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Aphrodisiac potentials of the aqueous extract of *Hibiscus asper* Hook. f. leaves (Malvaceae) in male Wistar rats

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ABSTRACT

Introduction: In general medicine, male sexual dysfunction is a major source of worry and consultation. Finding alternative substances like plants to regulate these disturbances is motivated by the side effects of standard aphrodisiac medicines. In the current investigation, male rats were used to test the aphrodisiac effects of an aqueous extract of *Hibiscus asper* leaves. **Methodology:** Aphrodisiac effect of the extract was evaluated following a single daily administration of *Hibiscus asper* at doses of 50, 100 and 200 mg/kg respectively. Sexual behavioural parameters such as mounting and intromission frequencies, mounting, intromission and ejaculatory latencies, sexual motivation were monitored on days 1, 4 and 8. At the end of the experiment, the grapping test was assessed, after which the animals were sacrificed and blood collected for the evaluation of cholesterol, fructose, proteins, acid phosphatase, testosterone, and other androgen-dependent enzymes. Some androgen-dependent organs, such as the testis, epididymis, seminal vesicle, prostate, and levator ani muscle, were also removed for biochemical and histological analysis. **Results:** The administration of the aqueous extract of *H. asper* leaves had a significant impact on sexual behaviour, increasing mounting frequency (MF), intromission frequency (IF), and ejaculatory latency (EL), and reducing mounting latency (ML), intromission latency (IL), and post-ejaculatory interval (PEI). There was also an enhancement in orientational activities, libido, arousal and potency. Cholesterol, acid phosphatase, vesicular fructose, proteins, nitric oxide and testosterone levels were all significantly increased in treated animals. **Conclusion:** These findings support the traditional use of *H. asper* leaves to enhance male sexual behavior by demonstrating the aphrodisiac potential of these plants.

Keywords: Aphrodisiac effect, *Hibiscus asper* leaves, Rats.

INTRODUCTION

Medicinal plants have been widely used in developing countries to manage myriad of health problems concern including reproductive disturbances [1, 2]. It has been acclaimed that aphrodisiacs with a healthy lifestyle can achieve a better sexual life. The sex is the most cherished, indispensable and an integral part of several people and can be a cradle of pleasure and satisfaction [3]. Globally, a happy and successful sex life is considered crucial to their quality of life, and physical and mental wellbeing. Aside from reproductive purposes, sex often serves as a catalyst for improving intimacy and developing the emotional bond between partners. It is certain that stressful life style has enhanced the number of subject's suffering from one form of sexual dysfunction or the other. Despite development of good progress in facilitating open discussions around sexual health in recent years, sexual myths are still embarrassed or feel ashamed for most people. The sexual myths could result in sexual dysfunctions, misery, silent suffering, distressed interpersonal relationships and even divorce. Sexual ignorance is a social disease and can be solved via compulsory all-inclusive sex education which boost awareness and improve people relationship [4]**.** Male sexual dysfunctions are more prevalence in sexually active men and seems to be a sexual complains with a noteworthy impact on the quality of life.

Studies on masculine sexual dysfunction have mainly focused on understanding erectile and ejaculatory disorders, which represent the major sexual problems [5]. The causes of sexual disturbance could result from various factors including age, lifestyle, alterations in psychogenic or some other physiological factors [6]. Despite the significant repercussions that these disorders trigger in social life such as anxiety, depression, loss of self-esteem or problems within the couple, very few people affected seek medical advice. The awareness of the importance of psychogenic factors as a cause of erectile dysfunction, little is known about impact of erectile dysfunction and the effect of its subsequent treatment on the lives and wellbeing of patients [7,8]. The management of this physiological dysfunction or to improve sexual performance and qualities is currently based on the use of aphrodisiac substances that amplify some facets of sensual experience and improve sensual consciousness, sexual stimulation and inclination [9].

The discovery of the drug viagra (sildenafil citrate) to improve male erectile dysfunction has caused a real revolution accompanied by a strong demand from men around the world. However, over the years, the misuse of the drug has sparked controversy and been associated with some side effects [10], that motivated people concerned to resort to other sources of aphrodisiac as food or medicinal plants. Human beings frequently turned to nature in search of solutions that help to improve the quality and frequency of the sexual relations focusing plants for erection or to improve sexual desire [11]. Some plant species from different cultures have been studied and traditionally used as natural aphrodisiacs, but not all of the scientific evidence was available. In Cameroon, several plants have been reported as aphrodisiac and/or to manage erectile disturbances [12–15]. Among these medicinal plants, *Hibiscus asper* (Malvaceae) which is traditionally used to control malaria, anaemia, and typhoid is also used to improve male sexual performance. However, scientific data on its pharmacological effects are not available yet. The present study aims to investigate the aphrodisiac properties of the aqueous extract leaf of *Hibiscus asper* (Malvaceae) in male Wistar rats.

MATERIALS AND METHODS

Plant Material, extraction procedure and phytochemical analyses

Plant parts (leafy twigs with fruit) of *Hibiscus asper* Hook F. (Malvaceae) were harvested, in March 2019, in Bangangté (West Region, Cameroon). The botanical authentication of the plant was done by a botanical staff of the national herbarium of Cameroon in Yaoundé in comparison with a voucher N ° 33987 / HNC. The leaves were collected from the rest of the plant, dried in the shade and powdered. The plant extraction was performed by macerating 500 g of powder into 5 L of distilled water for 24 h, with regular agitations. After filtration using Watman paper (No. 4) and drying in an oven (45 °C), the crude extract was stored at 4°C until use.

