THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 9, Number 2, 2003, pp. 257–265

Sexual Effects of Puncturevine (*Tribulus terrestris*) Extract (Protodioscin): An Evaluation Using a Rat Model

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ABSTRACT

Objective: Apart from its claims for improvement of sexual functions in men, the puncturevine plant (*Tribulus terrestris:* TT) has long been considered as an energizer and vitalizer in the indigenous system of medicine. Sexual behavior and intracavernous pressure (ICP) measurements were taken in rats to scientifically validate the claim of TT [containing protodioscin (PTN)] as an aphrodisiac.

Materials and methods: Forty sexually mature male Sprague–Dawley rats were randomly divided into four groups of 10 each. Group I served as a control group and groups II, III, and IV were treated with three different doses of TT extract (2.5, 5 and 10 mg/kg body weight, respectively), orally, once daily for 8 weeks. Weight was recorded and the rats from all four groups were subjected to sexual behavior studies with primed females and various parameters namely mount and intromission frequencies (MF and IF, respectively), mount, intromission and ejaculation latencies (ML, IL, and EL, respectively) as well as postejaculatory interval (PEI) were recorded. In addition, blood pressure and ICP were recorded for all rats at the end of study.

Results: Increases in body weight (by 9, 23, and 18% for groups II, III & IV) and ICP (by 43% and 26% for groups III and IV) were statistically significant compared to the control group. Increases in MF (by 27% and 24%) and IF (by 19% and 22%) for the groups III and IV were statistically significant. Decreases in ML (by 16%, 23%, and 22% for groups II, III, and IV) and PEI (by 20% for group III) were statistically significant compared to the control.

Conclusions: The weight gain and improvement in sexual behavior parameters observed in rats could be secondary to the androgen increasing property of TT (PTN) that was observed in our earlier study on primates. The increase in ICP which confirms the proerectile aphrodisiac property of TT could possibly be the result of an increase in androgen and subsequent release of nitric oxide from the nerve endings innervating the corpus cavernosum.

INTRODUCTION

Sexual behavior essentially reflects on the normal functioning of the hypothalamo–pituitary–gonadal axis. It encompasses sexual motivation (libido) as well as sexual performance. Sexuality in mammals helps species survival. In males, the sexual performance depends very much upon the integration and coordination of various anatomical and physiologic factors that finally brings about a rise in the corporal system and provides the requisite

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penile tumescence and rigidity for successful sexual activity. Apart from reproduction, sexuality is practiced in humans for purposes of providing pleasure, bolstering self-esteem, fostering intimacy, and reducing anxiety or tension. Both males and females typically experience normal sexual activity throughout adult life. Sexual and other physiologic functions decline with advancing years (Baulieu, 1996). Apart from aging people, healthy young people who are unable to carry out sexuals act normally suffer from psychogenic, organic, or mixed etiologies leading to erectile dysfunction (ED). In males, this is defined as the inability to achieve or maintain an erection that is sufficient for satisfactory sexual performance (NIH Consensus conference, 1993).

Unlike in the past, when such an affliction was considered to be a social stigma, people are more accepting of this inability, considering it to be like any other disease. This positive change has become possible owing to the extensive research that has provided better understanding of the physiopathologic processes of ED and development of effective treatment modalities. The fact that the phosphodiesterase inhibitor, sildenafil citrate, has been widely used clinically in the management of ED explains the acceptance of this disease entity in society and patients' willingness to undergo treatment as well. Although various therapeutic options are currently available for treating ED, an effective alternative oral therapy that could improve sexual functions and libido without any harmful side-effects will contribute tremendously to the therapeutic armamentarium.

