment of spermatogenesis.²² It has also been reported that reducing testicular weight and maturational arrest of the primary spermatocyte manifest androgen deficiency.²³

The results of the present study suggested that SFTT have a beneficial effect on male reproductive functions in rats. SFTT alter reproductive functions in male and improve the quality of spermatozoa. Sperm count, motility, viability and morphology are parameters considered as the determinants of the fertilizing capacity of sperm cells. Results of this study, which revealed significant increases in sperm parameters in the treatment groups, might lend credence to the potential of the SFTT to enhance the fertilizing ability of sperm cells. This was particularly based on the observation of the daily body weight increase and liver and kidney weights to body weight ratio which are not significantly different with the control. Although there was a slight increase in the reproductive organ weight to body weight ratio, reproductive organs in general were unaffected by oral administration of the SFTT. There was an obvious improvement in the spermatogenic activity.

The results also indicated that, the lumen of the seminiferous tubules was found to contain enormous number of spermatozoa and enhanced motilities compared with control group. Sertoli cells were found to enlarge and with dense granulated cytoplasm. Granulated cytoplasm of sertoli cells may provide nourishment to spermatozoans. A significant increase in the number and motility of sperm was observed in experimental groups, which could be due to the influence of the drug on the cell cycle, cell division and expression of genes necessary for the spermatogenesis. It is also possible that these changes might be the result of

changes in the favourable microenvironment of epididymis, thus influencing sperm count and motility. The alkaloids have been implicated in abnormal sperm morphology even in human adults²⁴, but on the other hand, the simple sugars seem to be involved in the observed increase in sperm motility and viability. This is due to the fact that the metabolism of simple sugars like glucose will lead to the production of pyruvate. Pyruvate is a substrate necessary for the activity and survival of sperm cells²⁵

Testosterone is required for the growth, development and maintenance of male reproductive organs²⁶ and in association with follicle-stimulating hormone, acts on the seminiferous tubules to initiate and maintain spermatogenesis. A high dose of 400 mg/kg of SFTT used in this study showed a significant decrease in the serum level of testosterone. It is probable that steroidogenic potentiation was responsible for the use of SFTT as an aphrodisiac in traditional medicine. In this process, protodioscin, increases the level of spermatogenesis by stimulating the Sertoli and germinal cells, resulting in the increased production of sperms. It also improves the conversion of testosterone to DHT, which in turn stimulates the production of Androgen Binding Protein in the Sertoli cells. Increased Androgen Binding Protein production results in increased formation of DHT-ABP complex, which stimulates spermatogenesis in the germinal cells. Another fraction of the DHT-ABP complex is transported to the epididymis, which increases the efficiency of the maturation of spermatozoa into fertile sperms. Increased DHEA level, in turn prevents the loss of epididymal cells which results in the improved spermiogenesis and increased fertility.

Protodioscin also increases the secretion of LH, but not that of FSH. It has been shown to increase the density of the Sertoli cells, without changing the density of the Leydig cells; and increase the number of spermatogonia, spermatocytes and spermatids without affecting the diameter of the seminiferous tubules. Protodioscin is the active ingredient of the plant extract of *Tribulus terrestris* L., is classified as a furostanol saponin, and is present in the extract at no less than 45% of the total weight. Protodioscin is physically characterized as a bitter-tasting amorphous powder, with a yellow-brown color.

The study suggests that SFTT may not have spermatotoxic effect, but rather it could improve sperm performance. Further studies aimed at elucidating the fertility activities of SFTT are worthwhile.

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