ASSESSMENT OF THE EFFICACY OF A POLYHERBAL FORMULATION YOUTHEN ON REPRODUCTIVE PERFORMANCE OF MALE RATS

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ABSTRACT: YOUTHEN is a polyherbal formulation. The present work was designed to establish the effect of YOUTHEN on androgenic and reproductive parameters of male albino rats. YOUTHEN at lower dose stimulated the haemopoietic function significantly. Higher dose of YOUTHEN significantly enhanced the sperm count, forward motility index and metabolic activity of testis spermatozoa of the experimental animals as compared to that of the control rats with marked improvement in sexual behaviour. The weight of accessory sex glands were increased on YOUTHEN treated rats. Concentrations of total protein and phosphomonoesterases were significantly higher, while the concentration of cholesterol in testicular tissues was lower in YOUTHEN treated rats as compared to placebo control group. The results of the present experiment lead to conclude that YOUTHEN increased the libido as well as the quality and quantity of spermatozoa in laboratory animals. The effects of YOUTHEN on haemopoietic system and on the primary and accessory sex organs suggest that the product may have androgenic effect.

INTRODUCTION

Potency is the physical capacity of the entire body to co-ordinate and perform the male’s normal role in coitus. Loss of libido and impotency occur mainly due to hormonal imbalance. This may take place following stressful conditions, chronic alcoholism, consumption of certain drugs, testicular malfunctioning and different gonadal and/or endocrine disturbances.

YOUTHEN* is a compound herbal formulation which contains the extracts of different plant materials (viz. Asparagus racemosus, Sida cordifolia, Pedalium murex, Asparagus adscendens, Mucuna pruriens, Gmelina arboria etc) in their optimum concentrations. These plant materials are reported to possess aphrodisiac action in ayurvedic literature. A similar polyherbal formulation (SAXOM) for veterinary practice was reported to produce stimulant action on male sexual characteristics in laboratory and domesticated animals (1-7). The present experiment was designed to observe the effect of YOUTHEN on reproductive system and performance of laboratory animals.

Materials and Methods

The present study was conducted in two different sets.

Set-1: Adult rats of wistar strain (100-120) days old; 175-200gm body weight were housed in colony cages in an air conditioned room (23 ±10C and 45-55% RH) with a 12 hour light and dark cycle. These were maintained on pellet diet and clean tap water
ad libitum. The animals were randomly allocated into three groups of six animals each. Group I served as vehicle treated control and received distilled water (orally) only for 21 days. The rats of group II and III were treated with YOUTHEN at the dose of 15mg and 30 mg per kg body weight (orally), respectively, dissolved in distilled water, the product was administered once daily for 21 days. All the animals were sacrificed 24 hours after last dose administration by decapitation. Blood samples were collected from each animal before sacrifice. Total Erythrocyte count (TEC), Haemoglobin (Hb) and packed cell Volume (PCV) were estimated using standard methods.

Sperm Count: Epididymis was dissected out quickly. Single cauda of the epididymis was weighed and chopped in 10ml of normal saline solution. The aliquot of sperm suspension was charged in a neubauer haemocytometer diluting in 3.8% sodium citrated (1:20). Sperm count was expressed in terms of number/100mg of cauda epididymis (8).

Sperm motility: Single cauda of the epididymis was punctured with a 21gauge hypodermic needle. Fluid content from the epididymal lumen was collected. Motility of the epididymal spermatozoa was determined in a haemocytometer chamber (9).

Metabolic activity of spermatozoa:

Metabolic activity of epididymal spermatozoa was determined by methylene blue reduction test as described by shastry (10) with some modifications. Spermatozoa from single cauda of epididymis was diluted in phosphate buffer (pH7.2). Cauda was chopped in phosphate buffer (1ml/100mg) and was taken in a small test tube . 0.03% methylene blue solution (in normal saline) was added to the test tube at the rate of 0.1ml/1ml of sperm suspension. Then liquid paraffin was layered over it (0.5 to 1cm thickness). The time taken by the sperm suspension to regain the original colour was recorded using a stoop watch.