Puncture vine (*Tribulus terrestris*; TT) Linn. is a perennial creeping herb with a worldwide distribution. The entire plant (and particularly the fruits) are used in an indigenous system of medicine for treating various ailments, such as urinary infections, eye infections, leucorrhoea, vitiligo, and impotence (Chemexcil, 1992; Chopra, 1958; Chopra et al., 1965; Wang et al., 1997). The extract obtained from the air-dried aerial parts of the plant TT contain mainly steroidal glycosides (saponins) of the furasatanol-type, isolated by preparative column chromatography on silica gel and elution with $CHCl_3$–CH_3OH–H_2O, the predominant active component being protodioscin (PTN, Fig. 1) which is 45% of dry weight (Dikova and Ognyanova, 1983; Tomova et al., 1981). Administration of this extract to humans and animals improves libido and spermatogenesis (Tomova et al., 1981). PTN is also found to increase the levels of testosterone, leutinizing hormone (Koumanov et al., 1982), dehydroepiandrosterone (Adimoelja and Adaikan, 1997), dihydrotestosterone and dehydroepiandrosterone sulphate (Adaikan et al., 2001; Gauthaman et al., 2000). Corpus cavernosal tissues obtained from New Zealand White rabbits following treatment with TT extract tested *in vitro* with various contractile and relaxing pharmacologic agents as well as electrical-field stimulation was found to have a proerectile effect (Adaikan et al., 2000).

To understand the stimulant effects of TT extract in relation further to sexual motivation and sexual activity, this current study was performed using a rat model in which sexual behavior and ICP were studied.

MATERIALS AND METHODS

Forty (40) adult male Sprague–Dawley (SD) rats, approximately 6 weeks' old and weighing between 200 g and 250 g were fed on standard rat pellets (Glen Forrest Stock Feeders, Glen Forrest, Western Australia) and water *ad libitum*. The animals were housed under standard laboratory conditions and maintained on a reverse light–dark cycle (10 PM–10 AM) for a minimum of 2 weeks prior to the study for adap-

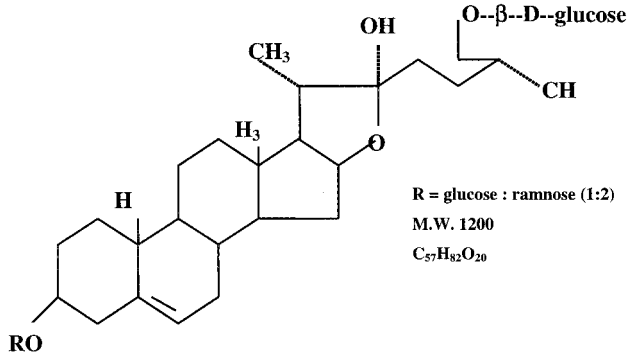

FIG. 1. Chemical structure of protodioscin.

tation. The rats were divided into four experimental groups of 10 each. Group I served as a control and the rats were given the vehicle (distilled water) only. The test groups (II–IV) were treated with the extract obtained from the plant TT (Sopharma Joint Stock Co., Sofia, Bulgaria). The extract, prepared in an aqueous solution, was administered orally in a once-daily regimen at three different doses (2.5, 5, and 10 mg/kg body weight for groups II, III, and IV, respectively) for a period of 8 weeks. The weight of the animals was recorded at 0 and 8 weeks.

Ovariectomized SD rats used for the copulatory studies were brought to estrus by sequential administration of estradiol benzoate (10 μg/100g body weight) and progesterone (500 μg/100g body weight), via subcutaneous injections, 48 hours and 4 hours before the copulatory studies, respectively (Srilatha, 1998). A baseline sexual behavior study was carried out in rats from all groups to render them sexually experienced and was then repeated following administration of the vehicle and extract to the male rats, both in the control and test groups at 0 and 8 weeks.

Sexual behavior studies were carried out in a separate room under dim red illumination according to the standard procedure (Dewsbury, 1972). Each male rat was placed in a rectangular plexiglass chamber, 10 minutes before the introduction of a primed female, for the male rat to get acclimatized to the chamber conditions. The primed female rat was then introduced into the chamber and the following sexual behavior parameters were recorded:

1. *Mount frequency (MF)*—the number of mounts without intromission from the time of introduction of the female until ejaculation
2. *Intromission frequency (IF)*—the number of intromissions from the time of introduction of the female until ejaculation
3. *Mount latency (ML)*—the time interval between the introduction of the female and the first mount by the male
4. *Intromission latency (IL)*—the interval from the time of introduction of the female to the first intromission by the male (characterized by pelvic thrusting and springing dismount)
5. *Ejaculation latency (EL)*—the time interval between the first intromission and ejaculation (characterized by longer, deeper pelvic thrusting and a slow dismount followed by a period of inactivity)
6. *Postejaculatory interval (PEI)*—the time interval between ejaculation and the first intromission of the following series.

