Saudi Journal of Pathology and Microbiology (SJPM) **ISSN 2518-3362 (Print)**
Scholars Middle East Publishers **ISSN 2518-3370 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

Acute Toxicity and Aphrodisiac Effect of the Aqueous Extract of the Trunk Bark of *Prunusafricana* (Hook F) Kalkman (Rosaceae) in Male Albino and Normal Rats of the Wistar Strain

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Original Research Article

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Article History
Received: 16.08.2018
Accepted: 26.08.2018
Published: 30.09.2018

DOI:
10.21276/sjpm.2018.3.9.1

Abstract: The objective of the study was to evaluate the aphrodisiac property of the aqueous extract of *Prunusafricana* trunk bark in albino and normal male rats of the Wistar strain. Colorimetric and precipitation methods were used to highlight secondary metabolites of the plant. The acute toxicity test was conducted according to OCDE guideline 423. Four lots of 6 animals were used to evaluate the aphrodisiac property of *P. africana*. The positive control received sildenafil citrate (5 mg/Kg) and the negative control, distilled water. The test lots received the extract at 100 and 200 mg/Kg for 8 days. The copulatory parameters were observed on days 1, 4 and 8. The phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponosides and tri terpenes in the extract. No changes in the general appearance of the rats and no mortality were recorded during the toxicity test, highlighting that *P. africana* is non-toxic. The extract significantly increased the sex parameters of the tested rats. This aphrodisiac effect of *P. africana* attributed to identify secondary metabolites justifies its use in traditional medicine as a sexual stimulant.

Keywords: *Prunusafricana*, aqueous extract,phytochemical screening, acute toxicity, aphrodisiac effect.

INTRODUCTION

Sexual disorders are a relatively important health problem, the consequences of which are harmful to man. They mainly include decreased sexual desire, erectile dysfunction and ejaculatory disorders [1].

Indeed, sexual abilities in humans vary according to age, physiological and psychological states [2]. However, many pathologies can influence human life and sexual activity. This is the case of sexual impotence or erectile dysfunction (ED), which is the persistent or recurrent failure for a man to obtain or maintain a sufficient penile erection, to allow sexual activity [3]. ED is a public health problem that affects the quality of life of patients and their partners [4]. Indeed, 20% of people suffer from it per year and this rate increases considerably by decade of age [5]. In addition, about 322 million men could be sexually disabled in the year 2025 [2]. Erectile dysfunction is a topical therapeutic subject, with the recent arrival of synthetic drugs stimulating erection [6]. However, the high cost of these drugs, as well as the recurring side effects are all reasons, which motivate researchers to be interested in substances of natural origin. Several authors including Kamchouing*et al*., [7] and Watcho*et al*., [8] studied plants known to create and stimulate sexual desire, such as *Piper guineense*, *Dracaena arborea* or *Pausinystaliayohimbe*. However, the studies relating to this activity remain embryonic and scattered. The present work was undertaken to evaluate the aphrodisiac property of the aqueous extract of *P. africana* trunk bark in albino and normal male rats of the Wistar strain. The specific objectives were to: identify the secondary metabolites present in the aqueous extract, study the acute toxicity of the aqueous extract and test the aphrodisiac properties of the aqueous extract.

MATERIALS AND METHODS

Material

Plant material

The bark of the trunk of *Prunusafricana* (Rosaceae) was harvested in Bamenda, North West Cameroon region, specifically in the Luh District, Donga-Mantum Department. The botanical identification of the species was carried out at the

Laboratory of Biology and Physiology of Plant Organisms of the Faculty of Science of the University of Douala.

Animal material

The acute toxicity test required young albino and normal female rats of the Wistar strain aged 8 to 12 weeks. For the aphrodisiac property, male and albino rats, of the Wistar strain, aged 12 to 20 weeks and weighing between 180 and 240 g were used. Female albino and normal rats, of the same strain (12 to 20 weeks of age), weighing between 120 and 170 g were chosen, to test the copulation performance.

