

Effect of *Sarcostemma acidum* stem extract on spermatogenesis in male albino rats

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Abstract Aim: To evaluate the possible antifertility activity of *Sarcostemma acidum* (Roxb) Voigt. stem extract in male rats. **Method:** Male rats were given 70% methanol extract of *S. acidum* stem orally at dose levels of 50 and 100 mg/kg/day for 60 days. Fertility was evaluated with mating test. Sperm motility and sperm density in cauda epididymides were also assessed. Biochemical and histological analyses were performed on blood samples and on the reproductive organs. **Results:** *S. acidum* stem extract resulted in an arrest of spermatogenesis without any systemic side effect. Sperm motility as well as sperm density was reduced significantly. Treatment caused a 80% reduction in fertility at the 50 mg dose and complete suppression of fertility at the 100 mg dose. There was no significant change in RBC and WBC count, hemoglobin, haematocrit, sugar and urea in the whole blood and cholesterol, protein and phospholipid in the serum. The protein and glycogen content of the testes, fructose in the seminal vesicle and protein in epididymides were significantly decreased. Cholesterol in the testes was elevated. Treatment at both of the doses caused a marked reduction in the number of primary spermatocytes (preleptotene and pachytene), secondary spermatocytes and spermatids. The number of mature Leydig cells was decreased, and degenerating Leydig cells was increased proportionately. **Conclusion:** *S. acidum* stem extract arrests spermatogenesis in male rats without noticeable side effects. (*Asian J Androl* 2002 Mar; 4 : 43-47)

1 Introduction

Sarcostemma acidum (Roxb) Voigt., a xerophytic plant of the family Asclepiadaceae, has several medicinal uses. Dried stem is an emetic employed in leprosy patients. Roots have been used in snake bite and rabies [1]. The

present study was undertaken to evaluate the effect of *S. acidum* stem extract on spermatogenesis of rats at an aim of developing a male fertility regulating agent of plant origin.

2 Materials and methods

2.1 Extraction of plant material

Sarcostemma acidum stems were collected from Ajmer (India). Shade-dried stems were crushed and pulverized. Five hundred grams of the plant material was boiled in a soxhalate apparatus with 1.5 litre of 70% methanol for 8 hours and was then filtered. The crude extract was obtained after removal of the solvent through vacuum distillation. The dry crude extract yield was 10% (10 g extract/100 g raw material).

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2.2 Animals and treatment

Male albino rats of Sprague-Dawley strain were provided by the animal house of the University. Animals were housed in plastic cages with proper aeration and 25 ; Å 2 ; æ and were maintained on standard rat pellet diet and tap water ad libitum. Male rats of proven fertility were divided at random into 3 groups of 10 animals each. The extract was freshly dissolved in distilled water and given orally through gastric gavage. Control animals received equal amount of vehicle. Group 1: Control; Group 2: Treated 50 mg/kg/day for 60 days; Group 3: Treated 100 mg/kg/day for 60 days.

After 55 days of treatment male rats were cohabited with pro-estrous females in the ratio of 1:2. The presence of sperm in the morning vaginal smear was the evidence of mating. On day 61, i.e., 24 hours after the last dose, animals were sacrificed. Blood was collected by cardiac puncture and serum was separated. Reproductive organs were dissected out and weighed.

2.3 Body weight, sperm motility and sperm density

Body weights of animals were recorded before the experiment and every 2 weeks thereafter. Sperm motility and sperm density were assessed in cauda epididymis by the method of Prasad et al. [2] .

2.4 Blood and serum analysis

Whole blood was analyzed for RBC and WBC count, hemoglobin [3], haematocrit, sugar [4] and urea [5], and serum was analyzed to estimate cholesterol [6], total protein [7], and phospholipids [8].

2.5 Tissue biochemistry

One testis from each rat was kept at -20 ; æ until assayed for cholesterol [9], glycogen [10] and proteins [7]. Fructose was determined in the seminal vesicles [11]. Protein was estimated in epididymides [7].

2.6 Testicular cell dynamics

Testes of each rat were fixed in Bouin's fluid, passed through ascending series of ethanol and then through xylene and, embedded in paraffin wax. Tissues were

sectioned at the thickness of 5 µm and stained with haematoxyline and eosin.

