

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X

JPHYTO 2019; 8(3): 117-123

May- June

Received: 14-05-2019

Accepted: 03-06-2019

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DOI: 10.31254/phyto.2019.8306

Rudy Valdès Simo

Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé1, P.O. Box 812 Yaoundé, Cameroon

Sylvin Benjamin Ateba

Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé1, P.O. Box 812 Yaoundé, Cameroon

Stéphane Zingué

i) Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé1, P.O. Box 812 Yaoundé, Cameroon

ii) Department of Life and Earth Sciences, Higher Teachers' Training College, University of Maroua, P.O. Box 55 Maroua, Cameroon

Constant Anatole Pieme

Department of Biochemistry and Physiological Sciences, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, P.O. Box 1364 Yaoundé, Cameroon

Dieudonné Njamen

Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé1, P.O. Box 812 Yaoundé, Cameroon

Correspondence:

Dieudonné Njamen

Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé1, P.O. Box 812 Yaoundé, Cameroon
Email: dnjamen[at]gmail.com

Baillonella toxisperma improves sexual performance and protects against stress-induced reproductive dysfunction in male Wistar rats

Rudy Valdès Simo, Sylvin Benjamin Ateba, Stéphane Zingué, Constant Anatole Pieme, Dieudonné Njamen

ABSTRACT

Baillonella toxisperma Pierre (Sapotaceae) is a medicinal plant widely used in Central Africa against several diseases including erectile dysfunction and male infertility. However, no study dealing with these male reproductive dysfunctions has been published until now. Accordingly, the present study was undertaken. It evaluated the capacity of an aqueous extract of stem bark of *B. toxisperma* (BT) at 75, 150 and 300 mg/kg/d to induce aphrodisiac effects and prevent the stress-induced reproductive dysfunction in males using normal non-experienced (an 8-day oral treatment) and stressed (a 6-h/day immobilization stress for 35 consecutive days) male Wistar rats. Both in normal and stressed animals, BT at the doses of 150 and 300 mg/kg decreased ($p < 0.05$) the mount and intromission latencies, and increased ($p < 0.05$) the number of total penile licking, mount and intromission as well as ejaculation frequencies. In stressed animals, a significant ($p < 0.05$) increase in sperm levels, sperm mobility as well as in the relative weight of androgen-dependent organs (testis, epididymis and seminal vesicles) was observed at the same doses compared with the stressed control. These results suggest that this aqueous extract of *B. toxisperma* might endow with aphrodisiac and androgenic properties in normal and stressed male Wistar rats.

Keywords: Stress-induced sexual dysfunction, *Baillonella toxisperma*, Aphrodisiac, sexual behaviour, male Wistar rats.

INTRODUCTION

Infertility is an inability for a couple to achieve pregnancy after a 1-year period of regular unprotected sex [1]. In several African countries like Cameroon, 20-30 % of couples suffer from infertility [2]. Around 50 % of infertility is attributable to a male factor alone [3]. Male sexual dysfunction (MSD) including reduced libido, erectile dysfunction, and orgasmic and ejaculation disorders are important causes of male infertility [4-6]. MSD has vascular, neurologic, psychological, and hormonal causes as well as concomitant morbidities and risk factors including chronic alcohol abuse, cigarette smoking, androgen deficiency, aging, psychological disorders, diabetes mellitus, hypertension, dyslipidemia, prostatic diseases, obesity and metabolic syndrome, side effects of some drugs and stress [5-7].

Stress is an unavoidable phenomenon and thus, every person faces stressful situations in day-to-day life [8]. In men, adverse effects of stress on reproductive system have been described and related to MSD and infertility depending on the type and duration of the stressor. Globally acute and chronic stress decrease sexual motivation [9], cause low plasma testosterone [10], germ cell loss and apoptosis [9, 11-13], alter spermatogenesis [14] and reduce sperm count and motility [15-16]. Immobilization stress model is an easy and convenient method [17] to evaluate the effects of stress on male reproductive function as it causes both psychological and physiological stress by the restriction of movement, aggression, feeling of distress and burn out [18]. This model has been associated with testicular tissue injury, decrease in testosterone secretion, reducing number of spermatogonia, primary and secondary spermatocytes and spermatids, alteration of sexual behavior and delay of testicular maturation, decreased weights of testis and male accessory sexual organs [19-21].

