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The hormonal effects of *Tribulus terrestris and* its role in the management of male erectile dysfunction – an evaluation using primates, rabbit and rat

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Abstract

Hormonal effects of *Tribulus terrestris* (TT) were evaluated in primates, rabbit and rat to identify its usefulness in the management of erectile dysfunction (ED). TT extract was administered intravenously, as a bolus dose of 7.5, 15 and 30 mg/kg, in primates for acute study. Rabbits and normal rats were treated with 2.5, 5 and 10 mg/kg of TT extract orally for 8 weeks, for chronic study. In addition, castrated rats were treated either with testosterone cypionate (10 mg/kg, subcutaneously; biweekly for 8 weeks) or TT orally (5 mg/kg daily for 8 weeks). Blood samples were analyzed for testosterone (T), dihydrotestosterone (DHT) and dehydroepiandrosterone sulphate (DHEAS) levels using radioimmunoassay. In primates, the increases in T (52%), DHT (31%) and DHEAS (29%) at 7.5 mg/kg were statistically significant. In rabbits, both T and DHT were increased compared to control, however, only the increases in DHT (by 30% and 32% at 5 and 10 mg/kg) were statistically significant. In castrated rats, increases in T levels by 51% and 25% were observed with T and TT extract respectively that were statistically significant. TT increases some of the sex hormones, possibly due to the presence of protodioscin in the extract. TT may be useful in mild to moderate cases of ED.

Keywords: *Tribulus terrestris*; Testosterone; Dihydrotestosterone; Dehydroepiandrosterone sulphate; Steroidal glycosides; Protodioscin; Erectile dysfunction

Introduction

Hormones are essential chemical mediators that are involved in the various physiological functions, including the sexual function of a living organism. Until the 8th week of gestation, the external genitalia are represented identically in both sexes (Conte and Grumbach, 1995). After this period, the development of the genital structures towards a particular identity depends mainly on the hormonal milieu that prevails. Furthermore, the mammalian reproductive axis is coordinated by the hypothalamic secretion and trophic effects of gonadotrophin releasing hormone, which is in turn controlled by negative feedback from the gonadal steroids.

Testosterone is the most important androgen secreted by the testis in humans. Approximately 8 mg of testosterone is produced daily, the major source (95%) being the interstitial cells of Leydig (Howell and Shalet, 2001). The adrenals contribute to the rest (5%) of testosterone. After puberty, the plasma level of this hormone in males is about 0.6 μg/dl of which, 97–99% is

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bound to sex hormone binding globulin, and approximately 1–3% remains free and readily available for physiological needs.

Dihydrotestosterone is the other potent androgen secreted by the testes. Testosterone is converted in many target tissues to the much active DHT by the enzyme 5α-reductase. The masculinization of the fetus occurs under the influence of DHT. It is noted that during 8–12 weeks of gestation, DHT stimulates the growth of genital tubercle, leading to fusion of the urethral folds and descent of the labioscrotal swelling which later forms the penis and scrotum respectively (Hinman, 1993). There is also a simultaneous inhibition of the descent and growth of the vesicovaginal septum and the vaginal differentiation in the male foetus.

The testes in addition to producing the above mentioned androgens also produce androstenedione and dehydroepiandrosterone that are considered to be weak androgens. DHEA, regarded as the 'fountain of youth', was isolated in 1934 and is the major secretory product of adrenal gland, although the testes produce a small quantity. After production and secretion from these glands, the potentiality of this hormone to enter the androgenic pathway depends on the individual's medical condition, age and sex, for every individual has a unique biochemical composition. DHEA is metabolized to form DHEAS, and both hormones are metabolically interconvertible by the action of the enzymes sulphotransferase for conjugation and sulphatase for hydrolysis, present in many tissues (Baulieu, 1996).

In general, androgens are essential for the development of the male external genitalia, the male secondary sexual characters and also in the regulation of erectile response. Sexual desire and activity as well as the nocturnal penile erections are dependent on the circulating androgen levels (Mills et al., 1996). Abnormalities in the synthesis and expression of androgens or its depletion by medical or surgical castration may cause a general decline in libido and sometimes in erectile and ejaculatory functions (Baskin et al., 1997). The incidence of sexual dysfunction resulting from hormonal imbalance is estimated to be 20–25% with hypogonadism (primary and secondary) being the most frequent cause (Manieri et al., 1997). In ageing, there seems to be a continuous decline in the levels of androgen leading to andropause a term akin to menopause in females (Burns-Cox and Gingell, 1997).

Androgen replacement helps to overcome the symptoms associated with andropause such as fatigue, nervousness, hot flushes, insomnia and also helps in restoration of bone density/turnover, muscle mass as well as the sexual function and libido (Vermeulen, 1991; Tenover, 1997). However, the hormonal preparations currently used as a replacement therapy can lead to hypofunction of the hypothalamo-hypophyseal-gonadal axis and also produce adverse effect on prostate gland and liver function, when used indiscriminately. A phytochemical with similar properties to that of the steroids that can bring about the changes necessary for restoration of general well being, sexual interest and activity without producing the undesirous side effects associated with the current hormone replacement therapy will contribute significantly to the management of erectile dysfunction (ED).

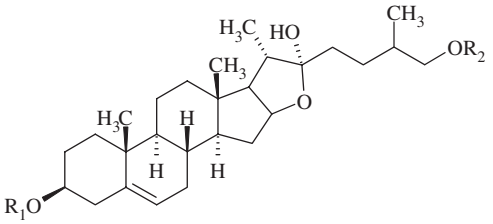

Fig. 1. Structure of protodioscin: R_1 = glucosyl-dirhamnosyl; R_2 = glucosyl.