The evaluation of the cell population was based on the calculation made for each cell type per cross section of the seminiferous tubule. The Sertoli cells, spermatogonia, preleptotene and pachytene spermatocytes, secondary spermatocytes and round spermatids were counted under ×100 magnification. The group counts of these cell types were designated as crude counts and these crude counts were corrected by using Abercrombie's formula [12]. Mature and degenerating Leydig cells were counted. Mature Leydig cells are those containing agranular clear cytoplasm and spherical nucleus and degenerating Leydig cell, containing residual bodies or dense bodies in abundance [13]. Leydig cell nuclear area was measured at × 800.

2.7 Statistical analysis

The mean and standard error of mean (SEM) were calculated and the significance of difference analyzed by applying Student's 't' test.

3 Results

3.1 Body weight, fertility and sperm dynamics

During the period of experiment the rats kept healthy, growing at normal growth rate. Their body weight gain was similar to that of control animals (Table 1). Treatment of *S. acidum* stem extract at 50 mg/kg/d for 60 days caused a 80% reduction in the fertility of rats and none of the rats was fertile after 100 mg dose (Table 2). Sperm motility was decreased by 50.5% and 43.4% at 50 and 100 mg dose levels, respectively. Significant reduction ($P<0.01$) was observed in sperm density after both the treatments (Table 3).

3.2 Blood and serum analysis

Blood variables, i.e., RBC and WBC counts, hemoglobin, haematocrit, sugar and urea were within the normal range. Cholesterol and protein did not changed significantly in any of the treatment but phospholipids were decreased only at the 100 mg dose level (Table 4).

Table 1. Effect of *S. acidum* stem extract on change in body weights of the rats. n=10 for each group, mean±SEM.

Treatment	Initial weight (g)	Weight after 2 weeks (g)	Weight after 4 weeks (g)	Weight after 6 weeks (g)	Weight after 8 weeks (g)
Control	218±14	230±18	245±20	256±28	285±16
<i>S.acidum</i> stem 50 mg/kg BW/d	200±18	223±12	242±19	260±21	280±20
<i>S.acidum</i> stem 100 mg/kg BW/d	230±21	248±13	257±14	290±18	305±25

3.3 Biochemical findings

Glycogen and protein contents in the testes were decreased significantly ($P < 0.01$) after treatment with *S. acidum* stem in comparison to the control. Cholesterol level in testes was elevated by 29.2% and 47.3% at 50 and 100 mg dose levels, respectively. Seminal vesicular fructose and epididymal proteins were decreased significantly ($P < 0.01$) after both doses (Table 3).

3.4 Histological observation

In the treated groups, there was a significant reduction in the number of primary and secondary spermatocytes and round spermatids, while the numbers of the spermatogonia and Sertoli cell remained unchanged. There was 49.6% and 40.7% reduction in the Leydig cell nuclear area at 50 and 100 mg doses, respectively. The number of mature Leydig cell was decreased significantly ($P <$

0.01), whereas degenerating cell number was increased in comparison to control animals (Table 5, Figures 1-3).

Table 2. Effect of *S. acidum* stem extract on fertility of the male rats. $n=10$ for each group.

Treatment	No of females cohabited with males (2 females/male)	No of females showed positive mating	Fertility (%)
Control	20	20	100
<i>S. acidum</i> stem 50 mg/kg BW/d	20	14	20
<i>S. acidum</i> stem 100 mg/kg BW/d	20	0	NIL

Table 3. Effect of *S. acidum* stem extract on sperm density and motility and some biochemical markers in the rats. $n=10$ for each group, mean \pm SEM, $^cP < 0.01$ vs control.

Treatment	Sperm motility (%)	Sperm density (million/mL)	Testes cholesterol (mg/g)	Testes protein (mg/g)	Testes glycogen (mg/g)	Seminal vesicle Fructose (mg/g)	Epididymides protein (mg/g)
Control	71.9 \pm 0.7	56.8 \pm 1.5	4.8 \pm 0.3	188 \pm 3	3.8 \pm 0.2	4.5 \pm 0.2	248 \pm 3
<i>S. acidum</i> stem 50 mg/kg BW/d	35.6 \pm 2.9 ^c	14.4 \pm 1.3 ^c	6.2 \pm 0.2 ^c	144 \pm 11 ^c	3.1 \pm 0.1 ^c	2.5 \pm 0.1 ^c	202 \pm 6 ^c
<i>S. acidum</i> stem 100 mg/kg BW/d	40.7 \pm 2.8 ^c	5.8 \pm 1.0 ^c	7.1 \pm 0.5 ^c	144 \pm 6 ^c	2.4 \pm 0.3 ^c	3.0 \pm 0.1 ^c	149 \pm 8 ^c

Table 4. Effect of *S. acidum* stem extract on some components in whole blood and blood serum of rats. $n=10$ for each group, mean \pm SEM, $^aP > 0.05$, $^cP < 0.01$ vs control.