Since decades, oral phosphodiesterase 5 inhibitors (PDE5Is) such as sildenafil are treatments of choice against erectile dysfunction (ED). However, they are expensive for several populations including Sub-Saharan Africa [22, 23], effective only in 60-70% of men [24] and associated with side effects such as headache, flushing, hypertension, dyspepsia and nasal congestion [25, 26]. Usually, immediate stronger efficacy is obtained at the cost of a higher risk of side effects, and tolerability of the drug at the cost of lower efficacy [27]. In this condition, various alternatives are being investigated and there is a growing

interest in plants/herbs with a long-standing traditional use and advocated for improving or curing MSD with varying degrees of success.

Baillonella toxisperma Pierre (Sapotaceae) is a plant distributed in primary tropical rain forests in hot and humid/wet climates of West and Central Africa [28-30]. Throughout this region the entire tree is used in traditional medicine, especially in the Cameroonian pharmacopeia to treat more than 50 diseases including toothache, hemorrhoids, sexually transmitted infections, diarrhea, malaria, vaginal and oral mycoses, rheumatism, anemia, female infertility and gynecological problems, intestinal worms, diabetes, kidney pain, back aches and itches [31-35]. Aqueous extracts of stem bark are used against male infertility and impotence [36, 37]. Antibacterial [38-40], antifungal [41, 42] and anti-protozoa [43] activities have been reported. Bark and seed oil are widely sold in local markets [44-46]. However, despite a long standing traditionally and widely use of this medicinal plant there is no scientific report dealing with the male physiological-related reproductive dysfunction. Accordingly, the present work was carried out to evaluate the potential of the aqueous extract of stem bark of *B. toxisperma* on male sexual behavior (aphrodisiac action) and reproductive system dysfunction using normal and immobilization-related stress Wistar rats and therefore, validate its traditional use.

MATERIAL AND METHODS

Chemicals

The 17 β -estradiol benzoate was obtained from Sigma-Aldrich (Hambourg, Germany). Progesterone was obtained from Sigma-Aldrich (St. Louis, USA). Sildenafil citrate (Viagra) was obtained from Micron® (Bangalore, India).

Animals

Healthy male (12 weeks old; 200-220 g) and female (10-12 weeks old; 160-180 g) Wistar rats (*Rattus norvegicus*) were obtained from the breeding facility of the Laboratory of Animal Physiology, University of Yaoundé I (Cameroon). Animals were housed in clean plastic cages placed in well-ventilated house conditions. They had free access to rat diet and tap water *ad libitum*. The experiments were conducted according to the guidelines of the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Innovation (Reg. no. FWA-IRD 0001954), which has adopted the guidelines established by the European Union on Animal Care (CEE Council 86/609).

Plant material, extraction and estimation of the doses

The stem barks of *Baillonella toxisperma* were harvested in September 2014 in Ekok (Department of Mefou Afamba, Central Region of Cameroon). The sample was identified and authenticated by Mr. Victor NANA, Botanist at the National Herbarium of Cameroon (HNC) in Yaoundé (Voucher specimen 14592SRF Cam).

The extraction was performed based on the traditional recipe used in Bansa (West Region of Cameroon) to treat male infertility and reported by Noumi *et al.* (2011) [37]. Air-dried powder (250 g) of stem bark of *B. toxisperma* was boiled in 2.5 L of water for 15 minutes. Cooled decoction was filtered using Wattman filter paper n°4 and lyophilized (Christ Beta 1-8 K, Bioblock scientific, Germany) to yield 20.22 g (8%) of extract.

Knowing the volume of decoction daily used to treat male infertility (250 mL, 2 times) [37] and after its lyophilization, the pharmacological dose in human was estimated to 24 mg/kg/day. Allometric calculations according to the U.S. Department of Health & Human Services [47] allowed the estimation of the equivalent dose in rat of 150 mg/kg. This dose was framed by the doses of 75 and 300 mg/kg.

Phytochemical screening

The concentrations of phytochemicals such as total phenols, flavonoids, as well as the antioxidant power were measured in the aqueous extract of *Baillonella toxisperma* as described previously [48-50].

Study design

Estrus induction

Sixty female Wistar rats were ovariectomized under ketamin and valium anesthesia (10 and 50 mg/kg BW, *i.p.*, respectively). Fourteen days after the bilateral ovariectomy, estrus was induced by subcutaneous injections of 17 β -estradiol benzoate (0.1 mg/100 g BW) and benzoate progesterone (0.5 mg/100 g BW) 48 h and 4 h, respectively, before introducing them to the male [51].