The afforded extract was subjected to qualitative phytochemical analysis using standard protocols [16].

Animals and experimental design

The study was carried out on male Albino Wistar rats aged of 10 to 12 weeks, and weighing from 180 to 220 g. The animals were bred in the animal house of the Laboratory of Animal Physiology at the University of Yaoundé I (Cameroon). They were kept at room temperature with natural nictemeral cycle. They were fed and watered *ad libitum*. All the experiments were carried out with approval of Institutional Ethical Committee, which adopted all procedures recommended by the European Union on the protection of animals used for scientific proposes (CEE Council 86/609; Ref N° FWA-IRD 0001954).

Assessment of male sexual behaviour

Identification of females in estrus cycle

The determination of the females in oestrus period for receptivity of female vis-a-vis to the male was done in nulligravid adult female rats based on the pap smear method as described by [17]. To perform the Pap smear, the vaginal fluid was collected using micropipette and placed on a slide that was examined under an optical microscope (Leitz, Germany) at 20x objective. The identification of the different phases of the oestrus cycle was based on vaginal cytology [18] . The vaginal environment of females in oestrus phase was characterized by the presence of superficial leaf cells. This female rat selection was carried out one hour before the copulation test.

Evaluation of the plant extract on the sexual behaviour in male rat

Thirty (30) healthy male rats were randomized by weight into 6 groups of 5 rats each which received distilled water (10 mL/kg) for the normal control group; Sildenafil Citrate (5 mg/kg) used as the reference substance for the Viagra control group, and the aqueous extract of *H. asper* at the doses of 50, 100, and 200 mg/kg considered as the test groups. The extract and water were administered as a single daily dose for 8 consecutive days whereas Sildenafil citrate was given on the days of evaluation of the male rats' sexual behaviour. During experimental period, the sexual behaviour of each male animal was evaluated on days 1, 4 and 8. The animals were treated one hour beforehand each copulation test according to the protocol described by Mbongue [19]. In brief, each male rat was placed in the copulation cage for 5 minutes for acclimatization then, a receptive female was introduced into the cage for a period of 20 minutes during which the following copulation parameters were recorded ; the number of urogenital sniffles, the latency time of the riding (ML), the latency time of the intromission (IL), the latency time of ejaculation (EL), the mean interval of copulation (MCI), the frequency of penile erections (EF), Mount frequency (MF), Frequency of intromissions (IF), Frequency of ejaculation (EF) and penile licking (PL). At the end of the treatment, the effects of the extract on sexual motivation and grip tests on male rats were carried out.

Sexual motivation test

The test was performed twenty-four hours later the end of the treatment, under a silent atmosphere and subdued light as described Waldinger *et al*. [20]. To conduct the test, each male rat was placed in a compartment side (A) of a cage divided into two compartments (A and B) separated by a metal grid. Following 10 minutes of acclimatization, a female in oestrus period was introduced in the other compartment side (B) for 5 min. The sexual motivation or attraction expressed as the number of times the male makes physical contact with the grid was recorded.

Grip test

The experimental animals were subjected to the grip test which consists in suspending the animals on a bar kept fixed and horizontal using an appropriate device [21] . The time spent at the helm by each animal was recorded in three assays and the mean time was calculated.

Evaluation of the effect of the extract on some physiological parameters

At the end of the sexual behavioural experiment, animals were sacrificed under anaesthesia diazepam (30 mg/kg) and ketamine (15 mg/kg). Blood sample was collected into dry tubes for hormonal and biochemical analysis. Some androgen-dependent sex organs (testis, prostate, epididymis, seminal vesicle and elevator ani muscle) were removed and weighted. A section of testis, epididymis and seminal vesicle was homogenized and the supernatant was used to perform some tissue biochemical analysis.

Testosterone assessment

The testosterone level was determined based on competition ELISA test using Calbiotech Elisa kit as described [22]. Briefly, goat anti-rabbit IgG-coated wells are incubated with testosterone standards, negative controls, serum samples, testosterone-horseradish Peroxidase (HRP) conjugate reagent and rabbit anti-testosterone reagent for 90 minutes. During the incubation, a fixed amount of HRP-labeled testosterone competes with the testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific testosterone antibody. Thus, the amount of testosterone-HRP immunologically bound to the well progressively decreases as the concentration of Testosterone in the specimen increases. Unbound testosterone-peroxidase conjugate was then removed and the wells washed, followed by addition of TMB Reagent resulting in the development of blue color. The color development was stopped and the absorbance was measured spectrophotometrically at 450 nm. The intensity of the color formed was proportional to the amount of enzyme present and was inversely related to the amount of unlabeled testosterone in the sample [22]. The testosterone concentration (ng/mL) of the specimens and controls run was determined by corresponding the means absorbance value for each specimen to the standard curve built from the following formula: Absorbance = log [concentration].

Evaluation of the effect of the plant extract on biochemical parameters

Serum was used to measure some biochemical parameters such as total protein [23], vesicular fructose [24] , and nitric oxide [25]. Total cholesterol level and acid phosphatase activity were determined using commercial Biolabo kits (France).

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM) and were analysed using analysis of variance (ANOVA) followed by Turkey's multiple range test through Graph-Pad Prism software 8.01. Difference was considered significant at $p < 0.05$.

