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Life Sciences 71 (2002) 1385–1396

Life Sciences

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Aphrodisiac properties of *Tribulus Terrestris* extract (Protodioscin) in normal and castrated rats

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Received 15 November 2001; accepted 1 May 2002

Abstract

Tribulus terrestris (TT) has long been used in the traditional Chinese and Indian systems of medicine for the treatment of various ailments and is popularly claimed to improve sexual functions in man. Sexual behaviour and intracavernous pressure (ICP) were studied in both normal and castrated rats to further understand the role of TT containing protodioscin (PTN) as an aphrodisiac. Adult Sprague-Dawley rats were divided into five groups of 8 each that included distilled water treated (normal and castrated), testosterone treated (normal and castrated, 10 mg/kg body weight, subcutaneously, bi-weekly) and TT treated (castrated, 5 mg/kg body weight, orally once daily). Decreases in body weight, prostate weight and ICP were observed among the castrated groups of rats compared to the intact group. There was an overall reduction in the sexual behaviour parameters in the castrated groups of rats as reflected by decrease in mount and intromission frequencies (MF and IF) and increase in mount, intromission, ejaculation latencies (ML, IL, EL) as well as post-ejaculatory interval (PEI). Compared to the castrated control, treatment of castrated rats (with either testosterone or TT extract) showed increase in prostate weight and ICP that were statistically significant. There was also a mild to moderate improvement of the sexual behaviour parameters as evidenced by increase in MF and IF; decrease in ML, IL and PEI. These results were statistically significant. It is concluded that TT extract appears to possess aphrodisiac activity probably due to androgen increasing property of TT (observed in our earlier study on primates).

Keywords: *Tribulus terrestris*; Protodioscin; Castration; Sexual behaviour; Intracavernous pressure; Androgens; Aphrodisiac

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0024-3205/02/$ - see front matter
PII: S0024-3205(02)01858-1

Introduction

The role of androgens in sexuality is unequivocal. Embryonic differentiation of the fetus into a male and its subsequent growth along this line is essentially due to the presence of physiological amounts of androgens (especially testosterone and its metabolite dihydrotestosterone) in the body [1]. Apart from these two major androgens, dehydroepiandrosterone secreted from the adrenals also contribute significantly to the overall androgenic status and therefore influence the sexual characteristics [2]. Reduction in the levels of these steroids (hypogonadism) at the early developmental phase leads to structural abnormalities of the penis [3,4]. Hypogonadism in adulthood may result in loss of libido and sexual activity and androgen replacement has been found to be effective in restoration of these conditions [5–8]. Androgens contribute to penile erection by acting in concert with the other determinants of penile erectile physiology. Androgen receptors mediate the effects of androgens and their presence in various tissues has been demonstrated [9–11]. Using the rat model it has been demonstrated previously that sexual behaviour and erection are androgen dependent (acting both centrally and peripherally) and that treatment with testosterone of the castrated rats helped in restoration of both sexual behaviour and penile erectile capacity [3,6,12]. It has also been demonstrated by various studies that androgens regulate corporal nitric oxide synthase (NOS) activity [13–15].

The aphrodisiac properties of *Tribulus terrestris* extract (TT) that contains protodioscin (PTN), a steroidal saponin that forms 45% (dry weight) of the extract was explored in castrated rats. The plant TT or its products have been extensively used both in the Chinese and Indian traditional medicine for the treatment of various ailments such as urinary, cardiovascular, and gastrointestinal disorders [16–18]. Administration of TT to humans and animals improves libido and spermatogenesis [19]. PTN is also found to increase the levels of testosterone, leutinizing hormone [20], dehydroepiandrosterone [21], dihydrotestosterone and dehydroepiandrosterone sulphate [22]. The corpus cavernosal tissues obtained from New Zealand White rabbits following treatment with TT were tested *in vitro* with various pharmacological agents and electrical field stimulation and was found to have a proerectile effect [23]. The present study was carried out to further understand the androgen releasing property of the TT extract (PTN) and its relation to sexual behaviour and intracavernous pressure (ICP) using castrated rats.