Aphrodisiac activity in normal rats

In this experiment 30 rats were randomly assigned into five groups of six animals each. Group I served as control and received distilled water; Group II received sildenafil citrate (reference) at the dose of 5 mg/kg BW. Group III to V received the aqueous extract of stem bark of *B. toxisperma* at doses of 75, 150 and 300 mg/kg BW, respectively. Treatments were given by gavage for 8 consecutive days. Male sexual behavior parameters such as copulation and male-oriented activity (latencies of first mount, intromission and ejaculation, numbers of mount, intromission, ejaculation and penile licking) towards an estrus female were recorded for 30 min by blinded observers on days 1 and 8 in a quiet room under dim light [52]. Animals were sacrificed by decapitation on day 9 and organs including right testis, epididymis, seminal vesicles, ventral prostate and penis were collected and weighed.

Effects of *B. toxisperma* on stress-induced male reproductive dysfunction

Thirty non-experimented Wistar rats were divided in 5 groups of 6 animals each and submitted to a 35-day oral treatment starting from the first day of stress induction. The first group serving as normal control was not exposed to stress and received distilled water. In the four other groups animals were individually submitted to the immobilization stress (6 hours/day from 8 to 14 a.m in Plexiglas cylinder (5 cm I.D x 16 cm) with holes for breathing) and put back in their home cages for treatment as follow: one group received distilled water and served as negative control; the remaining three groups received the aqueous extract of *B. toxisperma* at the doses of 75, 150 and 300 mg/kg/day, respectively. The process was repeated for 35 consecutive days. Water and food were withdrawn during the daily 6-h period of stress [53]. Animals were weighed once weekly and the above mentioned male sexual behavior parameters recorded for 30 min by blinded observers on days 1, 18 and 35 in a quiet room under dim light. Twenty four hours after the last administration, animals were sacrificed by decapitation and organs (testis, epididymis, seminal vesicles, ventral prostate and

penis) collected and weighed. These organ weights are reported as relative weights (g/100 g BW). The sperm count was determined as earlier described [54]. Regarding the sperm motility, it was assessed as described previously [55].

Histological analysis

Paraffin-embedded testis were cut to 5 µm sections and stained with hematoxylin and eosin. Following hematoxylin-eosin staining, sections were visualized (magnification: 200X) using a Zeiss Axioskop 40 microscope connected to a computer where the image was transferred and analyzed with the MRGrab1.0 and Axio Vision 3.1 softwares, all provided by Zeiss (Hall-bermoos, Germany).

Statistical analysis

Data from each experimental group were expressed as mean ± SEM. Two-way analysis of variance (ANOVA) followed by Dunnet’s post hoc test for multiple comparisons were performed using GraphPad Prism software version 3.10. Treated groups were compared to the normal and negative controls. The significance of the difference was fixed at p<0.05.

RESULTS

Phytochemical analysis

The concentration of total phenols and flavonoids in the aqueous extract of *B. toxisperma* as well as its antioxidant power are depicted in Table 1. Total phenols were the most abundant secondary metabolites in the dried extract with an amount of 290.54 ± 2.09 mg eququercetin/g. The antioxidant power was estimated to be 635.67 ± 0.60mg eququercetin/g of dried extract.

Table 1: Quantitative analysis of selected phytochemicals present in *B. toxisperma* aqueous extract

Phytochemical class	Concentration in <i>B. toxisperma</i> extract
Total phenols	290.54 ± 2.09
Flavonoids	0.03 ± 0.00
Antioxidant power	635.67 ± 0.60

Flavonoids and antioxidant power are expressed in mg eququercetin/g of dried extract. Total phenols are expressed as mg of gallic acid equivalent (GAE)/g of dry weight. Data are represented as mean ± SEM of triplicate from at least three independent experiments.

Effects of the aqueous extract of *B. toxisperma* on normal rats

Effects on male sexual behavior

Like sildenafil citrate (5 mg/kg), the aqueous extract of *B. toxisperma* at the doses of 150 and 300 mg/kg induced a significant (p <0.05) decrease of first mount, intromission and ejaculation latencies, while significantly increased the number of mount, intromission and penile licking on days 1 and 8 as compared to the control group (Table 2).

Effects of the extract on the relative weight of organs

While Viagra® (Sildenafil citrate) did not affect the relative wet weights of sex organs, the aqueous extract of *B. toxisperma* showed a significant increase (p < 0.01) of the relative weight of the testis at doses of 150 and 300 mg/kg as compared with the control group, while no change was found in other organs (Table 3).