The plant *Tribulus terrestris* (TT) popularly known as puncture vine is a perennial creeping herb with a worldwide distribution. Since ancient times it is regarded as an aphrodisiac in addition to its beneficial claims on various ailments such as urinary infections, inflammations, leucorrhoea, oedema and ascites (Chopra et al., 1958; CHEMEXCIL 1992). The extract (TT) (obtained from Sopharma, Bulgaria & Tegushindo, Indonesia) from the air-dried aerial parts of the plant contains steroidal glycosides (saponins) of furostanol type, the predominant furostanol being protodioscin (PTN) (Fig. 1), which constitutes about 5% of the extract (Dikova and Ognyanova, 1993). The levels of testosterone and lutinizing hormone are increased following treatment with PTN for a period of 30–90 days in patients with hypogonadism (Koumanov et al., 1982). Improvement in sperm count and motility has been reported in patients with low seminological indices following treatment with TT for 3 months (Balanathan et al., 2001). It also increases the sexual behaviour parameters in castrated rats treated with TT extract for 8 weeks compared to the normal rats (Gauthaman et al., 2002). It is also claimed to dilate coronary arteries and improve the coronary perfusion with no adverse effect on long-term use (Wang et al., 1990). In general, the plant TT or its products are consumed by people in different parts of the world for its effect of general well being and muscle building properties in addition to its popular claims as an aphrodisiac. In the present study, the effect of TT extract on some of the sex hormones namely, testosterone (T), dihydrotestosterone (DHT) and dehydroepiandrosterone sulphate (DHEAS) using three different animal models such as primates, rabbit and rat was evaluated to understand whether TT extract would be useful as an adjunct in the management ED.

Materials and methods

The animal models used in this study were the sub-human primates, rabbit and rat. All laboratory and experimental procedures were conducted in accordance with institutional guidelines for animal ethics.

Experimental design for acute study

For the acute study in primates, three baboons (*Papio anubis*) and two rhesus monkeys (*Macaca mullata*) were used. Briefly, after initial tranquilization with intramuscular ketamine (10–15 mg/kg body weight), the animal was intubated and connected to the Boyle's apparatus. The animal was maintained on oxygen (1–2 l/min) and halothane (1–3%) throughout the procedure and a pulse oximeter was used to monitor the oxygen saturation. The temperature was maintained between 36 and 38 °C by means of a thermo blanket. An intravenous line was established and the bladder catheterized. The femoral artery was then cannulated using a 20 G intravenous canula and connected to the already calibrated blood pressure transducer and Mac Lab apparatus for monitoring the blood pressure continuously. Electrocardiogram was also recorded throughout the procedure (Fig. 2A and B). Each animal was tested with three different concentrations of TT extract (7.5, 15 and 30 mg/kg body weight), and vehicle given as a bolus intravenous injection. Blood sample drawn at 15 min before administration of the extract served as control. Following drug administration, blood samples were collected at 15, 30, 60, 90, 120 and 180 min.

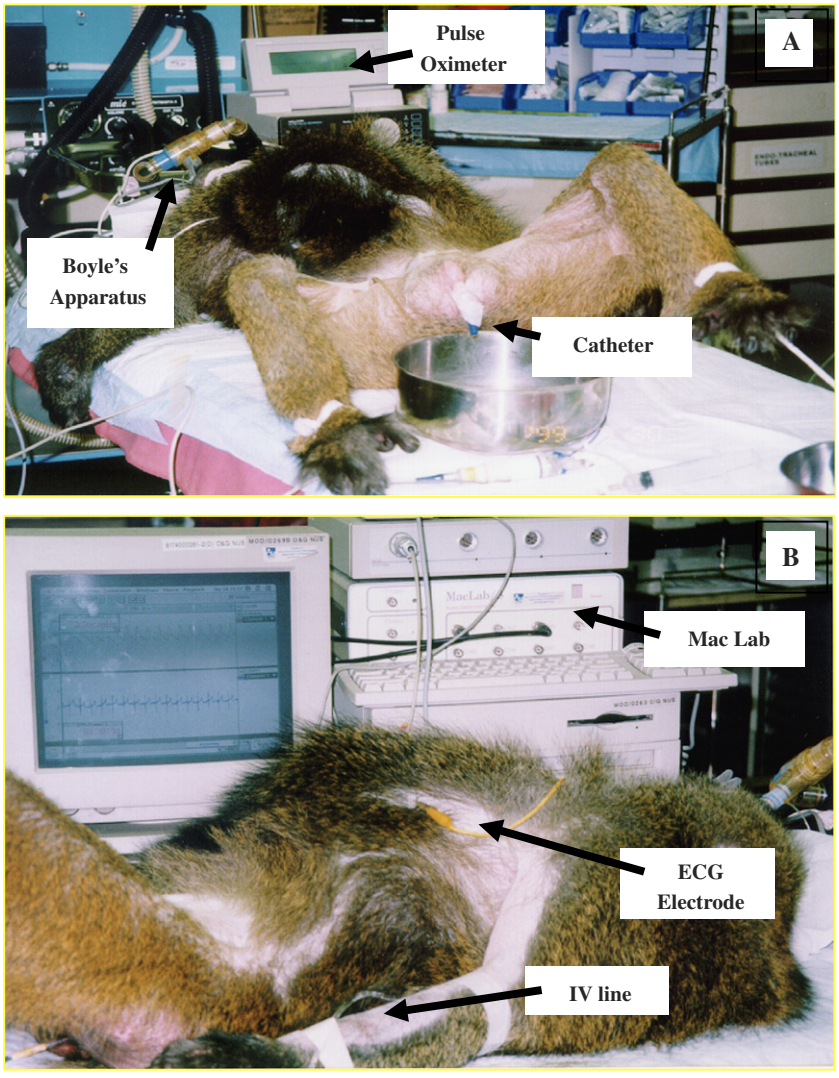

Fig. 2. The experimental set up for primates. The baboon was anesthetized and connected to Boyle's apparatus. Oxygen saturation, blood pressure, electrocardiogram, intravenous output and rectal temperature were monitored. Blood samples were obtained before and after intravenous administration of 7.5, 15 and 30 mg/kg body weight of TT extract. TT – *Tribulus terrestris*.