Set-2 : Sexual behaviour and biochemical profile:

Sexually active male Wister albino rats ageing between 100-120 days were used in this study. The animals were maintained in similar laboratory condition as stated before. These were divided into three groups (n=8) and treated with vehicle and YOUTHEN for 21 days as stated in the previous set of experiment. Sexual behaviour and biochemical profile of accessory sex organs were studied 24 hours after last dose administration of YOUTHEN.

Sexual behaviour was recorded in a dimly lit room in a specially constructed cage (50 x 30 x 30cm) with glass walls on all the four sides. The experimental rats were allowed to adapt in the cage for 15min. Two female rats treated with estradiole valerate (10 µg; S.C) and hydroxyl progesterone (1.5mg; S.C) 48 hours and 5 hours, prior to experimentation, respectively, were introduced to male rat in the mating cage. Sexual responses such as number of mounts, genital grooming and sniffing at females was visually monitored and recorded for 45 minutes (11).

The experimental animals were then sacrificed. Testes, prostate glands, seminal vesicles and epididymis were dissected out carefully, freed from adhering tissues, blotted and weighed to the nearest 0.1mg. The wet weight of organs was expressed as mg/100gm body weight.
Required amount of tissues from seminal vesicle, prostate and epididymis was homogenized in 0.2m phosphate buffer (pH 7.4) and centrifuged, the supernatant was used for the estimation of acid and alkaline phosphatase (12, 13), fructose (14) and citric acid (15). A portion of testicular tissue was homogenized in isopropyl alcohol (10% w/v). Cholesterol was extracted (overnight at 4c) and estimated (16). Another portion of testicular tissue was homogenized in phosphate buffer (pH 7.4) for the estimation of acid and alkaline phosphatase and total protein (17).

**Acute Toxicity Study**

Graded doses of YOUTHEN were administered orally to groups of ten mice kept at a room temperature of 26 ± 1oC. They were observed continuously for 2 hours and then at hourly intervals for the next 6 hours for gross behavioural changes. The animals were kept under observation for 72 hours to detect mortality, if any.

**Statistical Analysis**

The results of the experiment are expressed as mean ± SEM. Level of significance was calculated following student’s t-test.

**Results**

The results of set-I experiments are shown in Table –I,II. The rats of group II showed increased level of haemoglobin and packed cell volume as compared to control animals. There was a marginal increase on total erythrocyte count in YOUTHEN treated animals. The product did not produce dose-dependent effects. Significant increase in sperm count and forward progressive motility with decrease in methylene blue reduction time were recorded in epididymal spermatozoa collected from the rats of group III as compared to that of control group.

The results of set II experiments are shown in Table-III, IV, V, VI. Total sexual behavior and mounting index were significantly higher in YOUTHEN treated rats as compared to that of control animals. Relative weight of testis did not show any significant alterations in YOUTHEN treated rats (Table-IV)

Biochemical profile of primary and accessory sex organs of the experimental animals are presented in table-V and VI. The acid phosphatase activity was significantly increased in testis and prostate glands at both the doses while that of epididymis and seminal vesicles was increased in higher dose group animals only. The alkaline phosphatase activity was found to be increased significantly in testis, epididymis, prostate glands and seminal vesicles of the rats of group-III. Significant increase in fructose concentration was noted in epididymis, prostate glands and seminal vesicles of YOUTHEN treated rats of both the groups, while citric acid concentration were recorded in the rats of group-II and III as compared to control values.

Acute toxicity studies did not reveal any toxic signs and symptoms in albino mice treated with YOUTHEN (upto 500 mg/kg, orally). No mortality and CNS abnormality was detected in experimental animals, the animals did not show convulsion, tremor ataxia depression, hyperesthesia motor incoordination or any allergic reaction.

**DISCUSSION**

The results of the present study indicate probable androgenic effect of YOUTHEN. They effects of YOUTHEN on sperm count and methylene blue reduction time of
spermatozoa of the experimental animals indicate increased spermatogonensis and increased metabolic activity of the spermatozoa.

Increase in forward progressive motility of the spermatozoa in YOUTHEN treated rats reflects the fertility promoting effect of this product, earlier it was reported that SAXOM increased the volume and percentage of live sperms in rams(3). Our present study strengthens these findings.

