STUDY OF Andropic® ON ANDROGENIC AND ANABOLIC ACTIVITY IN MALE RATS

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Summary

Andropic® is a marketed preparation consisting of steroidal saponin fraction of *trigonella foenum* extract. *Trigonella foenum* (TF) has long been used in the traditional Indian systems of medicine for the treatment of various ailments and is claimed to contain steroidal saponins. The objective was to study the anabolic and androgenic activity of Andropic® in immature castrated male Wister rats. The animals (55+- 5 g) were castrated and divided into five groups of 6 rats each and treated with either vehicle, testosterone (10mg/kg s.c. bi weekly) or Andropic® (10 and 35 mg/kg p.o.) once daily for 4 weeks. Body weight, weights of male reproductive organs (viz. prostate, seminal vesicle and musculus levator ani) was recorded. Serum testosterone concentration and hematological parameters were measured after 4 weeks of treatment. Histopathological examination of testis was carried in immature noncastrated male Wister rats pretreated in a similar manner as above mentioned. In our study, increase in weight of levator ani muscle and body weight was shown by Andropic® (35 mg/kg p.o) as well as testosterone treatment. Andropic® (10 or 35 mg/kg p.o) did not change testosterone level in castrated rats. Both the compounds showed normal histopathology of testis. Andropic® exhibited anabolic activity but not androgenic activity in male castrated rats at a dose of 35 mg/kg p.o.

Keywords

Andropic®, Castration; Testosterone; Androgenic; Anabolic

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Introduction

Herbs have been used throughout history to enhance physical performance, but scientific scrutiny with controlled clinical trials has only recently been used to study such effects. Human consume herbs to enhance their long term endurance performance, to induce muscular hypertrophy and strength. Tonic herbs have been classified as 1) adaptogen 2) anabolic. Adaptogens (eg. Wild oat , vit c) improves strength, anaerobic power, endurance time and feeling of well being. Anabolic herbs are believed to provide or mimic testosterone like (anabolic) effects in humans because of their similarity of chemical structure. These herbs contain sterols or steroidal saponins. There is no evidence to support the conversion of plant sterols to testosterone in human body (1,2).

Trigonella foenum Linn.,(TF) also known as Fenugreek, is an aromatic annual plant, 30-60 cm tall, found wild in Kashmir, Punjab and the upper Gangetic plains and widely cultivated in many parts of India (3). Fenugreek seeds contain 50% fiber (30% soluble fiber and 20% insoluble fiber) (4).The major components of fenugreek seeds along with mucilaginous fiber are proteins, saponins, lipids and unavailable carbohydrate. The seeds also contain the major alkaloid trigonelline, flavonoids, carotenoids and coumarins (4,5) .Fenugreek has been used as cooking spice and flavoring agent for centuries(6).It is used as an abortifacient (7) antispasmodic, externally for abscesses, boils, galactagogue (8) appetite stimulant, blood cleansing, laxative, tonic (9) demulcent, emollient, expectorant, aphrodisiac (10) properties. The defatted seeds are rich source of steroids (11). However studies on fenugreek seeds (12) and its extract (13) are reported to have antifertility activity. No study has been carried out to evaluate the effect of steroidal saponins fraction from *Trigonella foenum* Linn on androgenic and anabolic status in laboratory animals. Andropic® consist of mixture of steroidal saponins and was provided by Indus Biotech PVT LTD.

The objective of the study was to evaluate androgenic and anabolic activity of Andropic®

Material and Methods

Chemicals

Testosterone (Himedia Laboratories, India), and anaesthetic ether (TKM Pharma, India) were purchased. Andropic® was given as gift sample by Indus Biotech, India.

Animals

Immature male Wistar rats of weight range 55±5 g and adult Swiss albino mice of either sex were purchased from National Toxicology Centre, Pune, India and used for the study. They were maintained at a temperature of 25 ± 1 °C and relative humidity of 45 to 55% under 12 :12-h light dark cycle. The animals had free access to food

pellets (Chakan Oil Mills, Pune, India) and water was given ad *libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India, constituted under Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Acute Oral Toxicity of Drug extract

Healthy adult Swiss mice of either sex weighing between 20-25 g were used for acute oral toxicity study. The study was carried out according to OECD (Organization for Economic Co-operation and Development) guideline no. AOT-425 (14).The mice were observed for 2 hrs for behavioral, neurological and autonomic profiles and for any lethality or death for the next 48 h.