RESULTS

Phytochemical screening

Phytochemical screening of the aqueous extract of *Hibiscus asper* leaves revealed the presence of alkaloids, flavonoids, catechin tannins, coumarins, glycosides, steroids, triterpenes and saponins.

Effects of the aqueous extract of *H. asper* on the relative weight of androgen-dependent sex organs

The Table 1 shows the effects of the aqueous extract of *H. asper* on the relative weight of some androgen-dependent organs. The daily administration of the extract for 8 consecutive days led to significant dose dependent increase ($p < 0.001$) in the weight of the testis by 16.54%, 23.14% and 23.31%, the prostate by 33.61% ($p < 0.01$), 36.41% ($p < 0.01$) and 45.21% ($p < 0.001$) at the respective doses of 50, 100 and 200 mg/kg compared to the normal control. The relative weight of the seminal vesicle also significantly increased ($p < 0.05$) of 10.65% with the extract (200 mg/kg). At a dose of 200 mg/kg of extract, a 32.77% rise in the elevator ani muscle was seen, which was significantly higher ($p<0.01$) than the usual control. The Viagra (5 mg/kg) administration induced significant rise in the testis weight of 22.58% ($p < 0.001$) and in seminal vesicle by 21.01% ($p < 0.001$) with regard to the normal control. No significant change in the relative weight of the overall organs was observed between animals receiving the plant extract and those treated with the Viagra.

Table 1: Effects of the *H. asper* leaves extract on the relative weight of some androgen-dependent organs

	Relative weight (%)				
	NC	VIA	HA 50	HA 100	HA 200
Testis	1.06±0.03	1.22±0.03[a]	1.23±0.05[a]	1.33±0.03[b]	1.30±0.05[b]
Prostate	0.24±0.01	0.24±0.02[b]	0.35±0.04[b]	0.32±0.03[b]	0.36±0.01[c]
Epididymis	0.38±0.03	0.60±0.17	0.39±0.02	0.46±0.02	0.43±0.01
SV	0.54±0.03	0.66±0.03[a]	0.55±0.04	0.51±0.02	0.60±0.05[a]
EAM	0.36±0.02	0.47±0.03	0.45±0.03	0.46±0.02	0.47±0.03[a]

Values are expressed as mean ± SEM, n = 5; [a]$p < 0.05$; [b]$p < 0.01$ and [c]$p < 0.001$: significant difference compared to the normal control; SV: Seminal vesicles; EAM: Levator Ani Muscle; NC: Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

Effect of the plant extract on sexual behaviour of animal

Effects of the aqueous extract of Hibiscus asper leaves on mount parameters

The effects of the aqueous extract of *H. asper* on the mount latency and number of mounts are summarized in Fig. 1. Daily administration of the aqueous extract of *Hibiscus asper* resulted in a significant decrease of the mount latency on day 1 by 45.91% ($p < 0.05$), 54.43% ($p < 0.01$) and 62.08 ($p < 0.001$), on day 4 by 65.09% ($p < 0.01$), 46.20% ($p < 0.05$) and 57.70% ($p < 0.01$) at the respective doses of 50, 100 and 200 mg/kg with respect to the normal control (Fig. 1A). On day 8, the decrease was of 63.42% ($p < 0.01$) and 54.28% ($p < 0.01$) at the doses of 100 and 200 mg/kg, respectively. Likewise, the treatment with sildenafil citrate (5 mg/kg) significantly reduced the latency time of riding by 64% ($p < 0.001$), 58.11% ($p < 0.01$) and 74.31% ($p < 0.001$) respectively, on days 1, 4 and 8 compared to the normal control group.

In the first day, the number of mounts significantly increased ($p < 0.001$) by 170.88%, 134.17% respectively in the extract doses of 100 and 200 mg/kg compared to the normal control. On day 4, the enhancement was of 54.70% ($p < 0.05$) and 98.29% ($p < 0.01$) at the same doses, respectively. Treatment with the extract at 50, 100 and 200 mg/kg showed significant increase in the mount frequency respectively, of 41.09% ($p < 0.05$), 44.88% ($p < 0.01$) and 68.18% ($p < 0.001$) on the 8th day as compared to the normal control. Comparing to the normal control, the administration of Viagra on the different

experimental days (day 1, 4 and 8) have resulted to a significant increase ($p < 0.001$) in the frequency of riding of 112.65%, 91.45%, and 111.65% respectively. No significant change was recorded between animal groups treated with plant extract and sildenafil citrate.

Effects of the aqueous extract of Hibiscus asper leaves on the intromission parameters

The Fig. 2 shows the effects of the aqueous extract of *H. asper* on the latency (Fig. 2A) and frequency of intromission (Fig. 2B). The aqueous extract of *H. asper* administration induced significant reduction in the lag time of intromission by 62.69% ($p < 0.001$) and 56.06% ($p < 0.01$) at doses 100 and 200 mg/kg respectively, compared to the normal control on day 1 (Fig. 2A). On day 4, the reduction in the lag time was of 51.39% ($p < 0.05$), 68.65% ($p < 0.001$) and 54.18% ($p < 0.01$) while on day 8 it was ($p < 0$, 001) by 58.68%, 67.24% and 53.34% at the respective dose of 50, 100 and 200 mg/kg compared to the normal control. Viagra treatment decreased the latency time ($p < 0.001$) by 84.37%, 74.23% and 78.78% on days 1, 4 and day 8, respectively with regards to the normal control. At the end of the treatment (day 8), a significant increase in the lag time of intromission ($p < 0.001$) was observed at the extract dose of 50 and 200 mg/kg by 168.75 % and 137.36 % respectively, compared to the Viagra control.