Materials and methods

Forty adult male Sprague-Dawley (SD) rats approximately 6 weeks old and weighing between 200–250 g were fed on standard rat pellets (Glen Forest Stock Feeders, 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 two weeks prior to the study to facilitate adaptation. The rats were divided into five experimental groups of 8 each. Of these, two groups were intact and three groups were castrated. The rats were treated either with distilled water, testosterone or TT extract as follows. Group I: Intact + distilled water, Group II: Intact + testosterone, Group III: Castrated + distilled water, Group IV: Castrated + testosterone and Group V: Castrated + TT extract. Testosterone cypionate (Pharmacia and Upjohn) was administered in the dose of 10 mg/kg body weight, subcutaneously, bi-weekly for 8 weeks. TT (Sopharma, Bulgaria), was administered in the dose of 5 mg/kg body weight (the optimal dose that had significant effect in our previous studies),

orally, once daily for 8 weeks. The weight of the animals was recorded at zero and eight weeks from all the groups.

Ovariectomised SD rats were used for the copulatory studies. The female rats were brought to oestrus by sequential administration of oestradiol benzoate (10 μg/100 g body weight) and progesterone (500 μg/100 g body weight), through subcutaneous injections, 48 hours and 4 hours before the copulatory studies respectively [24]. A baseline sexual behaviour study was carried out in rats from all groups to render them sexually experienced and were then repeated following the treatment schedule as mentioned above.

Sexual behaviour studies were carried out in a separate room under dim red illumination according to the standard procedure [25]. The male rat was placed in a rectangular plexiglass chamber, 10 minutes before the introduction of a primed female, for it to get acclimatized to the chamber conditions. The primed female was then introduced into the chamber and the following sexual behaviour parameters were recorded:

a) mount frequency (MF): the number of mounts without intromission from the time of introduction of the female until ejaculation,
b) intromission frequency (IF): the number of intromissions from the time of introduction of the female until ejaculation,
c) mount latency (ML): the time interval between the introduction of the female and the first mount by the male,
d) 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),
e) ejaculation latency (EL): the time interval between the first intromission and ejaculation (characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity),
f) post-ejaculatory interval (PEI): the time interval between ejaculation and the first intromission of the following series.

The experiment was terminated when the male rat begins to mount the female followed by intromission after a brief period of inactivity (which normally results following ejaculation).

The male rat at the completion of behaviour studies were taken up for investigation on cavernous nerve stimulation and measurement of ICP considered as a measure of penile erection [26]. The rats were anaesthetized using pentobarbital sodium 45 mg/kg body weight, intraperitoneally with 5–10 mg/kg intravenous supplements, if necessary. The temperature was maintained between 36°C–37°C using a heating source; an intravenous line established through the right external jugular vein, for saline infusion and intravenous supplements of the anaesthetic agent if needed. Tracheal cannulation was done to avoid respiratory embarrassment and to keep the animal stable throughout the procedure. The left internal carotid artery was cannulated and blood pressure was recorded. Through a perineal dissection, a 27 G needle filled with heparinised saline (250 units/ml) and fitted with PE10 tubing was inserted into the right crus for recording ICP. Through abdominal dissection, cavernous nerve was identified at its exit from the major pelvic ganglion on the medial aspect and traced towards the penis [27]. 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 volts and a frequency

of 20 Hz that produced consistent pressure recordings. The contact time was 45 seconds/stimulation. At the end of the study the animal was sacrificed and the wet weight of prostate measured from all the groups.

Statistical methods

The independent variables for subjects in the different experimental groups were compared and analyzed by two factors ANOVA with repeated measures of one factor and comparisons were made using Bonferroni procedure. All the results were expressed as mean ± SEM and the level of significance for comparisons set at $P<0.05$.