Treatment schedule

Immature male Wistar rats (55 gm ± 5gm) were castrated by method described by Ottani *et. el* (15) and were divided into 4 groups of six rats each. These groups received daily oral administration of either distilled water (vehicle control), Andropic® (10 or 35 mg/kg p.o), or testosterone (10 mg/kg in sesame oil suspension, s.c. bi-weekly). The treatment was given for 4 weeks and physiological, biochemical and histopathological studies were carried out on 30th day of treatment.

Anabolic and androgenic activity

Immature male Wistar rats weighing 55 gm (± 5gm) were anesthetized by anesthetic ether and castrated. After recovery period of 2 days the animals were divided into following groups I) Vehicle treated, II) Andropic® (10 mg/kg p.o.), III) . Andropic® (35 mg/kg p.o.) and IV) Testosterone (10 mg/kg in sesame oil suspension, s.c. bi-weekly). After 4 weeks of treatment, animals were sacrificed and the seminal vesicles, the ventral prostate, and the musculus levator ani were carefully dissected and weighed. Body weight of the animals was registered at the beginning and at the end of the experiment. Increase in weight of seminal vesicles and ventral prostate indicates androgenic activity, whereas gain in weight of musculus levator ani was considered to indicate anabolic activity (16).

Effect on serum testosterone and blood urea nitrogen

Immature male Wister rats weighing 55 gm (± 5gm) were anesthetized by anesthetic ether and castrated. After recovery period of 2 days the animals were divided into following groups I) Vehicle treated, II) .Andropic® (10 mg/kg p.o.), III) Andropic® (35 mg/kg p.o.) and IV) Testosterone (10 mg/kg in sesame oil suspension, s.c. bi-weekly). After 4 weeks of treatment, animals were sacrificed. The blood was withdrawn from the rats by retro orbital puncture method and analyzed for biochemical parameter blood urea nitrogen (BUN), using standard biochemical kits and serum testosterone was measured by radioimmunoassay (RIA).

Effect on histopathology of testis

Immature male Wistar rats weighing 55 gm (± 5gm) were divided into following groups I) Vehicle treated, II) Andropic® (10 mg/kg p.o.), III) Andropic® (35 mg/kg p.o.) and IV) Testosterone (10 mg/kg in sesame oil suspension, s.c. bi-weekly). After 4 weeks of treatment, animals were sacrificed. The testis from each group were excised quickly during the above studies using a separate group of noncasterated male rats and fixed in 10% buffered neutral formalin. The sections were stained with haematoxylin and eosin dye. Histopathological examination of testis was carried out.

Statistical analysis

Data for each of the parameter was analyzed by one-way ANOVA followed by Dunnett's test.

Results

Acute oral toxicity test indicates that the drug is safe up to 5000mg/kg orally**.**

Anabolic activity of Andropic®

Four-week treatment of Andropic® (10 mg/kg p.o) did not change body weights of the rats while testosterone (10 mg s.c biweekly) and higher dose of Andropic® (35 mg/kg p.o) group showed significant gain ($P < 0.01$) in body weight (Figure 1) as compared with control group. Andropic® (10 and 35 mg/kg) did not increase weights of organs of male reproductive system. Andropic® (35 mg/kg p.o) group and Testosterone group significantly ($P < 0.001$) increased the weight of musculus levator ani.

Effect on serum testosterone and Blood Urea Nitrogen

Andropic® (10 mg/kg p.o) produced Significant ($P < 0.01$) increase in BUN while no increase in BUN was observed in case of control (noncastrated vehicle treated) group, Andropic (35 mg/kg p.o) and Testosterone group. (Figure 3). No significant increase in serum testosterone level was observed after the once daily treatment of Andropic (10 or 35 mg/kg, p.o.).