Regarding the frequency of intromission, the plant extract at the doses of 50, 100 and 200 mg/kg resulted in a significant increase the parameter respectively, by 83.56% ($p < 0.01$), 121.91% ($p < 0.001$) and 128.76% ($p < 0.001$) on day 1 by 159.18% ($p < 0.01$), 234.18% ($p < 0.001$), and 234.69% ($p < 0.001$) on day 4 and 114.70% ($p < 0.001$), 123.52% ($p < 0.001$) and 126.57% ($p < 0.001$) on day 8 as compared to the normal control. The administration of Viagra also significantly increased the frequency of intromission ($p < 0.001$) by 152.05%; 226.53% and 173.52% on days 1, 4, and 8 respectively compared to the normal control.

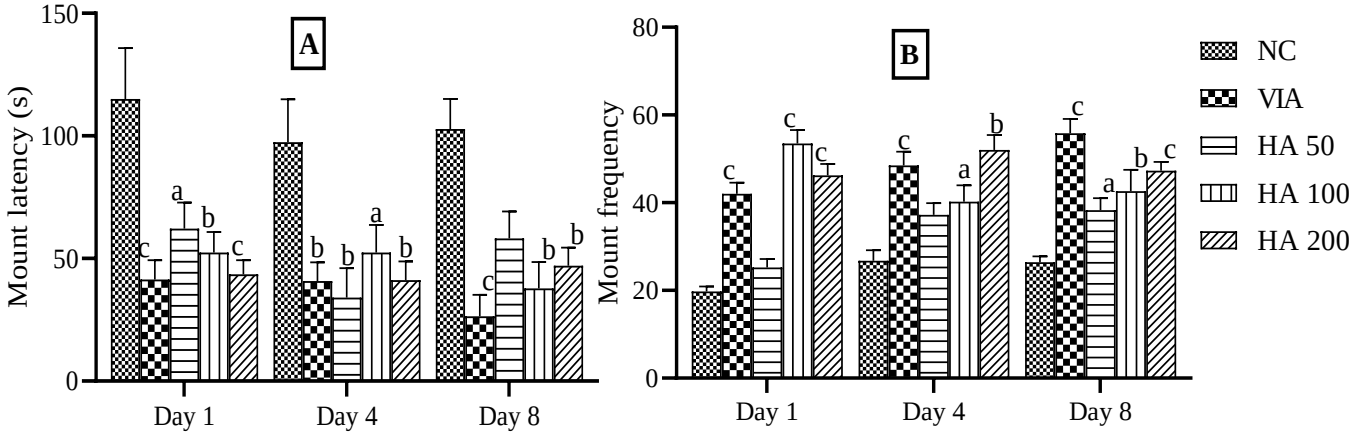

Fig. 1: Effects of the extract on mount latency (A) and on mount frequency (B)

Each bar represents the mean ± SEM, n = 5; $^{a}p < 0.05$; $^{b}p < 0.01$ and $^{c}p < 0.001$: significant difference compared to the normal control; NC: Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

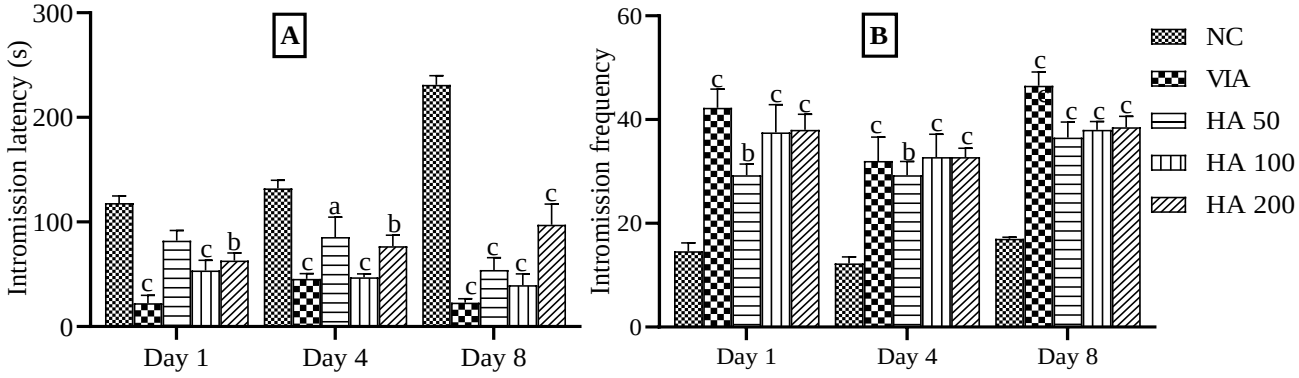

Fig. 2: Effects of the plant extract on the Intromission latency (A) and Intromission Frequency (B)

Each bar represents the mean ± SEM, n =5; $^{a}p < 0.05$; $^{b}p < 0.01$ and $^{c}p < 0.001$: significant difference compared to the normal control; Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