Table 2. Effects of an 8-day treatment with the aqueous extract of *B. toxisperma* on male sexual behavior

		Latency for first mount (sec)	Number of mount	Latency for first intromission (sec)	Number of intromission	Latency for first ejaculation (sec)	Number of ejaculation	Number of penilelicking
Control	Day 1	68.3 ± 4.3	21.4 ± 3.1	107.2 ± 8.3	18.8 ± 2.3	401.4 ± 31.3	1.4 ± 0.4	20.4 ± 1.9
	Day 8	89.6 ± 7.0	25.8 ± 1.0	119.4 ± 4.1	29.0 ± 1.3	411.8 ± 13.9	1.8 ± 0.5	23.2 ± 1.7
Sildenafil citrate (5 mg/kg)	Day 1	30.3 ± 3.2**	40.3 ± 2.7**	50.6 ± 6.1**	27.4 ± 1.7*	326.4 ± 40.7*	2.6 ± 0.2*	27.3 ± 3.1
	Day 8	25.7 ± 6.3***	45.7 ± 3.4**	62.9 ± 10.3**	38.6 ± 1.7*	335.2 ± 10.6*	2.2 ± 0.4	32.6 ± 2.4*
BT 75 mg/kg	Day 1	70.4 ± 7.8	19.2 ± 1.6	113.6 ± 12.6	17.6 ± 1.4	397.7 ± 27.9	1.2 ± 0.2	23.4 ± 2.1
	Day 8	67.3 ± 6.4	38.2 ± 4.5	108.4 ± 12.4	30.6 ± 2.1	399.9 ± 12.3	1.2 ± 0.2	25.4 ± 1.9
BT 150 mg/kg	Day 1	39.5 ± 6.2**	33.8 ± 1.9*	56.6 ± 8.0**	28.3 ± 2.8*	281.2 ± 69.4*	1.8 ± 0.4	32.4 ± 3.9*
	Day 8	30.2 ± 3.8**	49.0 ± 5.5**	41.6 ± 13.0**	48.9 ± 4.8*	297.9 ± 27.3*	2.6 ± 0.5	34.4 ± 2.5*
BT 300 mg/kg	Day 1	32.6 ± 2.5**	33.8 ± 1.6*	43.4 ± 13.5**	31.4 ± 1.5*	256.5 ± 34.3*	1.8 ± 0.4	31.8 ± 1.9*
	Day 8	25.4 ± 7.7***	50.2 ± 6.6***	50.2 ± 9.8**	49.2 ± 6.1*	265.6 ± 9.4*	2.8 ± 0.4	34.6 ± 1.8*

Control = normal rats receiving distilled water; BT = normal rats receiving *B. toxisperma* aqueous extract at doses of 75, 150 and 300 mg/kg. * p< 0.05, **p < 0.01, ***p < 0.001 versus Control.

Table 3: Effects of an 8-day treatment with an aqueous extract of *B. toxisperma* on the wet relative weights of androgenic-dependent organs

	Testis	Epididymis	Prostate	Seminal vesicles	Penis
Control	1.33 ± 0.03	0.40 ± 0.02	0.12 ± 0.01	0.45 ± 0.04	0.12 ± 0.01
Sildenafil citrate (5 mg/kg)	1.32 ± 0.01	0.40 ± 0.02	0.11 ± 0.02	0.43 ± 0.03	0.11 ± 0.01
BT 75 mg/kg	1.33 ± 0.03	0.38 ± 0.03	0.08 ± 0.01	0.36 ± 0.03	0.10 ± 0.01
BT 150 mg/kg	1.51 ± 0.03**	0.40 ± 0.01	0.10 ± 0.01	0.33 ± 0.07	0.13 ± 0.01
BT 300 mg/kg	1.44 ± 0.03**	0.38 ± 0.02	0.10 ± 0.01	0.40 ± 0.03	0.11 ± 0.01

Control = normal rats treated with vehicle (distilled water); BT = normal rats receiving the aqueous extract of *B. toxisperma* at doses of 75, 150 and 300 mg/kg. **p < 0.01 versus Control

Effects of the aqueous extract *B. toxisperma* on stress-induced male reproductive dysfunction