Experimental design for chronic study

For the chronic study, normal New Zealand white rabbits, normal Sprague Dawley rats and castrated Sprague Dawley rats were used.

Twenty-four New Zealand white rabbits were divided randomly into four groups of six each. Group I served as control and was treated with vehicle alone. Groups II, III, IV were treated with three different concentrations of TT (2.5, 5 and 10 mg/kg, respectively), orally for 8 weeks. Forty normal Sprague Dawley rats that were divided into four groups of ten animals each were also treated similarly. In addition, the castrated group of Sprague Dawley rats (5 groups of 8 animals each) were treated as follows: group I (normal control) and group III (castrated control) were treated with vehicle alone; group II (normal rats) and group IV (castrated rats) were treated with testosterone cypionate – 10 mg/kg body weight, subcutaneously, bi-weekly for 8 weeks; and group V (castrated rat) were treated with TT 5 mg/kg body weight, orally for 8 weeks. Blood samples were collected at the end of 8 weeks treatment from all animals and analyzed for hormones.

Castration

Twenty-four male rats were castrated in this study. Briefly, the rats were anaesthetized and a median skin incision of about 1 cm was made at the tip of the scrotum. The subcutaneous tissues were cleared by blunt dissection and the testis was reached by a small (about 5 mm) incision over the covering sacs. After firm ligation with a non-absorbable suture around the blood vessels and the vas deferens, these structures were severed distally thereby facilitating removal of the testis from both sides. Incisions were closed in layers and the rats were taken up for study after 3–4 weeks of convalescence.

The blood samples thus collected from both acute and chronic studies were allowed to clot at room temperature for 4–6 h. They were then centrifuged at 3000 rpm at 4 °C for 10 min. The separated serum was carefully transferred to labeled sterile polystyrene vials and stored at −70 °C until radioimmunoassay was done.

Hormone assessment

The sera obtained from the control and treated groups were analyzed for testosterone, DHT and DHEAS by radioimmunoassay. Briefly, the steroid hormones were extracted from the binding proteins using diethyl ether. The organic phase i.e., the ether layer was decanted into tubes containing the anti-bumping granules, and dried in a water bath at 60 °C. Gelatinised phosphate buffer in saline (0.3 ml) was added to each tube and mixed vigorously. In the case of DHT, in addition to the above extraction procedure, a second extraction (oxidation process) was carried out. The diluted standards (0.1 ml) of and the extracted samples (0.1 ml) were added to their respective assay tubes. Then 0.2 ml of 2-in-1 scintillation proximity assay (SPA) mixture followed by 0.1 ml of the respective tracer ($\{^3H\}$ testosterone, $\{^3H\}$ dihydrotestosterone) was added to all the tubes. For DHEAS, the assay procedure was similar to that of testosterone and DHT except for the volume of SPA (0.1 ml) and the tracer used ($\{^3H\}$ dehydroepiandrosterone sulphate). The content of each tube was mixed well and incubated at room temperature for 20 ± 4 h and read using a scintillation counter, Wallac 1410 and the results were computed using the MultiCalc software.

Drugs

The following drugs were used: *Tribulus terrestris* extract (Sopharma, Bulgaria & Tegushindo, Indonesia), ketamine, halothane (Sigma), standards for T, DHT and DHEAS (Sigma), Tracer ^3H (NEN Dupont), SPA reagent (Amersham).

Statistical methods

The variables from the different experimental groups were analyzed and compared by one-way ANOVA with Bonferroni's multiple comparisons. The differences in treatment within each group at different periods were analyzed and compared using Student's paired *t*-test. All the results were expressed as mean ± SEM and the level of significance for comparisons set at $p < 0.05$.

Results

Blood pressure in primates

Blood pressure was recorded from the primates throughout the experimental procedure. The systemic blood pressure recorded in the supine posture, from the lower limbs ranged from 70 to 140 mmHg. TT had only very minimal changes in the cardiovascular status. There was an initial, insignificant drop in blood pressure by 2–5 mmHg that returned to normal within few minutes (Fig. 3 – upper panel). The mean values of the blood pressure recordings from all the animals in the experiment for different doses of TT and vehicle are shown in Table 1.

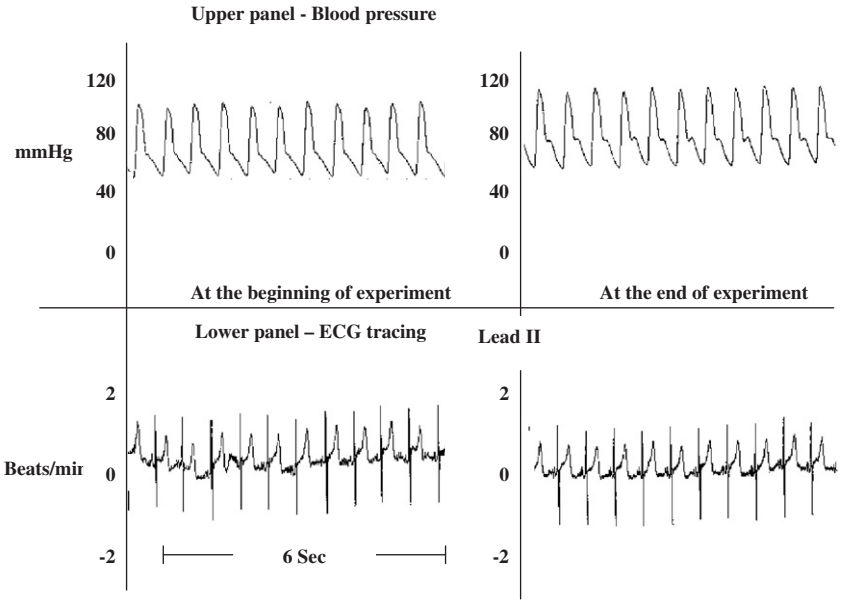

Fig. 3. The actual tracing of blood pressure (upper panel) and electrocardiogram (lower panel) recorded from baboon.