The experiment was terminated when each male rat begins to mount each female again after a brief period of inactivity (this period of refractoriness of the male rat to the female is usually observed after ejaculation) or following 30 minutes of sexual inactivity by the male rat from the time of introduction of the female into the testing chamber.

The male rats at the completion of behavior studies were investigated for cavernous nerve stimulation and measurement of intracavernous pressure (ICP), which is considered as a measure of penile integrity (Chen et al., 1992). The rats were anesthetized using pentobarbital sodium at a dose of 45 mg/kg body weight, intraperitoneally, with 5–10 mg/kg intravenous (IV) supplements, if necessary. The temperature was maintained between 36°C and 37°C, using a heating source. An IV line was established through the right external jugular vein for saline infusion and IV supplements of the anesthetic agent. Tracheal cannulation was performed to avoid respiratory difficulties and to keep the animals stable throughout the procedure. The left internal carotid artery was cannulated for continuous blood-pressure recording. Via a perineal dissection, a 27-ga needle filled with heparinized saline (250 U/mL) and fitted with PE10 tubing was inserted into the right crus for recording ICP. Via abdominal dissection, the cavernous nerve was identified at its exit from the major pelvic ganglion on the medial aspect and traced toward the penis (Matrinez-Pineiro et al., 1994). The cavernous nerve was gently "teased" from the prostatic capsule and hooked to a stainless-steel bipolar electrode for nerve stimulation. The two arms of the electrode were separated by a distance of 1 mm and each arm was 0.2 mm in diameter. The stimulation parameters were 2 V and a frequency of 20 Hz that produced consistent pressure recordings. The contact time was 45 seconds per stimulation.

TABLE 1. WEIGHT RECORDED IN DIFFERENT GROUP OF RATS ($n = 10$)

Group (n = *10)*	*Dose of TT mg/kg*	*Weight (g) at 0 and 8 weeks*	
I	Vehicle only	210 ± 4	513 ± 6
II	2.5	202 ± 4	557 ± 9*
III	5.0	207 ± 6	633 ± 10*
IV	10.0	207 ± 5	605 ± 5*

TT, *Tribulus terrestris*.

The results are compared between the control and the TT-treated groups and the values are expressed as mean ± standard error of the mean.

*Indicates significant difference ($p < 0.05$) from control.

STATISTICAL METHODS

The independent variables for subjects in the different experimental groups were compared and analyzed by one-way analysis of variance (ANOVA) and comparisons were made using Bonferroni procedure. All the results were expressed as mean ± standard error of the mean and the level of significance for comparisons set at $p < 0.05$.

RESULTS

Increases in weight were observed in the treated groups of rats (at 8 weeks) compared to the controls; this increase in weight was 9, 23, and 18%, respectively, for groups II, III, and IV. These results were statistically significant (Table 1).

Sexual behavior response

The male rats responded to the females by ano-genital sniffing, followed by pursuit and mounting behaviors. These acts of succession were followed by pelvic thrusting (intromissions) until ejaculation (confirmed by the relative inactivity of the male for a short period—a maximum of 6.54 ± 0.15 minutes). The responses among the rats in the control group were similar in all rats studied at different time intervals. The slight differences in sexual behavior parameters compared within the control group at different time intervals were not statistically significant.

The mount and intromission frequencies recorded from the TT-treated groups showed an increase compared to the rats in the control group (Figs. 2 and 3). The mean increases in MF were 12%, 27%, and 24% and the mean increases in IF were 16%, 19%, and 22% for the doses 2.5, 5, and 10 mg/kg of TT extract, respectively. However, only the increases in MF and IF for the doses 5 and 10 mg/kg of TT extract were statistically significant.