Effects on sexual behavior

Compared to normal rats, animals exposed to the 6-h/day immobilization stress showed a significant ($p < 0.01$) increase in the first mount, intromission and ejaculation latencies, while reducing ($p < 0.01$) mount, intromission and ejaculatory frequencies, and total penile licking (Table 4). Globally, these stress-induced unwanted effects on sexual behavior were prevented by aqueous extract of *B. toxisperma*. Compared to the stressed animals, extract significantly reduced mount latency (day 18 with 75 and 150 mg/kg, and throughout experiment

with 300 mg/kg) and intromission latency (days 18 and 35 with 75 and 150 mg/kg, and throughout experiment with 300 mg/kg), and increased the number of mount (throughout experiment with all tested doses) and intromission (days 1 and 35 with 75 and 150 mg/kg, and throughout experiment with 300 mg/kg). These parameters were closer or higher than those of the normal animals at the dose of 300 mg/kg. Extract exhibited difficulties to counteract the stress-induced increase ejaculation latency, while it increased ejaculation frequency at the dose of 300 mg/kg as compared to stressed controls. The values obtained at this dose were lower than those of normal animals except on day 1. Regarding the penile licking, increased numbers were noted throughout the study at all tested doses as compared with stressed controls. At the dose of 300 mg/kg, the values of this parameter were higher than those of normal animals on days 1 and 35.

Table 4: Effects of a 35-day immobilization stress (6-h/day) and treatment with the aqueous extract of *B. toxisperma* on the male sexual behavior

Treatment		Latency for first mount (Sec)	Number of mount	Latency for first intromission (Sec)	Number of intromission	Latency for first ejaculation (Sec)	Number of ejaculation	Number of penilelicking
Normal+ distilled water	Day 1	209.2 ± 3.2	37.0 ± 5.5	270.4 ± 22.0	25.0 ± 2.8	209.2 ± 3.2	0.2 ± 0.1	21.4 ± 2.8
	Day 18	15.2 ± 2.7	60.6 ± 2.3	16.0 ± 2.9	55.3 ± 1.1	290.2 ± 32.5	2.4 ± 0.6	54.8 ± 4.3
	Day 35	5.3 ± 1.0	42.8 ± 3.7	7.0 ± 1.1	42.8 ± 3.7	286.2 ± 35.6	3.0 ± 0.3	35.0 ± 1.4
Stress + distilled water	Day 1	207.1 ± 6.1	13.2 ± 1.9***	276.8 ± 24.3	11.8 ± 1.9*	707.8 ± 89.3***	0.2 ± 0.1	13.8 ± 2.9
	Day 18	24.6 ± 3.2*	33.0 ± 4.3**	22.4 ± 3.9	31.2 ± 3.5**	1420.4 ± 70.7***	0.8 ± 0.2*	28.2 ± 4.8***
	Day 35	12.1 ± 1.0**	23.2 ± 2.8**	16.0 ± 4.2*	22.8 ± 4.9**	479.5 ± 28.4*	1.2 ± 0.4**	30.0 ± 5.1
Stress + BT 75 mg/kg	Day 1	176.0 ± 29.4	29.6 ± 3.7###	178.2 ± 29.0	28.8 ± 3.6#	1124.1 ± 98###	0.4 ± 0.2	29.5 ± 3.1#
	Day 18	14.2 ± 2.7#	45.4 ± 4.2	14.2 ± 2.7	36.7 ± 5.4*	969.0 ± 59.5***###	0.4 ± 0.2*	36.7 ± 5.4***
	Day 35	9.1 ± 2.3	49.8 ± 4.1###	10.0 ± 1.1	41.4 ± 3.9#	897.2 ± 29.5***###	0.6 ± 0.2**	42.0 ± 1.9
Stress + BT 150 mg/kg	Day 1	206.0 ± 36.8	28.2 ± 1.5#	256.8 ± 32.2	19.4 ± 2.7	560.8 ± 60.8***#	0.8 ± 0.4	26.1 ± 3.4
	Day 18	13.2 ± 1.1#	42.0 ± 4.9*	13.2 ± 1.1	36.2 ± 5.1*	351.0 ± 32.6###	1.0 ± 0.4	35.2 ± 4.8***
	Day 35	11.0 ± 2.3*	43.0 ± 3.2	11.0 ± 2.3	31.6 ± 2.1	615.6 ± 73.9###	1.0 ± 0.4**	37.0 ± 1.8
Stress + BT 300 mg/kg	Day 1	58.8 ± 15.8***###	31.8 ± 2.1####	147.2 ± 32.5*#	29.6 ± 6.1#	787.5 ± 89.7**	1.6 ± 0.5*#	42.2 ± 6.5***#
	Day 18	7.6 ± 1.7###	54.8 ± 6.9#	7.6 ± 1.7***#	53.6 ± 6.2###	333.0 ± 52.2###	1.4 ± 0.4	42.6 ± 6.4***#
	Day 35	3.6 ± 0.4***###	65.0 ± 4.2***###	4.8 ± 1.4*#	60.8 ± 4.9***###	400.8 ± 57.1	2.2 ± 0.2*#	60.0 ± 6.1***###

BT = aqueous extract of *B. toxisperma* at doses of 75, 150 and 300 mg/kg. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus normal control. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus stressed control.