Table 1. The blood pressure and heart rate recorded in animals following treatment with different concentrations of TT extract

Animal	TT (mg/kg)	Mean BP (mmHg)		Heart rate/minute	
		Before TT	After TT	Before TT	After TT
Adult male primates (three baboons and two rhesus monkeys)	Vehicle	88.0±4.0	92.0±2.0	100.0±2.0	106.0±2.0
	7.5	84.0±6.0	89.0±4.0	98.0±2.0	104.0±4.0
	15.0	96.0±4.0	102.0±2.0	112.0±4.0	118.0±3.0
	30.0	96.0±2.0	99.0±4.0	83.0±3.0	90.0±5.0

TT – *Tribulus terrestris*.

Electrocardiogram in primates

The electrocardiographic (ECG) recordings showed only minimal changes. The rhythm was sinus, and all the values and intervals were within normal limits. There was a mild tachycardia with an increase in heart rate of about 18–20 beats/min (Fig. 3 – lower panel). This response was seen immediately after the administration of TT, which returned to the baseline values for all the concentrations and animals tested. The mean values and the intervals calculated from ECG tracings recorded from all the animals for the different concentrations of TT and vehicle are given in Table 1.

Effect on serum hormone levels in primates

Testosterone

Serum testosterone levels were increased for the different concentrations studied compared to the control. The rise was acute, observed especially in the samples taken at 15 and 30 min following administration of TT. This acute response was short-lived and the immediate rise gradually returned to the baseline level around 90–180 min. The mean maximal increase in the serum values were 52%, 49% and 55% for 7.5, 15, and 30 mg/kg body weight of the TT, respectively. The observed rise in testosterone for all the three concentrations was statistically significant.

Within the same concentration group at different time intervals the serum testosterone levels were increased compared to the baseline value. The mean maximal increases in serum testosterone level observed for the concentration 7.5 mg/kg (52% at 30 min) and for the concentration 30 mg/kg (55%, 17% and 10% at 30, 120 and 180 min, respectively) were statistically significant compared to the control (Fig. 4).

Dihydrotestosterone

The hormone DHT also showed a pattern similar to that of testosterone. There was an initial rise in concentration of DHT compared to the control sample for the three concentrations of TT studied. The rise in serum levels was observed at 15 and 30 min that

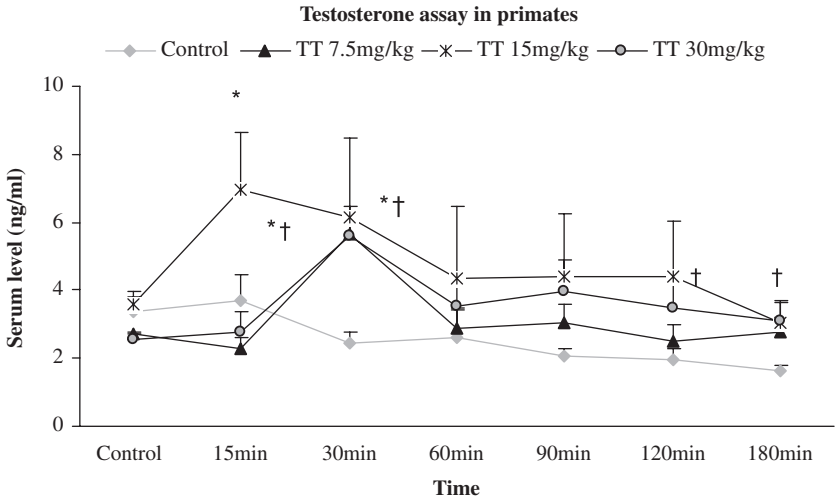

Fig. 4. Serum testosterone level following bolus intravenous administration of TT at three different concentrations in primates ($n = 5$). The results were analyzed and compared between the groups and within groups. The values are expressed as mean ±SEM. * and † indicate significant differences ($p < 0.05$) from control between groups and within groups, respectively. TT – *Tribulus terrestris*.

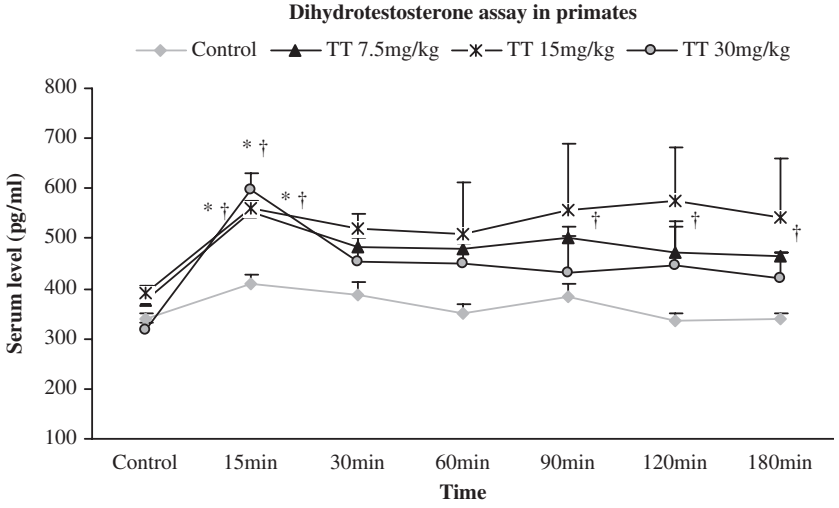

Fig. 5. Serum dihydrotestosterone level following bolus intravenous administration of TT at three different concentrations in primates ($n = 5$). The results were compared and analyzed between the groups and within groups. The values are expressed as mean ± SEM. * and † indicate significant differences ($p < 0.05$) from control between groups and within groups, respectively. TT – *Tribulus terrestris*.

returned to the base line values thereafter. The mean maximal increases in the serum values were 31%, 29% and 47% for the concentrations 7.5, 15 and 30 mg/kg of TT, respectively. These values were statistically significant compared to the control.