Results

Body and prostate weight

Considerable decreases in both the body weight (by 11.80%, 6.09% and 9.63%) and prostate weight (by 41.29%, 8.04% and 26.70%) for the groups III, IV and V respectively were observed among the castrated groups of rats compared to the intact group. These parameters were statistically significant. Within the castrated group the increase in prostate weight (by 43.28% and 29.85% for group IV and V respectively) were statistically significant compared to the castrated control (Table 1).

Sexual behaviour

The rats from both the castrated and intact groups were paired with females that were brought to oestrus prior to experimentation. The males from the intact group readily responded while the castrated group showed a diminished sexual response to the females. Unlike the intact group, the castrated rats were mostly withdrawn towards the common pre-copulatory behaviours such as anogenital sniffing, nosing and chasing.

Table 1
Weight recorded in different groups of rats (n=8)

Group (n=8 SD rats)	Body weight (G) at 8 weeks	Prostate Weight (G) at 8 weeks
	Mean ± SEM	Mean ± SEM
N + V	636.25 ± 15.80	1.02 ± 0.03
N + T	650.00 ± 29.22	1.24 ± 0.06
C + V	560.00 ± 11.80†	0.67 ± 0.01†
C + T	597.50 ± 11.91	0.96 ± 0.02*
C + TT	575.00 ± 8.66†	0.87 ± 0.01†,*

N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. The results 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 control for **a** and **b** respectively.

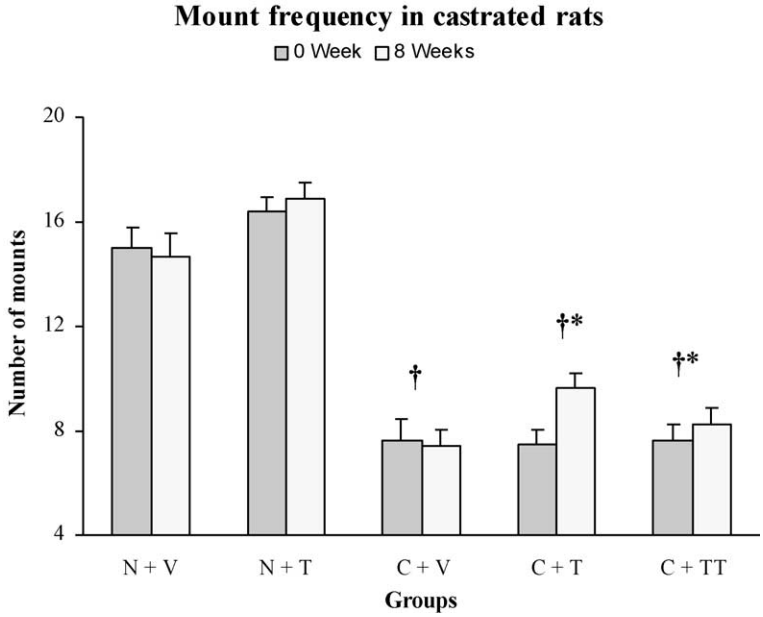

Fig. 1. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Number of mounts recorded in different groups of rats (n = 8). The results are compared between **a)** the intact control and rest of the groups, **b)** the castrated control and rest of the castrated groups. The values are expressed as mean ± SEM. † and * indicates significant differences ($p < 0.05$) from control for **a** and **b** respectively.