Male sexual behaviour is mainly regulated by circulating levels of androgens (18). Improvement in sexual behaviour which was noted in this experiment indicates that YOUTHEN probably acts by raising the circulating level of androgens. In a previous experiment, improvement in the libido and fertility amongst the guineapigs and mice following the treatment with SAXOM was noted (4) SAXOM was found to facilitate the growth of cockrel combs as also the libido in vasectomised rams and bulls(1). SAXOM treated rats and rabbits exhibited increased levels of urinary 17-keto steroids (1). Although the levels of urinary 17-ketosteroids do not have definite relationship with circulating levels of testosterone is converted into 17-Ketosteroids in the liver and are excreted through urine (19). So, the results of earlier and present studies lead to the assumption that YOUTHEN improves the libido and sexual behaviour of experimental animals by virtue of its androgenic effect. It has been reported that powdered seeds of *Mucuna pruriens* produced a striking and sustained increase of sexual activity in normal male rats (20). Similar results are reflected in this compound herbal formulation containing *M. pruriens*.

Androgens stimulate the growth and development of sex organs and improves the rate of haemopoiesis (16). In our present study the weight of accessory sex organs were higher in YOUTHEN treated rats; however gonado-somatic index (testicular weight: body weight ratio) did not show any significant alterations in these rats. YOUTHEN treated rats exhibited better haemopoietic activity as reflected in their PCV and haemoglobin concentrations.

Fructose and citric acid content in semen is directly influenced by circulating androgenic level. These are the indices of testicular activity (16). Seminal fructose is secreted by accessory sex glands. High fructose content in semen is associated with good libido and high fertility (23).

Freund and Murphree (24) observed the correlation between sperm concentrations, initial motility and fructose level. Fructose serves as the principal source of energy for ejaculated spermatozoa, where as citric acid significantly contributes towards spermatozoa e.g.; sperm motility, facilitation of semen coagulation and liquefaction, activation of prostatic acid phosphatase, maintenance of osmotic equilibrium of semen, promotion of hyaluronidase activity of semen etc (19). So, the observed increase in fructose and citric acid content of the accessory sex glands of YOUTHEN treated rats indicates its fertility promoting action.

Increase in testicular protein synthesis is chiefly regulated by FSH (25). So, the observed effect of YOUTHEN on testicular protein content in experimental animals indicated increased activity of FSH producing cells, the result correlates the observed improvement in sperm count in YOUTHEN treated rats which also indicates increased activity of FSH producing cells (18). Decrease in testicular cholesterol content, as seen in YOUTHEN treated rats indicates utilization of cholesterol towards
testosterone synthesis in the testicular tissues (26).

From our present study it can be concluded that Youthen effectively promotes sexual behaviour and increase the number activity of spermatozoa of experimental animals. Youthen.

### Table -1
**Effect of Youthen on Haemopoietic system of Experimental Animals (Values are mean ± SEM of six animals)**

| Group | TEC (X10/µl) | Haemoglobin (g/dl) | P.C.V. (%) |
|-------|--------------|--------------------|------------|
| I     | 4.89±0.20    | 12.63±0.17         | 44.1±0.8   |
| II    | 5.34±0.19    | 13.74*±0.26        | 49.2**±0.6 |
| III   | 4.99±0.16    | 13.03±0.25         | 46.1±0.7   |

Statistical significance:*P ≤ 0.05,** P ≤ 0.01 as compared to placebo control group(I).

### Table –II
**Effect of Youthen on Sperm Count, Motility and MBRT of Experimental Animals (Values are Mean ± SEM of six animals)**

| Group | Sperm count (X10) | Motility (%) | MBRT (Sec) |
|-------|-------------------|--------------|------------|
| I     | 8.28±0.42         | 57.5±4.4 (51.2±3.1) | 239.41±17.21 |
| II    | 8.91±0.91         | 61.8±5.9 (54.3±4.1) | 216.83±10.96 |
| III   | 9.90**±0.56       | 69.1±4.7 (65.7±3.9*) | 192.53*±6.96 |

Statistical significance:*P ≤ 0.05,** P ≤ 0.01 as compared to placebo control group(I). Figures in the parenthesis indicate forward motility.