Effects of the extract on body and organ weights

After 35 days of treatment, a significant ($p < 0.01$) loss of body weight as well as weight of testis, epididymis and seminal vesicles was observed in stressed rats as compared to normal group (Table 5). At the dose of 300 mg/kg the aqueous extract of *B. toxisperma* prevented the stress-induced body weight loss. A similar effect was observed at the

all tested doses on relative wet weights of testis, seminal vesicles, epididymis and penis. The relative weights of testis and epididymis were higher than those observed in normal animals. Stress did not alter the relative wet weight of the penis but the extract (at the doses of 150 and 300 mg/kg) increased it to higher values than those noted in normal animals.

Table 5: Effects of the 6-h/day immobilization stress and aqueous extract of *B. toxisperma* on body and organ relative wet weights in male stressed rats

Treatment	% increase in body weight	Testis	Epididymis	Prostate	Seminal vesicles	Penis
Normal + distilled water	37.40±5.52	1.25 ± 0.03	0.36 ± 0.01	0.10 ± 0.02	0.39 ± 0.02	0.11 ± 0.01
Stress + distilled water	18.00±5.87**	1.10 ± 0.02**	0.32 ± 0.01**	0.09 ± 0.01	0.28 ± 0.02**	0.10 ± 0.01
Stress + BT 75 mg/kg	20.72±3.17*	1.53 ± 0.02***###	0.42 ± 0.03***###	0.10 ± 0.03	0.36 ± 0.01#	0.12 ± 0.02
Stress + BT 150 mg/kg	14.66±1.44**	1.46 ± 0.03***###	0.41 ± 0.01***###	0.11 ± 0.02	0.40 ± 0.04###	0.13 ± 0.01#
Stress + BT 300 mg/kg	22.34±2.62	1.41 ± 0.03***###	0.40 ± 0.01***###	0.10 ± 0.01	0.39 ± 0.02##	0.14 ± 0.02***###

BT = aqueous extract of *B. toxisperma* at the doses of 75, 150 and 300 mg/kg. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus normal control. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus stressed control

Effects of the extract on sperm concentration and motility

After the 6-h/day immobilization stress for 35 consecutive days, a significant ($p < 0.05$) reduction in sperm count and sperm motility was

noted as compared to normal animals (Figure 1). The aqueous extract of *B. Toxisperma* protected animals against the decrease in sperm count and sperm motility induced by the stress.

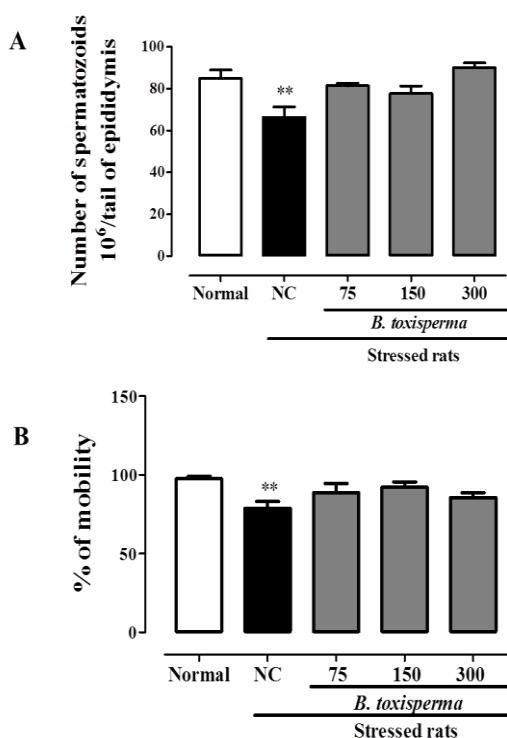


Figure 1: Effects of a 6-h/day immobilization stress and aqueous extract of *B. toxisperma* on sperm count and motility in adult male rats. Normal = normal rats receiving distilled water; NC = stressed rats receiving distilled water; BT = stressed rats receiving the aqueous extract of *B. toxisperma* at doses of 75, 150 and 300 mg/kg. * p<0.05 as compared with normal animals.