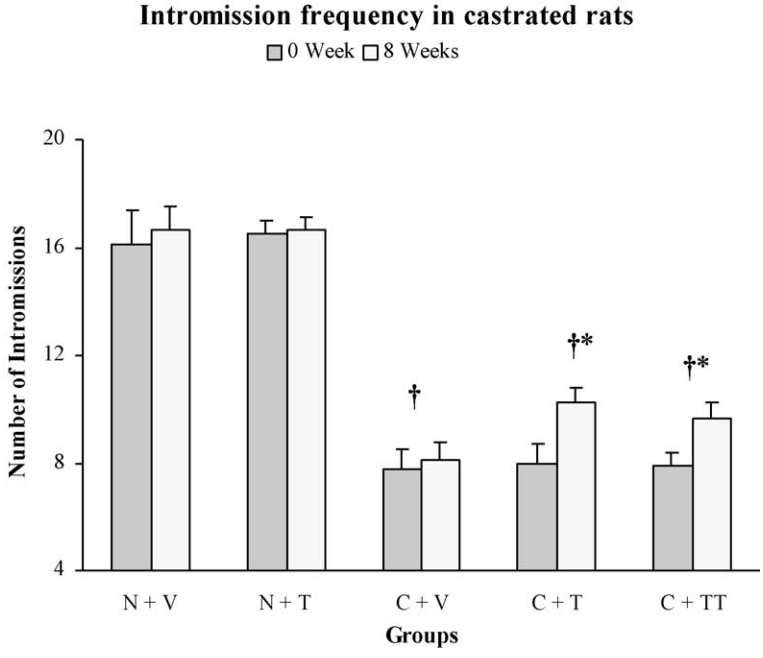

Fig. 2. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Number of intromissions recorded in different groups of rats (n = 8). The results are compared between **a)** the intact control and rest of the groups, **b)** the castrated control and rest of the castrated groups. The values are expressed as mean ± SEM. † and * indicates significant differences ($p < 0.05$) from control for **a** and **b** respectively.

Castrated groups showed a decrease in mount and intromission frequencies (Figs. 1 and 2). Decrease in MF (by 49.57%, 34.19% and 43.59%) and IF (by 51.13%, 38.35% and 42.11%) for the groups III, IV and V respectively were statistically significant. In addition within the castrated group, both testosterone and TT treated groups showed mild to moderate increase in both MF (by 30.51% and 11.86%) and IF (by 26.15% and 18.46%) for the groups IV and V respectively that were statistically significant.

Mount and intromission latencies showed an increase in both these parameters among the castrated groups of rats compared to the intact group that were statistically significant (Figs. 3 and 4). Increase in ML (by 70.76%, 39.95% and 52.71%) and IL (by 88.74%, 38.05% and 49.77%) were observed for the groups III, IV and V respectively. Within the castrated group, both the testosterone and TT treated groups showed minimal decrease in both ML (by 18.04% and 10.57%) and IL (by 26.86% and 20.65%) for the groups IV and V respectively that were statistically significant.

Ejaculation latency and the post-ejaculatory interval also showed considerable increase among the castrated groups of rats compared to the intact group (Figs. 5 and 6). The increase in EL (by 40.57%, 11.86% and 17.50%) and PEI (by 65.13%, 33.66% and 59.84%) for the groups III, IV and V were statistically significant. Among the castrated group, both the testosterone and TT treated groups showed moderate decrease in both EL (by 20.42% and 16.41% for the groups IV and V respectively) and PEI (by 19.06% for group IV) that were statistically significant.