The mount, intromission, and ejaculation latencies showed an overall decrease in the TT-treated groups compared to the rats in the control group (Figs. 4–6). Mean decreases in ML were 16%, 23%, & 22% for the doses 2.5, 5, and 10 mg/kg of TT extract, respectively. These results were statistically significant. Marginal decreases were observed for IL (by 2%, 7%, and 6%) for the doses 2.5, 5, and 10 mg/kg of TT extract, respectively. The mean decreases in EL were 12%, 27%, and 18% for the doses 2.5,

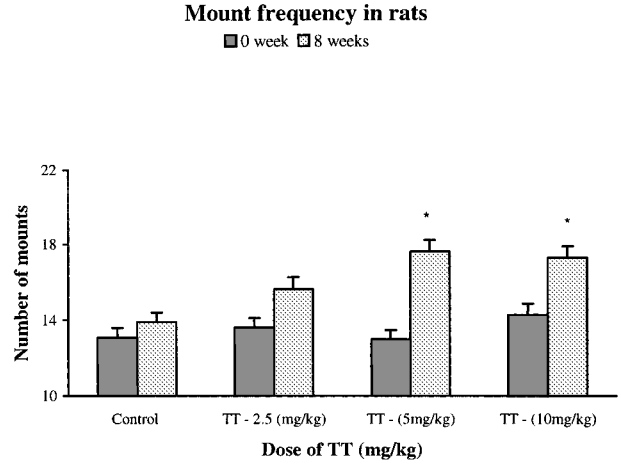

FIG. 2. The number of mounts recorded in different groups of rats ($n = 10$). The results are compared between the control and the *Tribulus terrestris* (TT)–treated groups. The values are expressed as mean ± standard error of the mean. *Indicates significant differences ($p < 0.05$) from control.

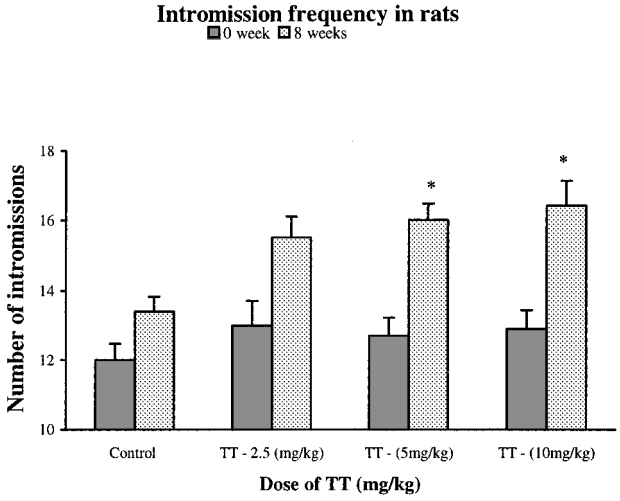

FIG. 3. The number of intromissions recorded in different groups of rats ($n = 10$). The results are compared between the control and the *Tribulus terrestris* (TT)–treated groups. The values are expressed as mean ± standard error of the mean. *Indicates significant differences ($p < 0.05$) from control.

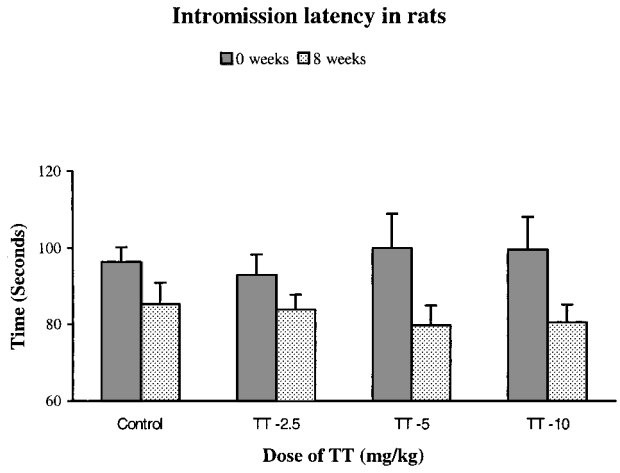

FIG. 5. The intromission latencies recorded in different groups of rats ($n = 10$). The results are compared between the control and the *Tribulus terrestris* (TT)–treated groups. The values are expressed as mean ± standard error of the mean.

5, and 10 mg/kg of TT extract, respectively. The decreases observed in EL for doses 5 mg/kg and 10 mg/kg were statistically significant compared to the control.

Postejaculatory interval also showed a decrease in the TT-treated groups compared to the control group (Fig. 7). The mean decreases in PEI were 13%, 20%, and 16% for the doses 2.5, 5, and 10 mg/kg of TT extract, respectively. However, only the decrease observed for the dose 5 mg/kg of the TT extract was statistically significant.