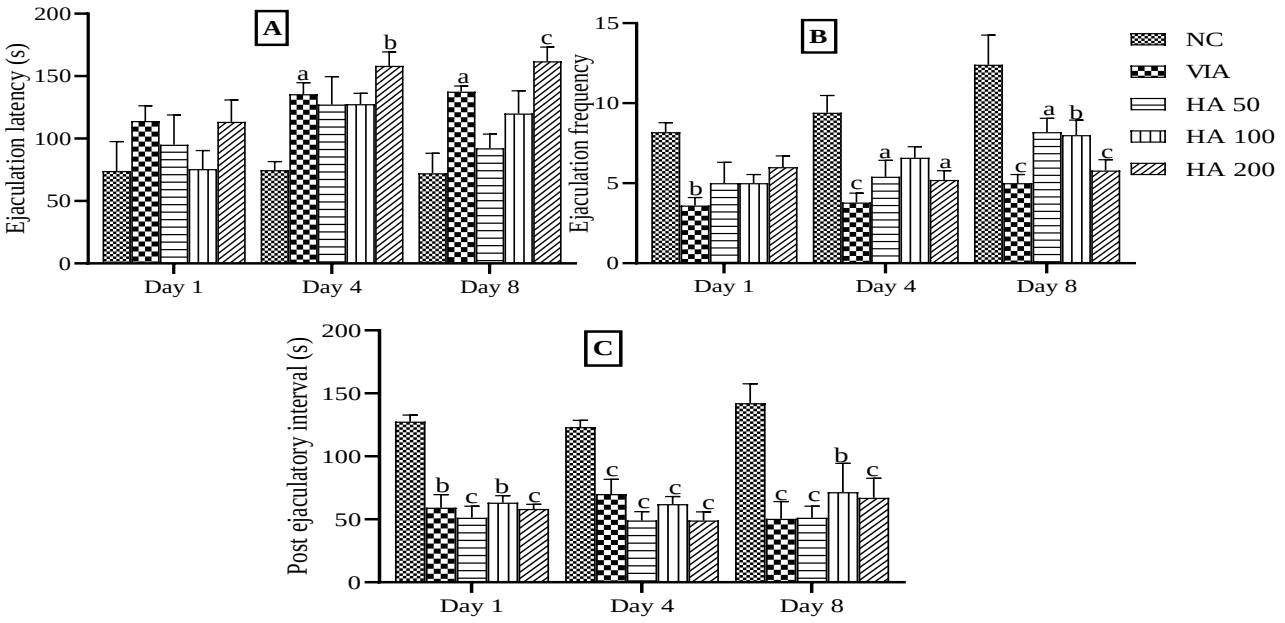

Fig. 3: Effects of the aqueous extract of *H. asper* leaves on Ejaculation latency (A), Ejaculation frequency (B) and post ejaculatory interval (C)

Each bar represents the mean ± SEM, n = 5; $^{a}p < 0.05$; $^{b}p < 0.01$, $^{c}p < 0.001$: significant difference compared to the normal control; Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

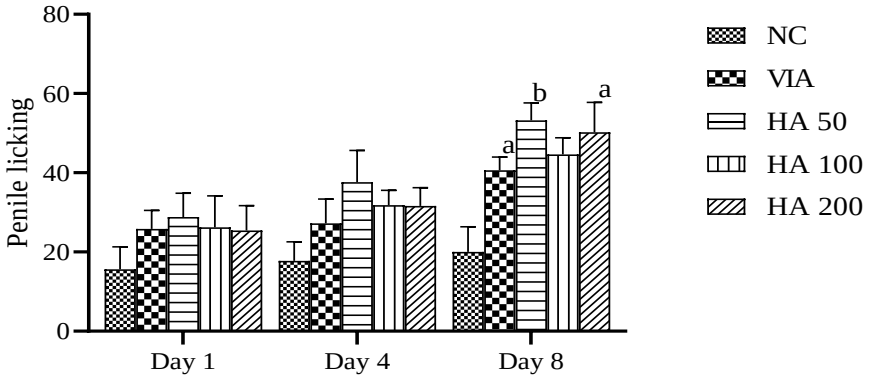

Fig. 4: Effects of the aqueous extract of *H. asper* leaves on penile licking

Each bar represents the mean ± SEM, n = 5 ; $^{a}p < 0.05$, $^{b}p < 0.01$: significant difference compared to the normal control ; Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

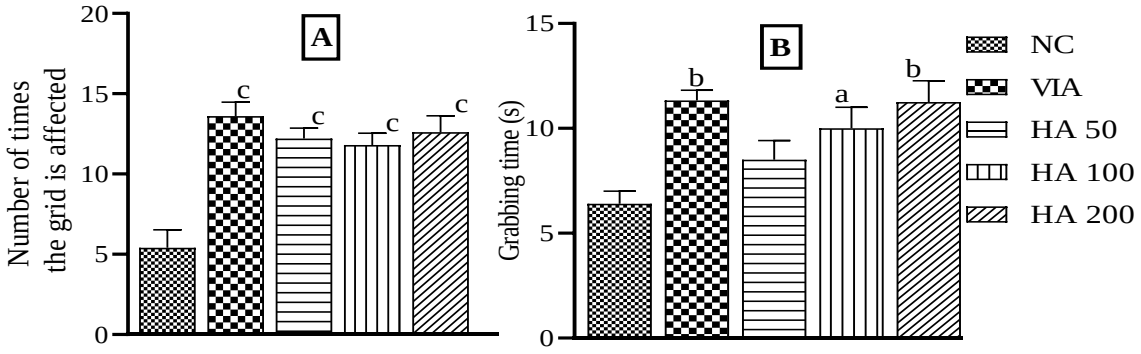

Fig. 5: Effects of the aqueous extract of *H. asper* leaves on sexual motivation (A) and grapping time (B)