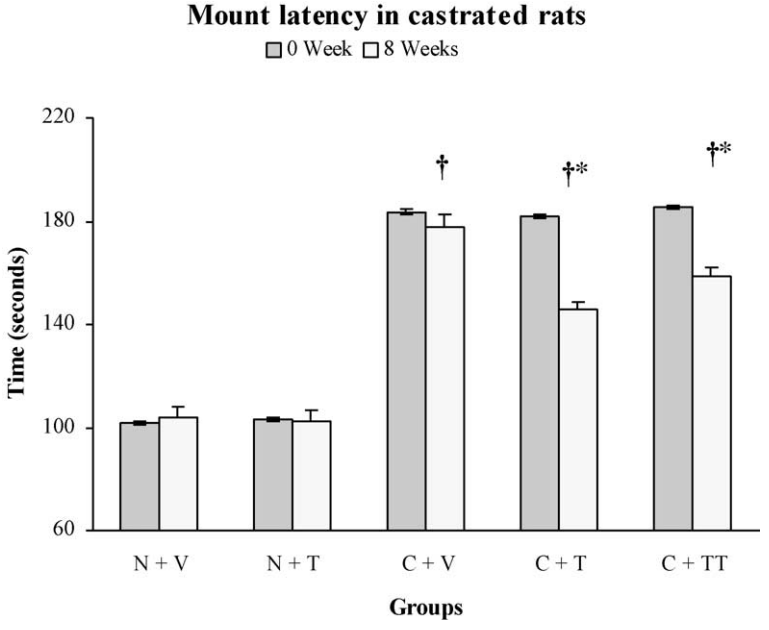

Fig. 3. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Mount latencies recorded in different groups of rats (n = 8). The results are compared between **a)** the intact control and rest of the groups, **b)** the castrated control and rest of the castrated groups. The values are expressed as mean ± SEM. † and * indicates significant differences ($p < 0.05$) from control for **a** and **b** respectively.

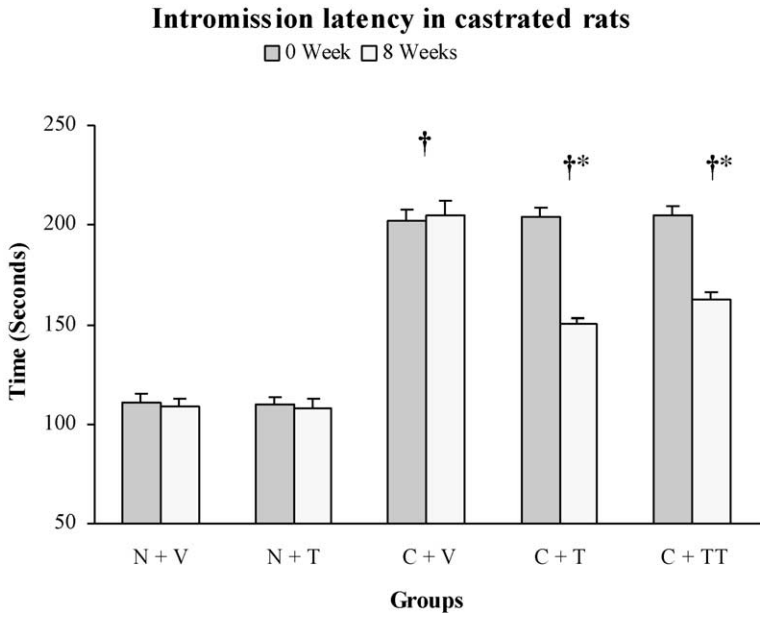

Fig. 4. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Intromission latencies recorded in different groups of rats (n = 8). The results are compared between **a)** the intact control and rest of the groups, **b)** the castrated control and rest of the castrated groups. The values are expressed as mean ± SEM. † and * indicates significant differences ($p < 0.05$) from control for **a** and **b** respectively.