Blood pressure response

Blood pressure (BP) was recorded in all animals from the control and treatment groups (Fig. 8). The mean increases in BP were 6%, 7%, and 11% for the doses 2.5, 5, and 10 mg/kg of TT extract, respectively. BP was stable through-

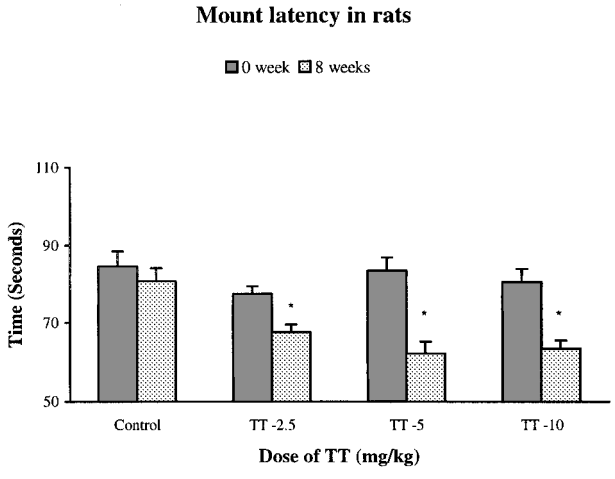

FIG. 4. The mount latencies recorded in different groups of rats ($n = 10$). The results are compared between the control and the *Tribulus terrestris* (TT)–treated groups. The values are expressed as mean ± standard error of the mean. *Indicates significant differences ($p < 0.05$) from control.

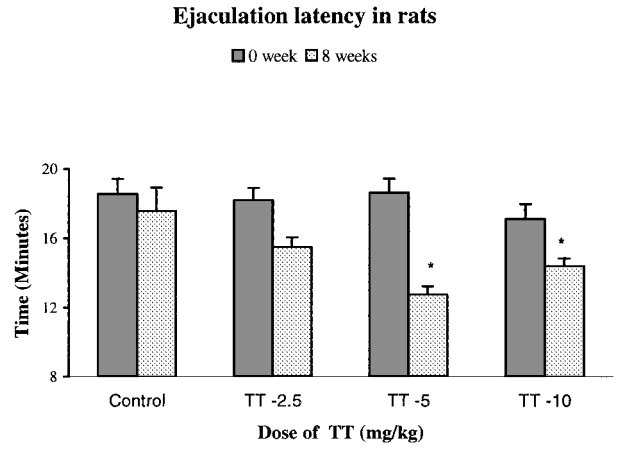

FIG. 6. The ejaculatory latencies recorded in different groups of rats ($n = 10$). The results are compared between the control and the *Tribulus terrestris* (TT)–treated groups. The values are expressed as mean ± standard error of the mean. *Indicates significant differences ($p < 0.05$) from control.

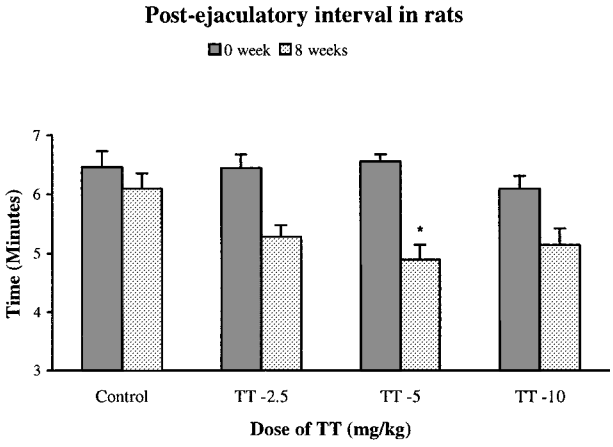

FIG. 7. The postejaculatory interval recorded in different groups of rats ($n = 10$). The results are compared between the control and the *Tribulus terrestris* (TT)–treated groups. The values are expressed as mean ± standard error of the mean. *Indicates significant differences ($p < 0.05$) from control.