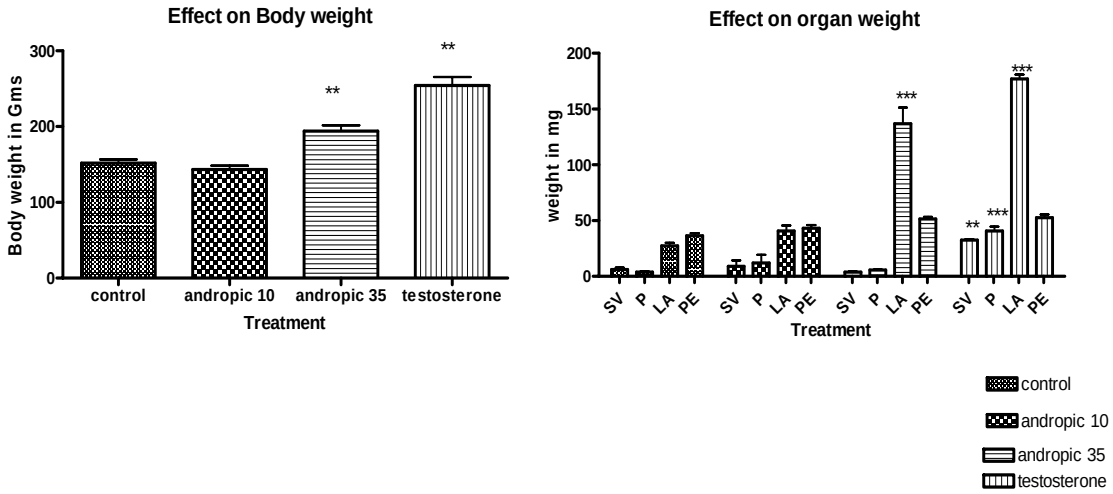


Figure 1: Effect of Andropic® (10 and 35 mg/kg,p.o daily) and Testosterone (testosterone, 10 mg/kg, s.c. bi-weekly) on body weight (g.) and organ weight of male castrated rats. Figures in bracket indicate dose in mg/kg. Data represented as mean weight (g.) of six rats ± S.E.M. ** P < 0.01, *** P < 0.01, as compared with castrated control group. SV- Seminal Vesicle, P- Prostate, LA-Levator ani muscle, PE-Penis.

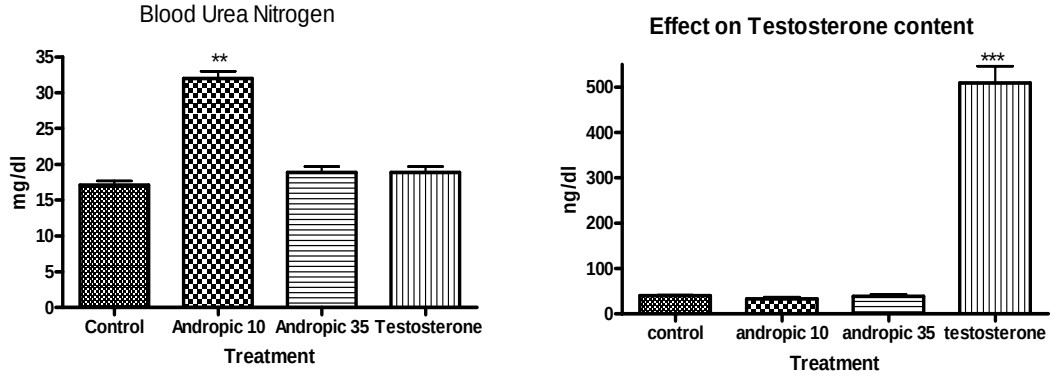
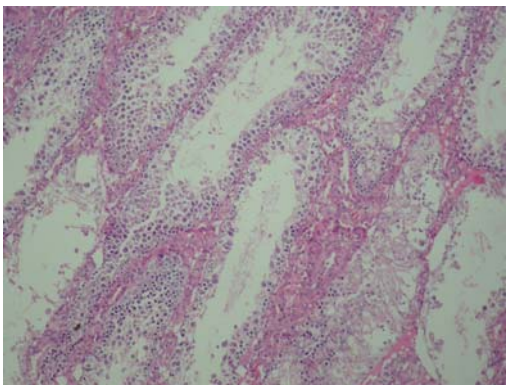