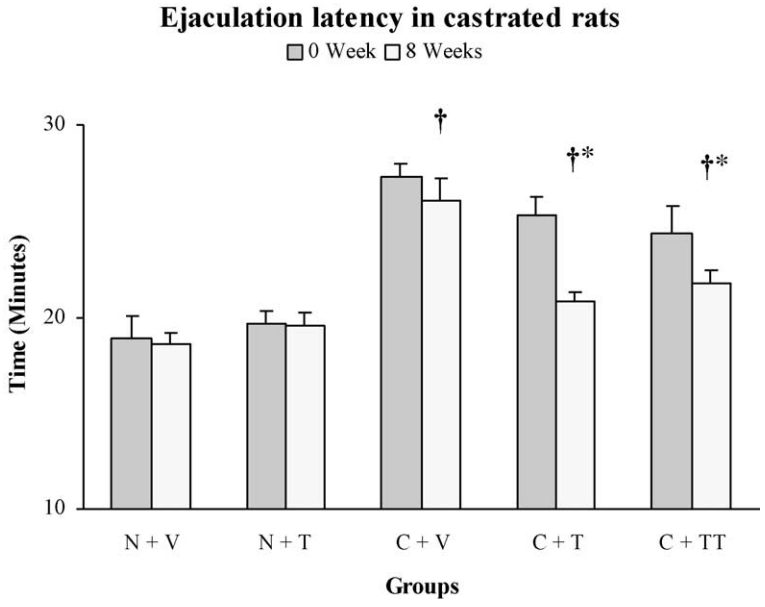

Fig. 5. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Ejaculatory latencies recorded in different groups of rats (n = 8). The results are compared between **a)** the intact control and rest of the groups, **b)** the castrated control and rest of the castrated groups. The values are expressed as mean ± SEM. † and * indicates significant differences ($p < 0.05$) from control for **a** and **b** respectively.

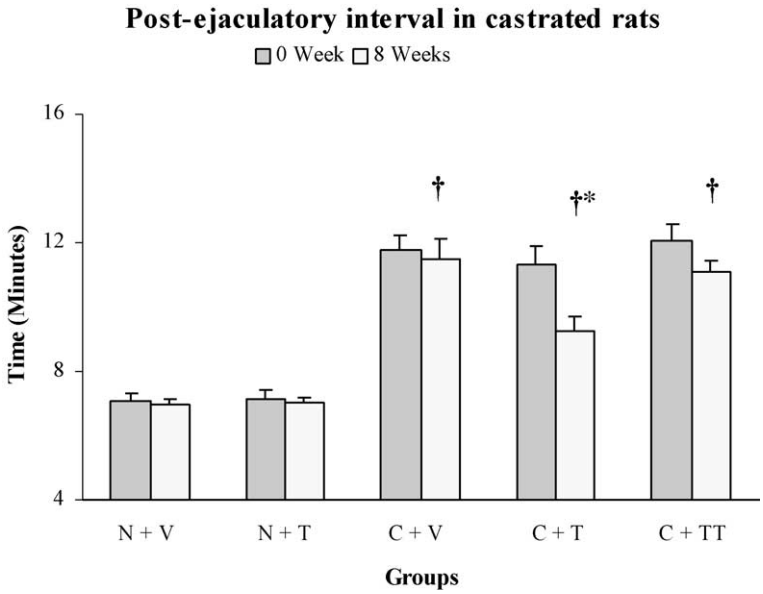

Fig. 6. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Post-ejaculatory interval recorded in different groups of rats (n = 8). The results are compared between **a)** the intact control and rest of the groups, **b)** the castrated control and rest of the castrated groups. The values are expressed as mean ± SEM. † and * indicates significant differences ($p < 0.05$) from control for **a** and **b** respectively.

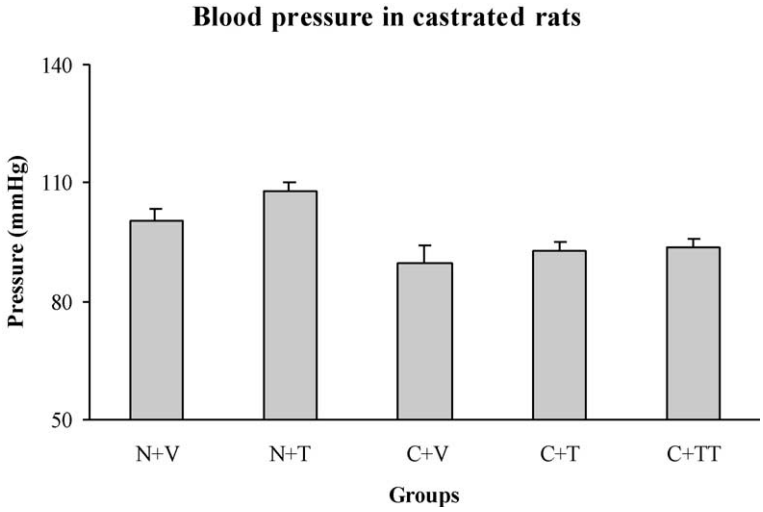

Fig. 7. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Blood pressure recorded in the control and treated groups of rats (n = 8). The results are compared between the groups and the values are expressed as mean ± SEM.