Effects of the extract on testicular histology

As depicted in figure 2 the 6-h/day immobilization stress for 35 consecutive days significantly reduced the amount of germ cells and spermatozoa in the lumen of seminiferous tubules as compared to normal rats. However, the stressed groups receiving the aqueous extract of *B. toxisperma* at all doses tested showed condensed seminiferous tubules filled with sperm cells; spermatogonia along the basal membrane with round and compact nuclei; and the presence of primary spermatocytes and spermatogonia compared to stress group receiving distilled water.

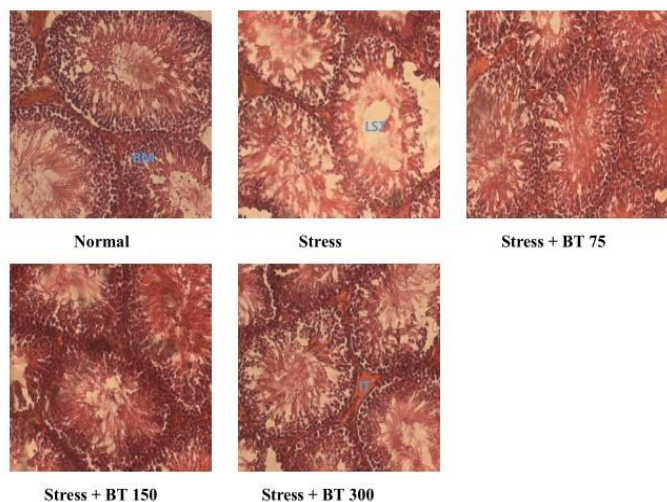


Figure 2: Effects of a 35-day treatment with *B. toxisperma* aqueous extract on testicular histological features in stressed rats (magnification: 200X). Normal = normal rats receiving distilled water; Stress = stressed rats receiving distilled water; BT = stressed rats receiving aqueous extract of *B. toxisperma*. LST: Lumen of seminiferous tubule; IT: Interstitial Tissue; BM: basal membrane

DISCUSSION

The present work aimed at evaluating the aphrodisiac potential of the aqueous extract of stem bark of *Baillonella toxisperma* and its capacity to prevent the stress-induced reproductive dysfunction in males.

First mount and intromission latencies are inversely related to sexual motivation [56] and then, well known indicators of sexual desire/motivation and potency [57, 58]. In our study, normal rats treated with the aqueous extract of *B. toxisperma* exhibited a significant reduction in mount and intromission latencies at the doses of 150 and 300 mg/kg indicating an aphrodisiac action of the extract. Chronic stress is widely recognized to impair/decrease sexual motivation [9, 13]. The 6-h/day repeated immobilization stress for 35 consecutive days increased the first mount, intromission and ejaculation latencies, while reducing mount, intromission and ejaculatory frequencies, and total penile licking. Treatment with 75 and 150 mg/kg *B. toxisperma* extract (during the period of stress) reduced the mount and intromission latencies on days 18 and 35, while increased the number of intromission on days 1 and 35. The dose of 300 mg/kg displayed rapid (from day 1) and sustained (throughout the study) effects. In other words no tachyphylaxis or decreased activity (a significant contributor to PDE5Is treatment failure) was noted with this dose during the 35-day treatment period; it markedly increased the mount and intromission latencies, mount, intromission and ejaculation frequencies and the number of penile licking compared to stressed controls throughout the study with values of parameters closer or higher than those observed in normal animals. This dose was also the only that prevented the decrease of ejaculatory frequency (on days 1 and 35) with a value higher (8-fold) than that of normal rats on day 1. Reduction of the first mount and intromission latencies are positive markers of sexual desire and motivation, while increased number of mount and intromission is associated with the improvement of penile erection process or enhancement of penile erection [59]. Through the reduction of the time of hesitation (mount and intromission latencies) and the increment of the erection process both in normal and stressful conditions, the aqueous extract of *B. toxisperma* exhibited aphrodisiac activities in both conditions. Testosterone is known to stimulate the brain and awaken the libido or sex drive [60, 61]. However, the adrenal production of glucocorticoids such as cortisol/corticosterone following a stress suppresses the secretion of testosterone (through the apoptosis of Leydig cells), libido and spermatogenesis [62-64]. Moreover, after examining many different animal models of stress, Andersen *et al.* [65] reported that immobilization stress model increases cortisol levels and decreases testosterone levels as the other models. In line with the major limit of this study is the lack of testosterone and cortisol levels. However, the improvement of sexual motivation and the enhancement of penile erection observed both in normal and stressful conditions suggest that the aqueous extract of *B. toxisperma* increased the levels of testosterone while decreasing that of cortisol/corticosterone.