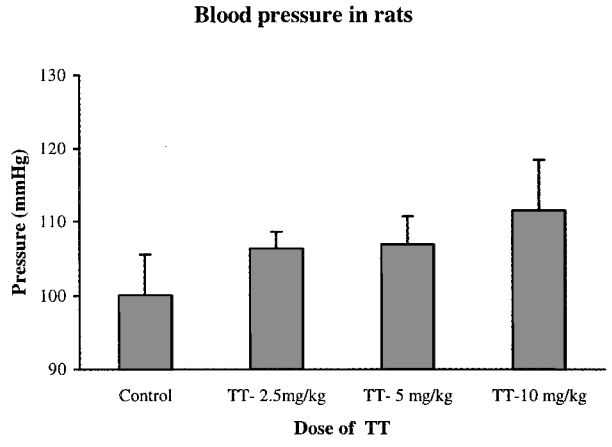

FIG. 8. The blood pressure recorded in the control and treated groups of rats ($n = 10$). The results are compared between the groups and the values are expressed as mean ± standard error of the mean. TT, *Tribulus terrestris*.

out the procedure and there were no significant variations between the control and treatment groups.

ICP response

The increase in ICP during electrical stimulation coincided with the increase in the length and girth of the penis of each male rat. This tumescent effect immediately returned to normal upon withdrawal of the stimulus. There was an associated decrease in BP during electrical stimulation in all of the animals. The mean increases in ICP were 17%, 43%, and 26% for the doses 2.5, 5, and 10 mg/kg of TT extract, respectively. The mean increase in ICP for the doses 5 and 10 mg/kg of TT extract was statistically significant (Fig. 9).

DISCUSSION AND CONCLUSION

In the present study, the rats showed an improvement in sexual behavior pattern following administration of TT extract at different concentrations. MF and IF were increased and EL, IL, ML, and PEI were decreased following continued TT administration for 8 weeks. The statistically significant increase in MF and IF and the decrease in ML and IL in the treated groups indicated the readiness of the TT-treated animals to respond to the estrus female. Desire is an essential component of sexual activity and TT increased motivation in rats as reflected by increase in MF, IF, and decrease in ML and IL. In addition, the TT-treated rats were able to achieve ejaculation much more quickly than the control rats as observed by decrease in EL. Although this appears to contribute to premature ejaculation, it reflects the increase seen in IF, indicating improved motivation. Moreover, the TT-treated animals were able to resume sexual activity much more quickly than the rats in the control group fol-

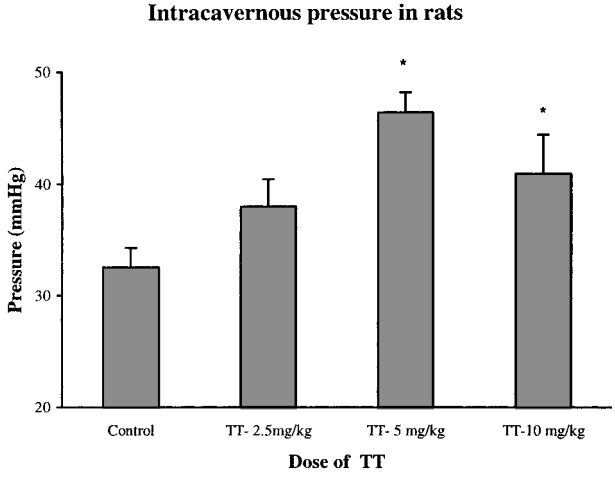

FIG. 9. The intracavernous pressure recorded in the control and treated groups of rats ($n = 10$). The results are compared between the control and the *Tribulus terrestris* (TT)–treated groups and the values are expressed as mean ± standard error of the mean. *Indicates significant differences ($p < 0.05$) from control.