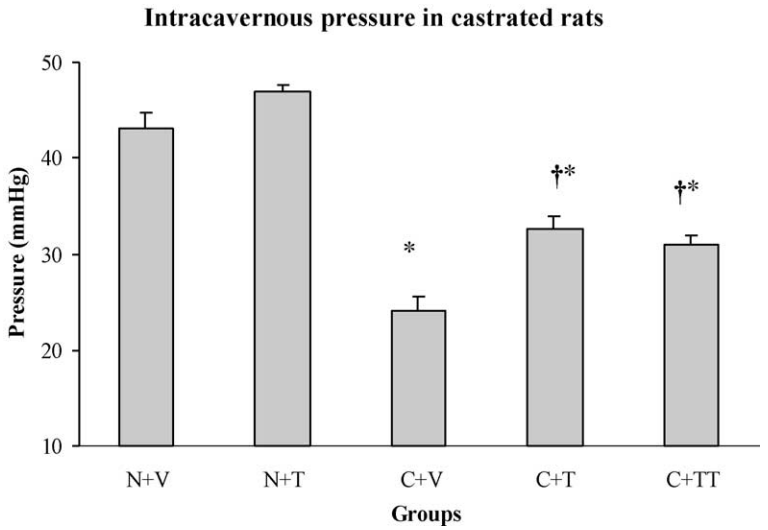

Fig. 8. N = Normal rat; V = Vehicle; C = Castrated rat; T = Testosterone; TT = Tribulus terrestris extract. Intracavernous pressure recorded in the control and treated groups of rats (n = 8). The results are compared between **a**) the intact control and rest of the groups, **b**) the castrated control and rest of the castrated groups. The values are expressed as mean ± SEM. † and * indicates significant differences ($p < 0.05$) from control for **a** and **b** respectively.

Blood pressure and intracavernous pressure

There was a decrease in blood pressure among the castrated group of rats compared to the control by 11.64%, 7.8% and 6.51% for the groups III, IV and V respectively. The decrease observed were however not statistically significant (Fig. 7). ICP was markedly decreased in the castrated group compared to the intact control. There was a decrease in ICP by 44.05%, 24.42% and 28.38% for the groups III, IV and V respectively (Fig. 8) that were statistically significant. Treatment with testosterone and TT recorded an increase in ICP (by 35.08% and 28.01%) for the groups IV and V that were statistically significant compared to the castrated control (group III).

Discussion and conclusion

Animal studies from various centres indicate that sexuality decreases with low androgenic status and is restored following androgen replacement. In this current study we attempted to investigate the effect of TT using the castrated rat, a convenient model for studying the effect of androgens in relation to the sexual characteristics [3]. In this study the overall sexual behaviour parameters were markedly reduced among the castrated groups of rats compared to the intact group. Castration leads to low androgenic status affecting structural, biochemical, pharmacological or any of these components of erectile physiology, which in turn cause reduction in erectile function as observed from studies on various animal models [28,29]. Decrease in MF and increase in both ML and IL in castrated rats of this study

implies reduction in the desire component of sexuality. Physiologically low levels of androgen as seen in hypogonadism is associated with decreased sexual desire and activity [30,31]. The observed decrease in IF as well as the increase in EL among the castrated group of rats indicates reduction in performance.