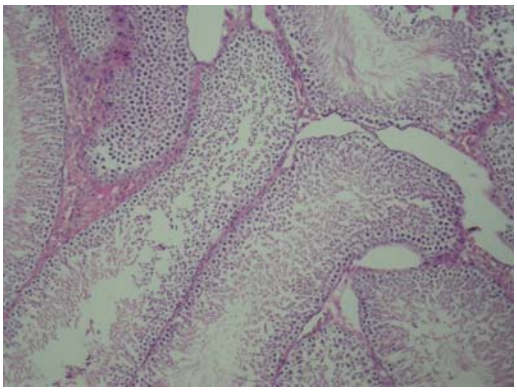
Figure 2: Effect of Andropic®c (10 and 35 mg/kg p.o daily) on BUN and serum Testosterone (ng/dl) in male rats after 4 weeks of treatment. Separate groups for vehicle and standard drug testosterone 10 mg s.c. twice weekly were also maintained. Figures in bracket indicate dose in mg/kg. Data represented are mean BUN (mg/dl) and testosterone level (ng/dl) ± SEM in castrated male rats (six per group) and analyzed by one-way ANOVA followed by Bonferroni post hoc test. ** P < 0.01, *** P < 0.001 as compared with normal (non castrated) untreated group.

Effect on histopathology of testis

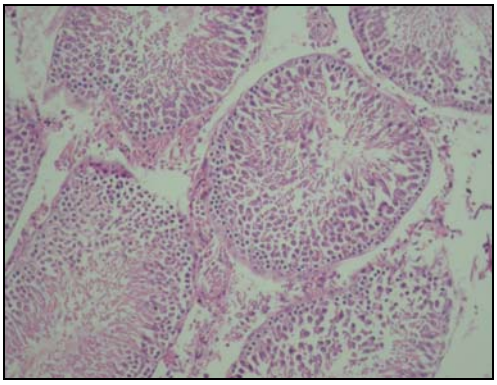
The testes of rats treated with vehicle, testosterone or Andropic showed normal features with successive stages of transformation of the seminiferous epithelium into spermatozoa with no sign of atrophy or toxicity with respect to pachytene spermatocytes, germ cells, Leydig cells, or Sertoli cells. (Figure 3)



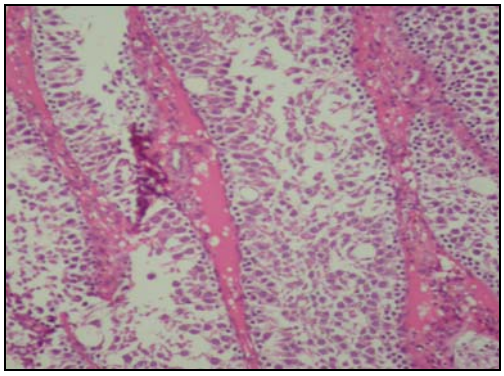
Control



Andropic® (10)



Andropic® (35)



Testosterone (10 mg/kg s.c. twice weekly)

Figure 3. Effect on histopathology of testis of male noncastrated animals pretreated for one month with vehicle, Andropic® (10or 35 mg/kg p.o.) and testosterone (10 mg/kg s.c. twice weekly) in male noncastrated male rats.

Discussion

It has been reported that fenugreek plant has a rich source of steroids (11). However, there is paucity of data on the effect of phytochemical constituents of *trigonella foenum* extract that are responsible for the activities in the *in vivo* experiments. Our study is an attempt to investigate the effect Andropic ^{R} which comprises of steroidal

saponins from fenugreek, in the castrated rat, which is a convenient *in vivo* model for studying the androgenic and anabolic effects.

Skeletal muscle is the largest pool of protein in the body. Maintenance of its mass involves delicate balance between protein synthesis and degradation. Muscle atrophy, defined as the unintentional loss of 5-10 %of muscle mass, is a frequent consequence of many catabolic conditions e.g. diabetes, sepsis and cancer. (17)

The anabolic study revealed by the gain in body weight and weight of levator ani is due to steroidal content in the Andropic®. As there is no increase in level of serum testosterone which indicated that the steroidal content of trigonella were not converted into testosterone or its analogue. Thus the drug doesn't exhibit any androgenic activity. The anabolic activity was further confirmed by BUN (18) in blood. The possible mechanism for anabolic activity could be due to the protein formation by either increasing growth hormone release or by stimulation of growth factors such as IGF-I. It has been reported that IGF-I and insulin are important determinants of muscle mass by virtue of their ability to promote growth and suppress protein degradation.

Conclusion

Results of this study may act as a proof of concept study on Andropic® from fenugreek as an anabolic compound. As it has not shown any increase in testosterone level thus can be used in disorders envolving muscle wasting without affecting the androgenic status of the body. Further study has to be done to elucidate the mechanism of Andropic®.

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