Within the same concentration group at different time intervals the serum DHT levels were increased compared to the baseline value. Mean maximal increases in serum DHT level observed for the doses 7.5 mg/kg (31%, 19%, 22% and 16% at 15, 60, 90 and 180 min, respectively), 15 mg/kg (29% at 15 min) and 30 mg/kg (47% at 15 min) were statistically significant compared to the control (Fig. 5).

Dehydroepiandrosterone sulphate

Serum DHEAS were also increased from control for all the three concentrations of TT tested. Unlike testosterone and DHT, the rise in DHEAS was more gradual and consistent and observed between 60 and 180 min following drug administration. The mean maximal increase in the serum DHEAS values were 29%, 13% and 36% for doses 7.5, 15, and 30 mg/kg body weight of the TT, respectively. However, only the increase observed for the concentration 7.5 mg/kg was statistically significant.

Within the same concentration group at different time intervals the serum DHEAS levels were increased

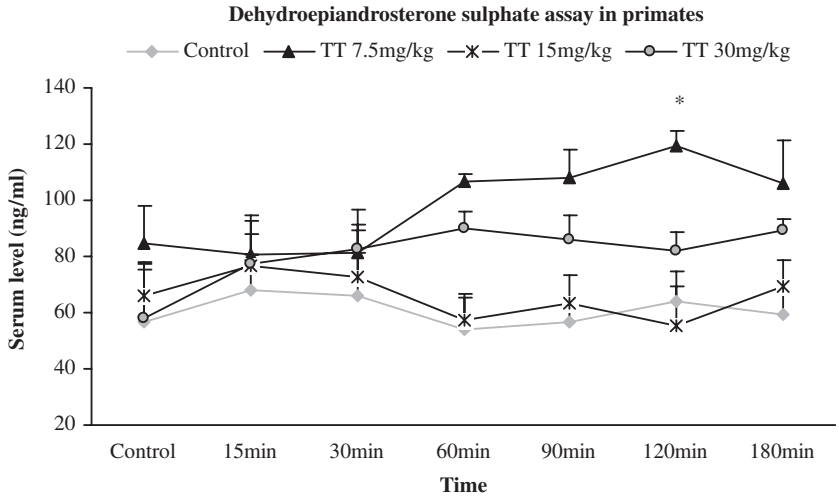

Fig. 6. Serum dehydroepiandrosterone sulphate level following bolus intravenous administration of TT at three different concentrations in primates ($n = 5$). The results were compared and analyzed between the groups and within groups. The values are expressed as mean ±SEM. * indicates significant difference ($p < 0.05$) from control between groups. TT – *Tribulus terrestris*.

compared to the baseline value. However, the results were not statistically significant (Fig. 6).

Effect on serum hormone levels in rabbit

Testosterone

Serum testosterone levels were increased compared to the control group for all the three concentrations of TT tested. There was a mean maximal increase in serum testosterone by 25%, 36% and 38% for the concentrations 2.5, 5 and 10 mg/kg of TT, respectively. However, the increases in values were not statistically significant (Fig. 7A).

Dihydrotestosterone

Serum dihydrotestosterone were increased compared to the control group for all the three concentrations of TT tested. There was a mean maximal increase in serum DHT by 9%, 30% and 32% for the concentrations 2.5, 5 and 10 mg/kg of TT, respectively. The increases observed for the doses 5 and 10 mg/kg of TT were statistically significant compared to the control (Fig. 7B).

Effect on serum hormone levels in normal rat

Testosterone

Serum testosterone was increased compared to the control group for the concentrations 15 and 30 mg/kg of TT tested. There was a mean maximal increase in serum testosterone by 21% and 23% for the concentrations 5 and 10 mg/kg of TT, respectively. However, the

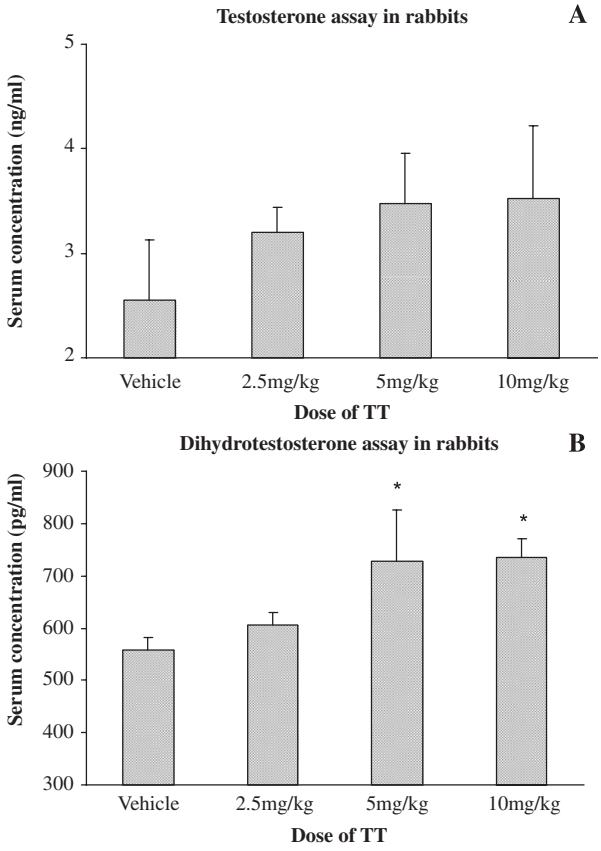

Fig. 7. (A) Serum testosterone, (B) serum dihydrotestosterone level following administration of TT at three different concentrations in rabbit ($n = 6$). The results are compared with control and the values are expressed as mean ±SEM. * indicates significant differences ($p < 0.05$) from control between groups.