Changes in body and organs' relative weights are well known as indicators of the health status of animals throughout the experiment. In our study, the aqueous extract of *B. toxisperma* exhibited an anabolic effect by increasing the relative wet weight of testes (gonadosomatic index) in normal rats. On the other hand, repeated immobilization stress resulted in significant decrease in both body and reproductive organs weight as reported in the literature [13, 18]. Interestingly, treatment with the extract of *B. toxisperma* (at the all tested doses) protected animals against stress-induced loss of relative weights of testis, seminal vesicles, epididymis and penis. The relative weight of testis, epididymis and penis were even higher than those of normal rats following treatment. It is well established that the development and growth of male reproductive tract (penis, testis and accessory sexual organs) is androgen-dependent, and testosterone is required to sustain their functionality. Therefore, the increase of the relative weight of these organs observed both in normal and stressed animals indicates the androgenic properties of *B. toxisperma*.

Beyond the sexual behavior and sex organs' weights, the evaluation of the male fertility/infertility emphasizes on sperm quality (count, motility

and morphological defects) and the histopathologic examination of testis (area of the seminiferous epithelial, aspects of germ cells and magnitude of spermatogenesis). In the literature, acute (for 6h) and chronic (5-6h/day for 60 days) immobilization stress decreased sperm count, viability and motility [66-68]. Similarly in this study, the 6-h/day immobilization stress for 35 consecutive days reduced sperm motility and concentration in the tail of epididymis. Our results showed that the aqueous extract of *B. toxisperma* at all tested doses prevented these immobilization stress-induced adverse effects. Sperm count in the tail of epididymis is positively related to the degree of spermatogenesis. As reported early, immobilization stress reduced spermatogenesis characterized by the reduced number of spermatogonia, primary and secondary spermatocytes, as well as spermatids [69]. Histopathological examination of the testis of rats treated with the aqueous extract of *B. toxisperma* showed an increased spermatogenesis as evidenced by increase in number of spermatogonia, primary and secondary spermatocytes and spermatids. In other words abundance of spermatozoa in seminiferous tubules of animals treated with the aqueous extract of *B. toxisperma* compared to the untreated controls clearly indicates spermatogenesis. According to Nirupama *et al.* [70], most of the stress-induced alterations in the spermatogenesis and antioxidant status of the testis are irreversible indicating the importance of the prevention. However, stress is an unavoidable phenomenon and people have to face it day-to-day life. Therefore, the aqueous extract of stem bark of *B. toxisperma*, that prevented all these alterations, appears to be quite interesting and promising to overcome/prevent stress-induced alterations of male sexual behavior and reproductive dysfunction.

Phytochemical screening showed that the aqueous extract of *B. toxisperma* contains flavonoids that are probably responsible for the androgenic properties and the high antioxidant power observed *in vivo* and *in vitro*, respectively. Indeed, flavonoids are well-known as antioxidant compounds. Stressful conditions are responsible for the imbalance in the oxidant/antioxidant system by increasing reactive oxygen species (ROS) production and lipid peroxidation, and decreasing antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities, especially in organs like testis rich in polyunsaturated fatty acid and poor in antioxidant defense [71-73]. Accordingly, our study suggests that the aqueous extract of *B. toxisperma* probably improved oxidative status in the testis and therefore, prevented the reduced mobility and concentration of sperm in the tail of epididymis as well as testicular alterations induced by the stress.

CONCLUSION

The data suggest that the aqueous extract of *B. toxisperma* has aphrodisiac properties both in normal and stressed rats. It can also prevent the stress-decreased spermatozoa count and motility, and germinal cells in the tubule lumen. To the best of our knowledge this study provides the first substantial scientific background for the traditional use of this plant against various physiological-related reproductive dysfunctions and would justify the use of *B. toxisperma* as aphrodisiac and against infertility in males.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank M. Chegang Benjamin and all members of the research unit of Pr. Njamen Dieudonné for their help during the experiments.

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HOW TO CITE THIS ARTICLE

Simo RV, Ateba SB, Zingué S, Pieme CA, Njamen D. *Baillonella toxisperma* improves sexual performance and protects against stress-induced reproductive dysfunction in male Wistar rats. J Phytopharmacol 2019; 8(3):117-123.