Treatment	RBC count (mill/mm ³)	WBC count (no./mm ³)	Hemoglobin (gm%)	Haematocrit (%)	Blood sugar (mg/dL)	Blood urea (mg/dL)	Serum cholesterol (mg/dL)	Serum phospholipid (L)	Serum protein (mg/dL)
Control	4.7 \pm 0.1	8540 \pm 81	13.4 \pm 0.3	44.6 \pm 4.4	86.0 \pm 1.6	35.6 \pm 2.5	89 \pm 5	86 \pm 6	13530 \pm 594
<i>S. acidum</i> stem 50 mg/kg BW/d	4.7 \pm 0.2 ^a	8317 \pm 78 ^a	11.9 \pm 0.4 ^a	40.1 \pm 4.4 ^a	76.9 \pm 6.3 ^a	42.0 \pm 3.3 ^a	81 \pm 4 ^a	81 \pm 4 ^a	13000 \pm 444
<i>S. acidum</i> stem 100 mg/kg BW/d	4.6 \pm 0.1 ^a	8300 \pm 100 ^a	12.6 \pm 0.2 ^a	43.6 \pm 0.8 ^a	75.5 \pm 8.9 ^a	40.5 \pm 3.0 ^a	69 \pm 6 ^a	55 \pm 4 ^c	11556 \pm 544

Table 5. Effect of *S. acidum* stem extract on spermatogenesis and Leydig cells in the rats. $n=10$, mean \pm SEM, $^bP > 0.05$, $^cP < 0.01$ vs control.

Treatment	Sertoli cell	Spermatogonia	Preleptotenes spermatocytes	Pachytene spermatocytes (No/CS of tubule)	Secondary spermatocytes	Round spermatids	Mature Leydig cells (%)	Degenerating Leydig cells (%)	Leydig cell nuclear area (μ m ²)
Control	3.2 \pm 0.4	5.9 \pm 0.6	19.9 \pm 2.1	24.6 \pm 2.6	44.8 \pm 3.4	32.2 \pm 1.6	63.1 \pm 0.5	36.9 \pm 3.3	28.0 \pm 1.2
<i>S. acidum</i> stem 50 mg/kg BW/d	3.3 \pm 0.2 ^b	3.4 \pm 0.5 ^b	6.9 \pm 0.3 ^c	19.3 \pm 0.1 ^b	25.9 \pm 1.7 ^c	18.5 \pm 2.1 ^c	45.8 \pm 1.3 ^c	54.2 \pm 0.3 ^c	14.1 \pm 0.8 ^c
<i>S. acidum</i> stem 100 mg/kg BW/d	2.7 \pm 0.1 ^b	5.7 \pm 0.4 ^b	12.4 \pm 0.7 ^c	14.3 \pm 1.0 ^c	28.4 \pm 1.7 ^c	12.6 \pm 1.2 ^c	37.9 \pm 2.2 ^c	62.1 \pm 2.1 ^c	16.6 \pm 0.7 ^c