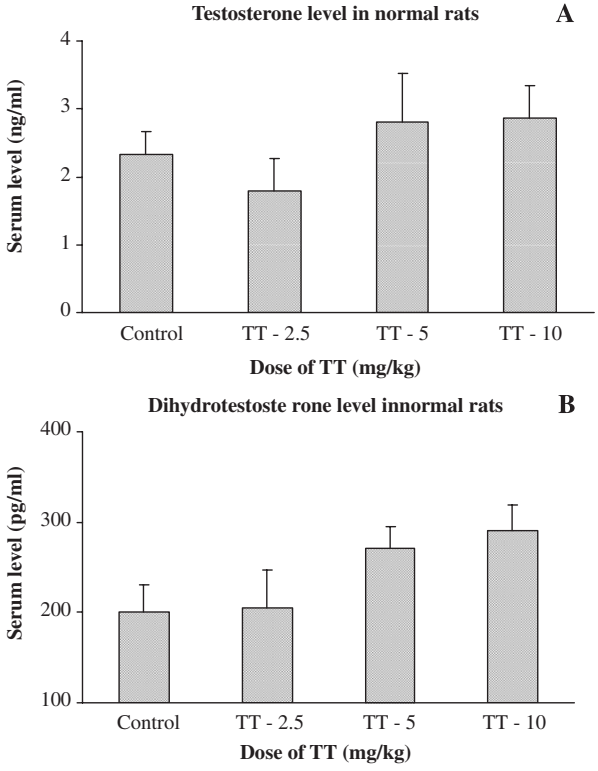

Fig. 8. (A) Serum testosterone, (B) serum dihydrotestosterone level following administration of TT at three different concentrations in normal rat ($n = 10$). The results are compared with control and the values are expressed as mean ±SEM.

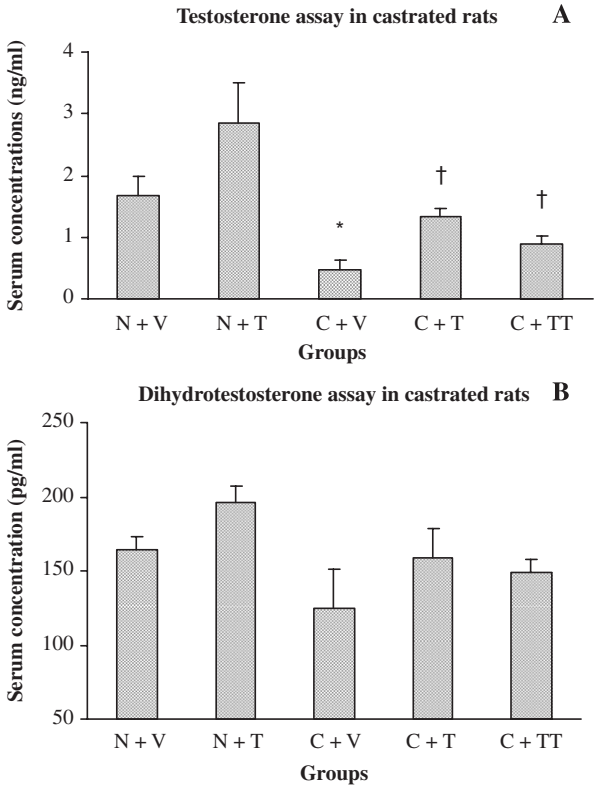

Fig. 9. N = Normal rat; V = vehicle (distilled water); C = castrated rat; T = testosterone; TT = *Tribulus terrestris* extract. (A) Serum testosterone, (B) serum dihydrotestosterone level following administration of TT in castrated rat ($n = 8$). The values are compared between (a) the intact control and rest of the groups, (b) the castrated control and rest of the castrated groups. The values are expressed as mean ±SEM. * and † indicates significant differences ($p < 0.05$) from normal control and castrated control, respectively.

increases in values were not statistically significant (Fig. 8A).

Dihydrotestosterone

Serum dihydrotestosterone showed an increase in TT treated groups compared to control group for all the three concentrations tested. There was a mean maximal increase in serum DHT by 3%, 36% and 45% for the doses 2.5, 5 and 10 mg/kg of TT, respectively. However, the increases in values were not statistically significant (Fig. 8B).

Effect on serum hormone levels in castrated rat

Testosterone

Serum testosterone levels were decreased in the castrated group of rats compared to the intact control. There was a mean maximal decrease in serum testosterone by 71%, 20% and 46% for the groups III, IV and V, respectively. However, only the decrease in value for group III was statistically significant. Within the castrated group there was an increase in serum testosterone concentrations by 51% and 25% for the groups IV and V, respectively. These results were statistically significant (Fig. 9A).

Dihydrotestosterone

Serum dihydrotestosterone levels were also decreased in the castrated group of rats compared to the intact control. There was a mean maximal decrease in serum DHT by 24%, 4% and 9% for the groups III, IV and V, respectively. Within the castrated group there was an increase in serum DHT concentrations by 20% and 15% for the groups IV and V, respectively. These results were not statistically significant (Fig. 9B).

Discussion

Testosterone is secreted in the testes and is the main androgen in the plasma of man. The normal daily production of T is 2.5–11 mg/day. It is reduced at the 5α positions to DHT, which serves as the intracellular

mediator of most actions of T. The pattern of T levels in male shows peak at about 8 weeks of gestation, the neonatal and at puberty, which continues through adult life and later declines. The influence of androgens on androgen receptors and penile growth (Baskin et al., 1997), the erectile function, the responsiveness of the vascular smooth muscle in the corpus cavernosum (Reilly et al., 1997) as well as the fact that DHT is the active androgen in the maintenance of nitric oxide mediated penile erection (Lugg et al., 1995) has been reported earlier.

In the present study, both acute and chronic administration of TT at various concentrations was found to increase the hormone levels. The hormones testosterone and DHT were increased significantly, which could possibly be due to the presence of steroidal glycosides, among them PTN, as one major active principle in TT. The steroidal nature of this compound may facilitate its role as an intermediary in the steroidal pathway of androgen production. It may act either by binding to hormone receptors or to enzymes that metabolize hormones. Most biological actions of plant-derived compounds are brought about by these mechanisms (Baker, 1995). The rise in the level of DHT was proportionate to the increase observed with testosterone. Since DHT is the reduced form of testosterone, the observed increase could probably be due to a primary increase in testosterone level. The administration of Tribestan® a commercial product containing 250 mg of TT to humans and animals for a period of 60–90 days was found to improve testosterone levels, libido, and promote spermatogenesis (Tomova et al., 1981; Koumanov et al., 1982).