Apart from desire that is essential for initiation of sex, penile tumescence and rigidity as well as the accessory muscles that helps in providing additional penile rigidity and ejaculation are also dependent on androgen for a normal sexual activity. Various neurotransmitters and their inter/intracellular signaling are responsible for the relaxation of corpus cavernosal smooth muscle (CCSM). Androgens influence these neurotransmitters and contribute to the regulation of penile erection. Restoration of apomorphine-induced erections in castrated rats following administration of exogenous testosterone indicates the central influence of androgens in penile erection. However, the exact mechanism of action is still to be elucidated [6]. The peripheral effects of androgens appear to be better understood. The decrease in density of nonadrenergic noncholinergic fibres following castration and its reversal to near normal levels as well as differential inhibition and restoration of NOS activity following testosterone supplementation explains the association of androgens especially testosterone with nitric oxide (NO) mediated erectile activity [13,15].

In the present study, upon treatment with either testosterone or TT the sexual behaviour in the castrated groups of rats improved compared to the castrated control. The early loss of ejaculatory capacity followed by cessation of intromission and mounting in castrated rats and the restoration of these parameters with testosterone supplementation explain the role of testosterone in sexual function [32,33]. As regards to TT, its active ingredient PTN improves libido and spermatogenesis in humans and animals [19]; increase the levels of testosterone, leutinizing hormone [20] and dehydroepiandrosterone [21]. The improvement in sexual behaviour as noted by increase in MF and IF, and decreases in ML, IL, EL and PEI following TT administration to the castrated group of rats for eight weeks correlate well with that of testosterone replacement although at the given dose it is not as effective as testosterone itself.

In addition the increase in body weight and prostate weight in both testosterone and TT treated castrated groups compared to the castrated control confirms the role of androgens and a possible contribution by TT via similar mechanism. The role of androgens and its regulatory effect on prostate have been studied earlier [34]. There were no significant changes as regards to blood pressure (BP) following treatment with TT.

The increase in ICP among the castrated group following testosterone and TT treatment implies their role on NO and erectile function. It has been studied earlier that stimulation of cavernous nerve leads to increase in NO and cGMP signaling resulting in CCSM relaxation. The subsequent arteriolar dilatation leading to increased arterial inflow and impaired venous return (due to engorgement of the cavernosum) builds up a pressure system within the corpora that results in penile tumescence and rigidity. Restoration of ICP has been observed in castrated rabbits and rats following testosterone replacement [15,35]. Inclusion of pharmacological agents such as prostaglandin E_1, papaverine or sildenafil citrate (that regulate the cAMP/cGMP levels needed for the initiation of erection) in this study could have provided additional information to better understand the mechanism for the observed effects of TT. Blood pressure remained more or less stable; mild decrease observed in the castrated group of rats implies that BP to some extent is regulated by androgens. It has earlier been observed in dogs that the mean arterial pressure decreased following castration [36].

The continued administration of the extract for eight weeks in this study could have possibly increased the androgenic status both centrally and peripherally. DHEA is a major circulating steroid in human plasma, mainly synthesized by the adrenal glands and to a lesser degree by the gonads; it is a

common precursor for both androgens and oestrogens [37]. TT containing PTN, has been shown to increase the DHEA level in man [21]. DHEA is considered as a neurosteroid; it acts centrally as a gamma amino butyric acid antagonist to facilitate sexual function [38]. Possible increase in DHEA and its subsequent conversion to testosterone and its metabolites may account for the observed effects in this study. It is far from clear how such increase in DHEA is brought about by this plant extract; the mechanism for this effect needs to be clarified. Much detailed studies on the hormonal aspects as well as the levels of intracellular nucleotides need to be studied to better understand the clinical usefulness of TT. As such TT may still contribute as a conditioner for the treatment of mild to mid-level erectile dysfunction. However, this study provides additional information as regards to androgens and its regulation of penile erection and that the plant *Tribulus terrestris* is found to exert similar properties to that of androgens. Results of this study provide evidence for the claimed role of this plant as an aphrodisiac in the traditional medicine.

Acknowledgements

We would like to thank Sopharma Joint Stock Co., Bulgaria, for providing the *Tribulus terrestris* extract used in this study.

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