Each bar represents the mean ± SEM, n = 5 ; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$: significant difference compared to the normal control ; Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

Effects of the extract of Hibiscus asper leaves on ejaculatory parameters

Fig. 3 summarizes the effects of the aqueous extract of *H. asper* leaves on ejaculatory parameters of the animals. It appears that the extract dose of 200 mg/kg significantly increased the ejaculation latency time by 48.30% ($p < 0.01$) and 47.67% ($p < 0.001$) on day 4 and 8 respectively, comparing to the normal control (Fig. 3A). Similarly, the administration of Viagra also enhanced the latency of ejaculation by 54.10% ($p < 0.05$) and 45.14% ($p < 0.05$) on days 4 and 8 respectively. An improvement of this frequency of riding was observed on day 8 compared to day 1 and 4. No significant difference was observed between animals treated with plant extract compared to those treated with Viagra

The daily treatment of animals with the extract at doses of 50 and 200 mg/kg resulted in significant decrease the frequency of ejaculations ($p < 0.05$) by 42.55% and 44.68% respectively, on day 4 compared to the normal control (Fig. 3B). On day 8, the reduction was of 33.87% ($p < 0.05$), 35.48% ($p < 0.01$) and 53.22% ($p < 0.001$) at the respective doses of the extract of 50, 100 and 200 mg/kg compared to the normal control. Viagra administration resulted in a significant decrease in the frequency of ejaculations by 56.09 % ($p < 0.01$), 59.57% ($p < 0.05$) and 59.67% ($p < 0.05$) in days 1, 4 and 8 respectively, compared to normal control. No significant change was observed in treated animals.

The treatment with the extract led to the reduction in the post ejaculatory interval ($p < 0.001$), of 59.87%, 50.47% and 54.38 % on day 1; by 45.22% ($p < 0.001$), 62.00% ($p < 0.001$) and 69.97% ($p < 0.001$) on day 4; then, of 58.50% ($p < 0.001$), 33.41 ($p < 0.01$) and 52.88% ($p < 0.001$) on day 8 at the respective doses of 50, 100 and 200 mg/kg compared to the normal group (Fig.3A). The Viagra treatment resulted in significant decrease in the post ejaculatory interval by 53.60% ($p < 0.05$) by 57.10% ($p < 0.001$) and 58.33% ($p < 0.001$) respectively, on days 1, 4 and 8 with regard to the normal control group. No significant change in the parameter was noticed in animals treated with plant extract compared to the Viagra treatment.

Effects of the aqueous extract of Hibiscus asper leaves on the number of erections

The Fig. 4 shows the effects of the aqueous extract of *Hibiscus asper* on the number of erections ("Penile licking") during the experimental period. It appears that the treatment of rats with the aqueous extract of *Hibiscus asper* at the doses of 50 mg/kg and 200 mg/kg, resulted in on day 8 to significant increase ($P < 0.01$) in “Penile licking” by 166.00% ($P<0.01$) and 151.00% ($P<0.05$) respectively, compared to the normal. Treatment of rats with Viagra (5 mg/kg) resulted in a significant increase ($P<0.01$) in this parameter by 103.00% on day 8 compared to the normal control.

Effects of the aqueous extract of Hibiscus asper leaves on sexual motivation and grabbing time

The effect of the aqueous extract of *H. asper* leaves on sexual motivation and muscle strength is presented in Fig. 5. The aqueous extract of the leaves of *Hibiscus asper* at the doses of 50, 100 and 200 mg/kg caused significant increase ($p < 0.001$) in the sexual motivation of rats respectively, by 125.92%, 118.51% and 133.33 % compared to the normal control batch (Fig. 5A). Similarly, the treatment with Viagra (5mg/kg) significantly increased the number of times the rat hit the grid by 151.85% ($p < 0.001$) compared to the normal group.

The daily administration of the aqueous extract of *H. asper* resulted in significant increase in the gripping time of 56.25% ($P<0.05$) and 75.78% ($P<0.01$) at the respective doses of 100 and 200 mg/kg, compared to the normal control (Fig. 5B). In rats treated with Viagra (5 mg/kg), significant increase in the grip time by 77.08% ($P<0.01$) was recorded as compared to the normal control. No significant change in muscle strength was observed in animals treated as well with plant extract as with Viagra.

Effects of the aqueous extract of *Hibiscus asper* on biochemical parameters

Effects of the aqueous extract of Hibiscus asper leaves on some androgenic parameters