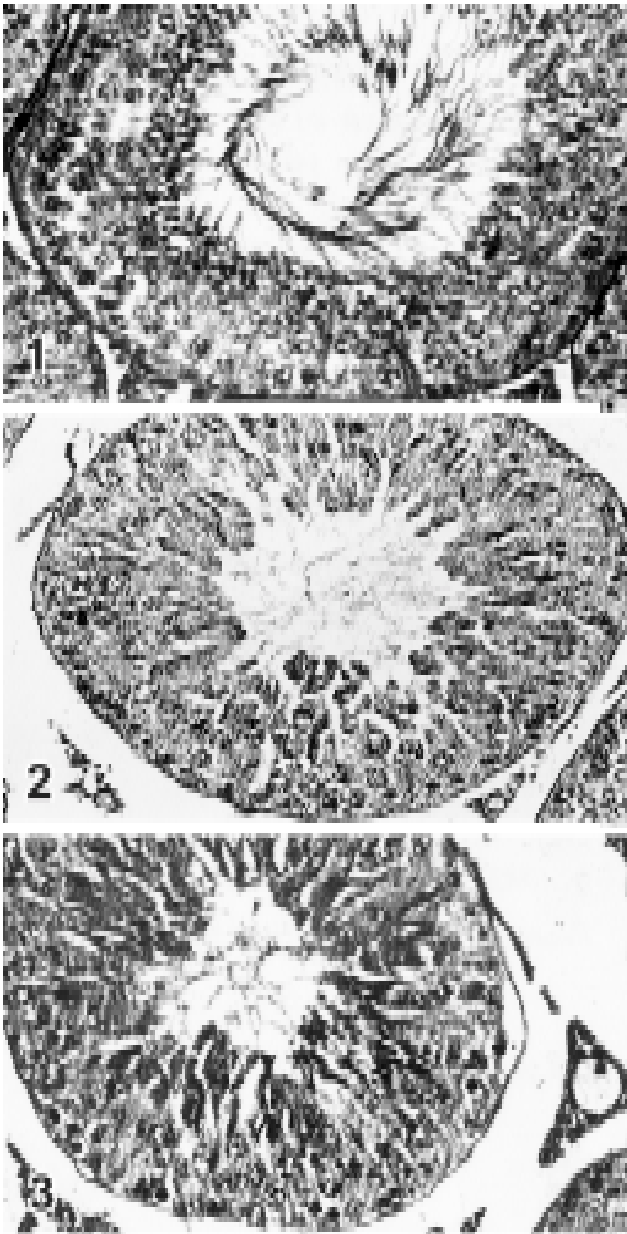


Figure 1. Control rat testis showing normal spermatogenesis. $\times 200$ HE.

Figure 2. *S. acidum* stem extract 50 mg/kg BW/d. Microphotograph of testis showing reduced number of spermatogenic cells. $\times 200$ HE.

Figure 3. *S. acidum* stem extract 100 mg/kg BW/d. Microphotograph of testis showing arrest of spermatogenesis at spermatocyte level and degeneration of Leydig cells. $\times 200$ HE.

4 Discussion

Sarcostemma acidum stem suppressed sperm production, as evidenced by the reduction in the number of spermatogenic cells. Similar results were found by the administration of *Colebrookia oppositifolia* in rats [14] and *Mentha arvensis* in mice [15]. The principal cells of epididymis synthesize proteins which have im-

portant role for maturation of spermatozoa [16]. Alteration in secretion and function of these proteins caused incomplete maturation of spermatozoa with a decline in sperm motility. In the present study the epididymal protein was also decreased by *S. acidum* stem extract feeding. Low fructose concentration may be another cause of low sperm motility. Chinoy and Bhattacharya [17] reported reduced sperm motility after aluminium chloride administration in mice with decreased seminal vesicular fructose, as the latter supplies energy for sperm motility. Reduced glycogen reflects decreased number of post-meiotic germ cells, which are thought to be the sites of glucose metabolism [18].

Cholesterol is involved in steroidogenesis in testes. It is most important precursor in synthesis of steroid hormones and its level is related to fertility of individuals [19]. Increased level of cholesterol may be due to decreased androgen production, which resulted in accumulation of cholesterol in testes, hence impaired spermatogenesis [20].

The impairment of Leydig cell function was evinced by its reduced nuclear area and lower number of mature Leydig cells. The number of mature Leydig cells has a direct bearing on spermatogenesis [21]. Deformation of Leydig cells further indicates the inefficiency of these cells to synthesize testosterone [22].

Reduction in number of spermatogenic cells may be due to insufficient amount of testosterone. Spermatogenesis is activated by testosterone which is synthesized by Leydig cells and act on Sertoli cells, and peritubular cells [23]. Similar results were reported by Gupta et al [21] in the *Barleria prionitis* root extract treated rats. The number of Sertoli cells and spermatogonia (stem cells) were not changed after the treatment, so 100% fertility could be retained after cessation of the treatment.

It has been observed that the blood and serum parameters were within the normal range, indicating non-toxicity of *S. acidum* stem on general body metabolism. Our results reflect antispermatogenic/antiandrogenic effects of *S. acidum* stem in male albino rats, without affecting general body metabolism.

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