lowing ejaculation as evidenced by the decreases in PEI. These observations indicate that TT extract has the capacity to improve central and peripheral effects in rats. Sexuality declines with age and supplementation with androgenic hormones are known to restore libido (Read, 1999; Tenover, 1999). The levels of testosterone, dihydrotestosterone, and dehydroepiandrosterone sulphate were also found to be increased in primates following intravenous administration of the TT extract (Gauthaman et al., 2000). Hence, it appears that steroidal pathways with stimulation of the androgenic targets may be involved in the mechanism of these actions of TT in rats. Considerable weight gain observed in this study could also be the result of the androgenic effect of TT, secondarily contributing to appetite stimulation. Androgenic action of testosterone increases lean-muscle mass (Forbes, 1992) and the weight gain is predominantly the result of enhancement of appetite (Wilson, 1996). Steroidal saponins from fenugreek [*Trigonella foenum-graecum*) seeds have been shown to enhance appetite and reduce the cholesterol level in rats (Petit et al., 1995). It is suggested that the steroidal pathway is a possible mechanism for the improvement in sexual behavior as well as the weight gain observed in rats following TT. Further studies are necessary to understand the mechanism for the increase in weight. The minimal increase in BP in the treated group could also have been secondary to the androgenic effect of the TT, because testosterone and dehydroepiandrosterone sulfate have been reported to increase systolic BP in humans (Giorgi et al., 1999; Schunkert et al., 1999). The transient fall in BP observed during the electrical stimulation of the cavernous nerve could possibly have been the result of the release of nitric oxide (NO) and the resultant vasodilatation.

ICP measurement in rats is widely accepted as an index of penile erection (Chen et al., 1992). The cavernous nerve stimulation increased ICP in all of the rats following treatment with TT at various doses. Cavernous nerves are responsible for erection, and correlation of behavioral response with electrophysiologic studies in animals confirm the role of these nerves in penile erection (Dahlof and Larsson, 1976). Cavernous tissue from rabbits treated with TT has been shown to improve nitrergic neurotransmission *in vitro* (Adaikan et al., 2000). The increase in ICP observed in the present investigation was probably the result of the release of NO from the nerve endings innervating the *corpus cavernosum* (CC), which in turn caused relaxation of the penile smooth muscle allowing blood to flow in with a subsequent rise in ICP. The increase in systemic androgen level observed in this study may have also played a role in NO release at the CC level and could possibly have contributed to the increase in ICP, because androgens are known to influence the penile erection reflex and nitric oxide synthase activity in the penis (Seo et al., 1999).

CONCLUSIONS

In conclusion, TT extract appears to possess sexual enhancement properties as reflected from the sexual behavior studies and the ICP measurements. None of the treated rats had any negative, antierectile responses. The proerectile responses, however, were mild in these normal rats. It is possible that such responses would be more marked if the rats were had a low androgenic status before treatment with TT. The results were more pronounced at a lower dosage level, and increasing the dose of TT up to 40 mg/kg body weight did not produce dose-related increases in response (data not shown). This may possibly have been the result receptor saturation and drug uptake. Further study may reveal additional information on the androgenic effect of the TT extract.

The present study has provided scientific evidence in rats for the age-old claims for TT as a sexual stimulant. The probable mechanisms leading to these effects are:

(1) Direct conversion of the active ingredient present in the extract namely, PTN to dehydroepiandrosterone (DHEA) in the body and its central effect as a neurosteroid. This possibility is supported by the fact that PTN is structurally related steroids and undergoes significant biotransformation in the body (Dikova and Ognyanova, 1983; Tomova et al., 1981)
(2) The increase in DHEA following TT extract may bring about an antagonistic effect on

gamma aminobutyric acid (GABA), thereby facilitating sexual function. It is known that DHEA has antagonistic reactions to GABA (Majewska, 1995). GABA produces an inhibitory effect on intromission patterns of male sexual behavior and motor activity (Agmo and Contreas, 1990; Agmo and Paredes, 1985); concentration of GABA has been found to be increased during the post-ejaculatory interval that marks the period of sexual inactivity in rats (Fernandez-Guasti et al., 1986)

(3) Increase in the levels of testosterone and its reduced form, dihydrotestosterone, both acting centrally and peripherally to promote sexual behavior, because androgenic hormones are known to restore libido (Tenover, 1999) and improve nitrergic transmission in the cavernous tissue of rabbits (Adaikan et al., 2000).

(4) The final common pathway could be an upregulation of NO synthesis in the medial preoptic area, which, in turn, may increase dopamine within the areas of sexual arousal to mediate the increased libido and sexual activity (Hull et al., 1997, 1999) and at the peripheral level via nitrergic neurotransmission (Adaikan et al., 2000).

Further studies are in progress to investigate these possibilities.

ACKNOWLEDGMENTS

The authors thank Sopharma Joint Stock Co., Bulgaria, for providing the *Tribulus terrestris* extract that was used in this study.

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