Both testosterone and DHT are very essential for a normal sexual function. Mean serum testosterone decreases approximately 1%/year after age 50 years (Morales et al., 2000). Although decline in the levels of testosterone and DHT occurs during male ageing there is no significant changes in the level of DHT compared to testosterone. However, penile erection improves following transdermal administration of DHT in men (Kunelius et al., 2002). The increased sexual behaviour patterns in rats following administration of TT extract observed in our earlier study (Gauthaman et al., 2002) correlates well with the increase in the levels of these androgens in the present study.

Various pharmacological properties of DHEAS, which include anti-obesity effect (Cleary, 1991) and cardioprotective action (Nafizer et al., 1991) have been demonstrated using animal models. However, adrenal production of DHEA and DHEAS are negligible or absent in most laboratory animals including rats and rabbits (Guillemette et al., 1996). Therefore much research on these steroids depends upon human and other close primates. DHEA is a weak androgen precursor and about 1–2 mg/day is produced. DHEA exerts its androgenic activity after its conversion to T/DHT. It is sulphated in the liver to form dehydroepiandrosterone sulphate (DHEAS), which is more stable than DHEA throughout the day owing to its slow clearance (Longscope, 1995).

The hormone DHEAS was increased in the present study on primates. This could probably be due to direct conversion of the extract to DHEA in the system or due to increased synthesis via the steroidal pathway. As mentioned earlier DHEA is mainly secreted by the adrenals. TT may increase the cAMP levels directly thereby leading to increase in DHEA. It is reported that increase in the cAMP level can activate an esterase leading to conversion of cholesterol to pregenalone via cytochrome P450 side chain cleavage (Miller, 1988; Granner, 2000) and DHEA production. In an earlier study, it was reported that PTN increased the level of DHEA (Adimoelja and Adaikan, 1997). Based on the fact that PTN undergoes significant biotransformation in the body (Dikova and Ognyanova, 1983) increasing the level of DHEA, it is suggested that this steroidal saponin is involved in DHEA biosynthesis.

Apart from the hormones studied, the blood pressure recorded in primates had a transient fall immediately following the intravenous administration of the extract that returned to normal baseline recordings in less than a minute. A similar response was found to occur in mongrel dogs, when the aqueous extract was given in the dose of 80 mg/kg (Bose et al., 1963). In another study on dogs, a dose of 20 mg/kg produced a sharp fall in blood pressure (20–50 mmHg) that lasted for about 3 min before returning to baseline values (Chakraborty and Neogi, 1978).

Similar to the changes in blood pressure, there was a transient increase in heart rate by 18–20 beats/min that returned to normal within the next 2–3 min. The PR interval, QRS complex and the QT interval were also within normal limits and the rhythm was always sinus. However, infusion of the extract at a rate of 40 mg/kg in guinea pig has been reported to increase the R voltage, the PR interval and depress the RS-T segment (Seth and Jagadeesh, 1976).

Although we understand from various studies that androgens have a definite role in sexual function and their decline with age leads to andropause and associated symptoms, great caution must be exercised when replacement is to be considered. Replacement therapy is not always efficacious and a good therapeutic outcome is seen only in the hypogonadal states (Howell and Shalet, 2001). This correlates well with the results observed from the present study where increase in hormones were more pronounced in the castrated group of rats than in normal rats following treatment with TT. Estimation of serum levels of luteinizing hormone and follicle-stimulating hormones that would reflect the status of hypothalamo-hypophyseal-gonadal axis would

have provided much clarity. However, androgen substitution in all aging males is not justified.

Unlike the synthetic hormonal preparations that are available today for the treatment of ED which at times can cause potential side effects, the use of a phytochemical that can increase the body's natural androgens will be most after-sought. The plant *T. terrestris* containing PTN is one such product and various studies on PTN indicate that it increases libido and erection, spermatogenesis, sperm motility, ejaculatory volume, muscle mass and testosterone in animal as well as human models. In the present study too it is confirmed that the PTN containing TT-extract increases the levels of T, DHT and DHEAS and that the effect was more pronounced in hypogonadal state. Such increase in androgen levels could be the responsible factor for the age-old claims of PTN as an aphrodisiac and therefore TT may be useful as an adjunct in mild to moderate cases of ED. Further studies on cellular events may shed light on the mechanisms leading to increased androgen levels following the administration of TT. Furthermore it is important to investigate the toxicological potential of the steroidal glycosides and of protodioscin (PTN) in particular.

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References

Adimoelja, A., Adaikan, P.G., 1997. Protodioscin from herbal plant *Tribulus terrestris* L improves male sexual function possibly via DHEA. Int. J. Impot. Res. 9, S64.

Baker, M.E., 1995. Endocrine activity of plant-derived compounds: an evolutionary perspective. Proc. Soc. Exp. Biol. Med. 208 (1), 131–138.

Balanathan, K., Omar, M.H., Zainul Rashid, M.R., Ong, F.B., Nurshaireen, A., Jamil, M.A., 2001. A clinical study on the effect of *Tribulus terrestris* (Tribestan) on the semen profile in males with low sperm count and low motility. Malay. J. Obstet. Gynaecol. 7 (3), 69–78.

Baskin, L.S., Sutherland, R.S., DiSandro, M.J., Hayward, S.W., Lipschultz, J., Cunha, G.R., 1997. The effect of testosterone on androgen receptors and human penile growth. J. Urol. 158, 1113–1118.

Baulieu, E.M., 1996. Dehydroepiandrosterone (DHEA): a fountain of youth? J. Clin. Endocrinol. Metab. 81 (9), 3147–3151.