Table 2 resumes the effects of aqueous extract of *Hibiscus asper* leaves on some androgenic parameters. The aqueous extract of *Hibiscus asper* leaves significantly increased ($p<0.001$) the testosterone level by 84.34% and 77.12%, at the respective dose of 100 and 200 mg/kg as compared to the normal control. No significant modification was observed in animals treated with Viagra (5 mg/kg) compared to the normal control. Likewise, the treatment of rats with *H. asper* extract at the doses of 50, 100 and 200 mg/kg significantly increased the vesicular fructose concentration by 36.44% ($P<0.01$), 38.47% ($P<0.01$) and 54.74% ($p < 0.001$) respectively, compared to the normal control. Treatment of rats with Viagra do not resulted in significant increase in the vesicular fructose level compared to the normal control. It was also observed significant increase ($p < 0.001$) in the level of nitrites of 124.54%, 113.53% and 140.53%, at the respective doses of extract of 50, 100 and 200 mg/kg compared to the normal control (Table 2). Significant increase by 111.26% ($p < 0.01$) was also noticed in animals treated with Viagra (5 mg/kg) compared to the normal control. The extract administration for 8 days exhibited significant increase in the acid phosphatase activity by 120.03% ($P<0.01$), 121.14% ($P<0.01$) and 275.95% ($P < 0.001$) at the respective doses of 50, 100 and 200 mg/kg compared to normal control (Table 2). From overall parameters analysed, no significant change was observed between in animals receiving the sildenafil citrate (5 mg/kg) and those treated with *H. asper*.

Effects of the extract on total serum and testicular cholesterol levels

The effects of the aqueous extract of *H. asper* leaves on serum and testicular cholesterol levels are summarized in Fig. 6. It was observed significant increase in serum cholesterol levels by 39.66% ($P<0.01$) and 47.50% ($p < 0.001$) and in the testis ($p < 0.001$) by 221.84% and 116.40% after plant administration at the respective doses of 100 and 200 mg/kg compared to the normal control. The testicular cholesterol level significantly increased by 79.11% ($P<0.01$) after treatment at the dose of 50 mg/kg, compared to the normal control. No significant change was observed between animals treated with Viagra and those treated with the extract.

Effects of Hibiscus asper on total protein levels

Table 3 presents the effects of the aqueous extract of *H. asper* on protein levels in serum, epididymis and seminar vesicle after 8 days of treatment. It was noted that the treatment of animals with the extract resulted in significant increase of total proteins level in serum by 36.70% ($p < 0.001$); 29.27% ($p < 0.01$) and 36.18% ($p < 0.001$); in the testis by 65.79% ($P<0.01$), 97.56% ($p < 0.001$) and 57.40% ($P<0.05$) and in the epididymis by 100.22% ($P<0.01$), 149.47% ($p < 0.001$) and 121.35% ($P<0.01$) at the respective doses of 50, 100 and 200 mg/kg

compared to the normal control. The administration of Viagra (5 mg/kg) significantly enhanced the protein level by 94.80% (p <0.001) and 32.66% (P<0.05) in the serum and the epididymis, respectively, compared to the normal control group.

Table 2: Effects of the aqueous extract of *H. asper* leaves on some androgenic parameters

Parameters	NC	VIA	HA 50	HA 100	HA 200
Testosterone (ng/mL)	0.21±0.01	0.38±0.04^{b}	0.32±0.05	0.38±0.01^{b}	0.36±0.03^{a}
VF (µmol/g of tissue)	3.55±0.11	5.19±0.25	4.84±0.21^{b}	4.92±0.18^{b}	5.49±0.28^{c}
NO (µmol/g of tissue)	0.04±7x10^{-3}	0.09±4x10^{-3c}	0.08±6x10^{-3c}	0.08±8x10^{-3c}	0.09±5x10^{-3c}
PAC (UI/L)	0.94±0.38	2.94±0.37	2.55±0.88^{b}	2.55±0.50^{b}	4.34±0.38^{c}

Results are expressed as the mean ± SEM, n = 5 ; $^{a}p < 0.05$, $^{b}p < 0.01$: significant difference compared to the normal control ; VF: vesicular fructose , NO: Nitric oxide, PAC: acid phosphatase; Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

Table 3: Effects of the aqueous extract of *H. asper* leaves on total Proteins level

	NC	VIA	HA 50	HA 100	HA 200
Serum (mg/L)	0,8±0,05	1,06±0,02^{c}	1,09±0,03^{c}	1,03±0,04^{b}	1,09±0,08^{c}
Testis (mg/g of tissue)	0,33±0,02	0,45±0,08	0,54±0,03^{b}	0,65±0,05^{c}	0,52±0,04^{a}
Epididymis (mg/g of tissue)	0,2±0,02	0,38±0,02^{a}	0,39±0,04^{b}	0,49±0,02^{c}	0,43±0,04^{b}

Values are represent mean ± SEM, n = 5 ; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$: significant difference compared to the normal control ; Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

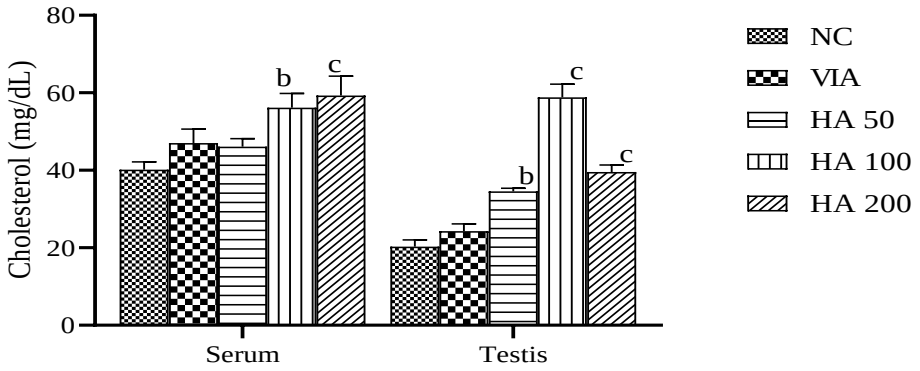

Fig. 6: Effects of the aqueous extract of *H. asper* leaves on total Cholesterol level

Each bar represents the mean ± SEM, n= 5; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$: significant difference compared to the normal control; Normal Control= animals treated with distilled water (10 mL/kg); VIA: animal receiving Sildenafil citrate (5 mg/kg); HA 50, HA 100, HA 200: animals treated with the *Hibiscus asper* extract at the respective doses of 50, 100 and 200 mg/kg.