### Table – III
**Effect of Youthen on sexual performance of Experimental Animals (Values are Mean ±SEM of eight animals)**

| Group | Mounting index | Total Sexual behaviour |
|-------|----------------|------------------------|
| I     | 7.19±0.29      | 190.00±6.67            |
| II    | 11.29**±0.57   | 228.11*±6.81           |
| III   | 12.25**±0.59   | 240.13*±11.51          |

Statistical significance:*P ≤ 0.05,** P ≤ 0.01 as compared to placebo control group(I).
Table – IV
Weight of Primary and Accessory Sexual Organs in response to Youthen administration in experimental animals (values are Mean ±SEM of Eight animals)

| Group | Organ weight (g/100gm body weight) |
|-------|-----------------------------------|
|       | Testis | Epididymis | Prostate gland | Seminal vesicles |
| I     | 1.026 ±0.04 | 0.342 ± 0.003 | 0.372±0.009 | 0.510 ± 0.007 |
| II    | 1.046 ±0.07 | 0.360**±0.002 | 0.395 ± 0.010 | 0.527 ± 0.009 |
| III   | 1.047 ±0.08 | 0.362**±0.003 | 0.425*±0.011 | 0.562**±0.007 |

Statistical significance:*P ≤ 0.05,** P ≤ 0.01 as compared to placebo control group(I).

Table – V
Effect of Youthen on Biochemical profile of male accessory sex organs (values are Mean ±SEM of Eight rats)

| Groups | Acid phosphatase (U/gm) | Alkaline phosphatase (U/gm) | Fructose (mg/gm) | Citric acid (mg/100gm) |
|--------|-------------------------|-----------------------------|------------------|-----------------------|
|        | E | P | SV | E | P | SV | E | P | SV | E | P | SV | E | P | SV |
| I      | 1.23 ± 0.05 | 1.94 ± 0.07 | 1.40 ± 0.12 | 1.37 ± 0.04 | 4.61 ± 0.17 | 0.59 ± 0.05 | 1.61 ± 0.13 | 0.89 ± 0.07 | 1.09 ± 0.07 | 61.90 ± 3.12 | 25.2 ± 3.12 | 31.4±2.7 |
| II     | 1.39 ± 0.09 | 2.43**±0.13 | 1.62 ± 0.13 | 1.39 ± 0.07 | 4.76 ± 0.12 | 0.67± 0.05 | 2.46*±0.19 | 1.19*±0.07 | 1.39*±0.09 | 66.71 ±2.15 | 30.7 ±2.1 | 34.3±2.19 |
| III    | 1.45*±0.00 | 2.69 ± 0.11 | 1.86*± 0.11 | 1.67**±0.07 | 4.99**±0.11 | 0.87**±0.06 | 2.61**±0.17 | 1.24*±0.19 | 1.41*±0.11 | 69.21 ±2.17 | 29.7*±1.0 | 37.1±2.17 |

Statistical significance:*P ≤ 0.05,** P ≤ 0.01 as compared to placebo control group(I).

E=Epididymis, P=Prostate glands, SV = Seminal Vesicles.

Table – VI
Effect of Youthen on Testicular Biochemical profile of Experimental Animals (Values are Mean ±SEM of eight animals)

| Group | Protein (mg/gm) | Cholesterol (mg/gm) | Acid Phosphatase (u/gm) | Alkaline phosphatase (u/gm) |
|-------|-----------------|---------------------|-------------------------|-----------------------------|
| I     | 34.83 ± 3.14 | 2.15 ± 0.12 | 2.14±0.23 | 0.276±0.027 |
| II    | 55.94***±1.04 | 1.72*±0.11 | 3.98***±0.25 | 0.298±0.035 |
| III   | 56.84***±1.97 | 1.52***±0.06 | 4.11***±0.18 | 0.486***±0.04 |

Statistical significance:*P ≤ 0.05,** P ≤ 0.01, P ≤ 0.001 as compared to placebo control group(I).
Probably induces androgenic action in treated animals. Further experiments are necessary to study the effect of YOUTHEN on circulating levels of testosterone and FSH.

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