Bose, B.C., Saifi, A.Q., Vijayvargiya, R., Bhatnagar, J.N., 1963. Some aspects of chemical and pharmacological studies of *Tribulus terrestris* Linn. Indian J. Med. Res. 17 (4), 291–293.

Burns-Cox, N., Gingell, C., 1997. The andropause: fact or fiction? Postgrad. Med. J. 73, 553–556.

Chakraborty, B., Neogi, N.C., 1978. Pharmacological properties of *Tribulus terrestris* Linn. Indian J. Pharm. Sci. 40, 50–52.

CHEMEXCIL, 1992. *Tribulus terrestris* Linn. (N.O.-Zygophyllaceae). In: Selected Medicinal Plants of India (A Monograph of Identity, Safety and Clinical Usage). Compiled by Bharatiya Vidya Bhavan's Swamy Prakashananda Ayurveda Research Centre for CHEMEXCIL. Tata Press, Bombay. pp. 323–326 (Chapter 10).

Chopra's Indigenous drugs of India, 1958. Second edition, revised by Chopra, R.N., Chopra, I.C., Handa, K.L., and Kapoor, L.D. U.N. Dhur & Sons Private Ltd., Calcutta, pp. 430–431.

Cleary, M.P., 1991. The antiobesity effect of dehydroepiandrosterone in rats. Proc. Soc. Exp. Biol. Med. 196, 8–16.

Conte, F., Grumbach, M., 1995. Pathogenesis, classification, diagnosis and treatment of anomalies of sex. In: De Groot, L.J. (Ed.), Endocrinology, third ed. W.B. Saunders Co., Philadelphia, PA, pp. 618–670 (Chapter 29).

Dikova, N., Ognyanova, V., 1983. Pharmacokinetic studies of Tribestan. Anniversary Scientific Session'35 Chemical Pharmaceutical Research Institute, Sofia.

Dikova, N., Ognyanova, 1993. Pharmacokinetic studies of Tribestan. Anniversary Scientific Session'35 Chemica.

Gauthaman, K., Adaikan, P.G., Prasad, R.N.V., 2002. Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in castrated rats. Life Sci. 71 (12), 1385–1396.

Granner, D.K., 2000. Hormones of the adrenal cortex. In: Barnes, D.A., Ransom, J., Roche, J. (Eds.), Harper's Biochemistry. Appleton and Lange, Stamford, CT, pp. 575–587.

Guillemette, C., Hum, D.W., Belanger, A., 1996. Levels of plasma C19 steroids and 5 alpha-reduced C19 steroid glucuronides in primates, rodents, and domestic animals. Am. J. Physiol. 271 (2 Pt 1), E348–E353.

Hinman, F.J., 1993. Penis and male urethra. In: UroSurgical Anatomy. W.B. Saunders Co., Philadelphia, PA, p. 418.

Howell, S., Shalet, S., 2001. Testosterone deficiency and replacement. Horm. Res. 56 (1), 86–92.

Koumanov, F., Bozadjieva, E., Andreeva, M., Platonova, E., Ankova, V., 1982. Clinical trial of Tribestan. Exp. Med. 4, 211–215.

Kunelius, P., Lukkarinen, O., Hannuksela, M.L., Itkonen, O., Tapanainen, J.S., 2002. The effects of transdermal dihydrotestosterone in the aging male: a prospective, randomized, double blind study. J. Clin. Endocrinol. Metab. 87 (4), 1467–1472.

Longscope, C., 1995. The metabolism of DHEA. Dehydroepiandrosterone (DHEA) and aging. Ann. NY Acad. Sci. 774, 143–148.

Lugg, A.J., Rajfer, J., Gonzalez-Cadavid, N.F., 1995. Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. Endocrinology 136, 1495–1501.

Manieri, C., Di Bisceglie, C., Tagliabue, M., Fornengo, R., Zumpano, E., 1997. Hormonal control of sexual behaviour

in males and endocrinologic causes of sexual dysfunction. Minerva Endocrinol. 22 (2), 37–43.

Miller, W.L., 1988. Molecular biology of steroid hormone synthesis. Endocr. Rev. 9, 295–318.

Mills, T.M., Reilly, C.M., Lewis, R.W., 1996. Androgens and penile erection: a review. J. Androl. 17, 633–638.

Morales, A., Heaton, J.P., Carson III, C.C., 2000. Andropause: a misnomer for a true clinical entity. J. Urol. 163 (3), 705–712.

Nafizer, A.N., Harrington, D.M., Bush, T.L., 1991. Dehydroepiandrosterone and dehydroepiandrosterone sulphate: their relation to cardiovascular disease. Epidemiol. Rev. 13, 267–293.

Reilly, C.M., Stopper, V.S., Mills, T.M., 1997. Androgens modulate the alpha-adrenergic responsiveness of vascular smooth muscle in the corpus cavernosum. J. Androl. 18, 26–31.

Seth, S.D., Jagadeesh, G., 1976. Cardiac action of *Tribulus terrestris*. Indian J. Med. Res. 64 (12), 1821–1825.

Tenover, J.L., 1997. Testosterone and the aging male. J. Androl. 18, 103–106.

Tomova, M., Gjulemetova, R., Zarkova, S., Peeva, S., Pangarova, T., Simova, M., 1981. Steroidal saponins from *Tribulus terrestris* L. with a stimulating action on the sexual functions. In: International Conference of Chemistry and Biotechnology of Biologically Active Natural Products, Varna, Bulgaria, September 21–26, 1981, vol. 3, pp. 298–302.

Vermeulen, A., 1991. Androgens in the aging male. J. Clin. Endocrinol. Metab. 73, 221–224.

Wang, B., Ma, L., Lim, T., 1990. 406 cases of angina pectoris in coronary heart diseases treated with saponin of *Tribulus terrestris*. Chung His Chieh Ho Tsa Chih 10 (2), 85–87.