DISCUSSION

The use of plant to stimulate sexual desire or to enhance performance and enjoyment is almost as old as the human race itself. Therefore, several plants have been used to improve sex desire and sexual performance enhancer in traditional systems of medicine of different countries [26, 27]. The present study aimed to explore the effects of the aqueous extract of *H. asper* leaves on the sexual behaviour of male rat. The plant extract intake significantly modified major of the sexual behaviour parameters in the treated animals compared to the normal. The modification was expressed by an enhancement of the number of urogenital sniffles, the frequency of penile erections (EF), the mount frequency (MF), the frequency of intromissions (IF), the frequency of ejaculation (EF) and penile licking (PL) which are some markers of libido and potency while the reduction of the latency time of the riding (ML), intromission (IL), ejaculation (EL) and the mean interval of copulation (MCI) are indicators of sexual arousal. The overall sexual behaviour modifications testify the aphrodisiac properties of the extract [28] . The aphrodisiac effects of the plant may be assigned to the presence of some bioactive components in the plant; alkaloids are known to increase penile erection and induce dilation of blood vessels in the sexual organs while saponins act on the central nervous system and gonadal tissues leading to enhancement of libido and copulatory performance through facilitation of penile erection. These overall activities induce vasodilatation and relaxation of the penile corpus cavernosum via a NO-dependent mechanism [29, 30]. In the present study, the level of NO in the penis of the animals treated with the plant extract was significantly high compared to the normal control animals which could be evidence the role of nitrites in the increase of sexual desire in animals. These findings so far reported the role of NO as a major neuronal messenger [31]. Particularly, it is a physiological mediator of penile erection in the brain; NO synthase is

highly concentrated in structures involved in sexual behaviour as olfactory bulb, supraoptic and paraventricular nuclei, amygdala, septal structures, etc. [31] .

Moreover, this enhancement of sexual behaviour was also accompanied by a significant increase of the testosterone level in animal treated with the plant extract. It is known that, testosterone remains the important male gonadal hormone synthetized and secreted by the interstitial Leydig cells of the testis through luteinizing hormone (LH) [32] . Psychosexual effects and physiological stimulation of the sexual desire of testosterone have been demonstrated and, a proven causal relationship between testosterone level, sexual desire and behaviour in male is well established [33]**.** Testosterone exerts a stimulating role on sexual desire by cerebral action, in particular through receptors located at the level of the median preoptic area of the hypothalamus and the limbic system [34]. These observations indicate stimulating effects of the plant extract on the hypothalamic-pituitary-gonadal axis [32]. These effects could assign to some metabolites such as terpenoids, steroids and alkaloids detected in the extract and involved into the increase of blood testosterone concentration [17, 35, 36]. In addition, saponins may boost the level of testosterone in the body and trigger libido enhancement [15] .

In the present study, significant elevation of the cholesterol and total proteins concentrations was recorded in serum, testis and epididymis in the animals treated with the plant extract. It is well known that cholesterol is the precursor of steroid synthesis which is crucial for normal sperm production. Moreover, high dependent on cholesterol homeostasis has been demonstrated in male reproductive function [37]. This elevation in the cholesterol level could result from stimulation by alkaloids compounds present in the extract [35]. The increase in total proteins level could indicate the potential anabolism effect of androgens hormone as testosterone which insures improvement proteins synthesis. Anabolic effects are those associated with increased protein synthesis and decreased protein catabolism, as well as retention of nitrogen products, leading to tissue growth, especially muscle; testosterone is essential for protein metabolism muscle, bone metabolism, maintenance of sexual and cognitive functions, and many other functions [38–40]

Administration of the aqueous extract of *H. asper* resulted in a significant increase in acid phosphatase, a useful enzyme for evaluation of prostatic function in which the prostatic fluid is implicated to the motility, survival and metabolism of spermatozoa. Indeed, positive correlation was reported between acid phosphatase and sperm concentration [41]. It was also observed significant increase in the level of vesicular fructose. Fructose is an indicator for the function of the seminal vesicles; it is the only sugar secreted by the seminal vesicles which plays an important energetic role in the maturation of sperm. It is also involved in the mobility of these sex cells. The increase of fructose in animals treated with the extract may be due to the stimulation of its synthesis placed into vesicular cells, triggered by some bioactive substances as flavonoids and terpenoids contained in the plant.

CONCLUSION

The study demonstrated the benefit effects of the aqueous extract of *Hibiscus asper* leaves in improvement of libido and copulatory performance, monitored by some biochemical factors involved in triggering and maintaining erection. These overall results testify the aphrodisiac potentials of *Hibiscus asper* leaves and supports its traditional claim in the reproductive function.

Competing of interest

The authors declare that they have no competing interest

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Authors' contributions

MJTN, RF, LW carried out the experiments. MJTN, ANMB and PEO collected the data. MJTN and RGK drafted the manuscript. RGK, NTF and TD designed the study. All authors have gone through and approved the manuscript.

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