Preparation of the aqueous extract

The freshly harvested bark was cleaned and then dried out of the sun for 15 days at 25 °C. The dry matter obtained was pulverized with the aid of an electric mill, in order to render it suitable for extraction. A 500 g powder of *Prunusafricana* was mixed with 3 L of distilled water. The resulting mixture was homogenized and allowed to stand for 48 H then, sifted. The collected solution was filtered using a Wattman paper N°1, before evaporation at 45 °C in an oven, for 3 weeks.

Phytochemical Screening

The major chemical families (alkaloids, flavonoids, tannins, anthocyanins, coumarins, tannins, saponosides, sterols and triterpenes) have been investigated in the aqueous extract of *P. africana* using the characteristic tests of each family [9, 10, 11].

Acute toxicity study

The acute toxicity test was conducted following the protocol of OCDE Test Guideline 423 [12]. Young female rats (18 divided into 6 lots of 3 rats) were randomly selected and fasted for 12 hours before the test, but receiving water at will. These batches were treated as follows: the control group received distilled water at 10 mL/Kg of body weight and lots 1, 2, 3, 4 and 5 were treated with the aqueous extract of *P. africana* at doses of 5, 50, 300, 2000 and 5000 mg /Kg body weight. After administration of the substance orally to the different batches, the young female rats were observed individually during the first 24 H after treatment, with particular attention during the first 4 hours. The observation then continued for 14 days following the administration of the substance. These observations concerned the behavior and general condition of young rats. Batch mortality was assessed for 48 H after administration of the products. In addition, these young rats underwent, during the study period, weighing respectively every two days (D0, D2, D4, D6, D8, D10, D12, D14), in order to evaluate the weight variation.

Evaluation of the aphrodisiac activity

The evaluation of the aphrodisiac activity of the aqueous extract of *Prunusafricana* on the sexual behavior of albino and normal male rats of Wistar strain was conducted according to the protocol of Mbongue [13]. Four batches of 6 male rats each, fasted for 24 H, were formed and treated for 8 days as follows: the negative control group received distilled water at a rate of 5 mL/Kg of body weight; the positive control group treated with the reference molecule, sildenafil citrate at a dose of 5 mg/Kg body weight. Two other batches were treated with the extract respectively at doses of 100 and 200 mg /Kg body weight. All products were administered orally daily for 8 days. After gavage on days 1, 4 and 8, the rats were placed in a cage each for 10 min, for acclimation. A female ovariectomized and made receptive by injecting a single subcutaneous dose of 15 µg/Kg of estradiol benzoate 48 H before the experiment, followed by the injection of a 60 µg/Kg dose of progesterone 6 H before the experiment, was introduced into the cage for a period of 30 min [13]. During these 30 min, copulation parameters were recorded: the latency time of the ride, the intromission latency time, the frequency of ascents, the frequency of intromissions, the frequency of ejaculations and the number of erections [14].

Statistical analyzes

The data were presented as mean ± Standard Mean Error (ESM) in histograms. The one-way order of variance analysis (ANOVA) was used to compare the mean values of the copulatory parameters between the different animal groups of the experiment. Subsequently, the Dunnett test was used to make even comparisons between the test and control groups (negative and positive). The repeated measures ANOVA was used to study the weight change of each group during the follow-up time, while the non-parametric Kruskal-Wallis test was used, to compare the average weight of the different groups according to each day of follow-up. The threshold of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Screening phytochimique

Qualitative phytochemical screening based on colorimetric and precipitation reactions by specific chemical reagents has shown that the aqueous extract of *P. africana* trunk bark is rich in alkaloids, flavonoids, triterpenes, saponosides, tannins and reducing compounds (Table-1).

Table-1: Screening phytochemical of the bark extract from the *Prunusafricana* trunk

Secondary metabolites		Remarks	Results
Phenolic compounds	Gallic tannins	Appearance of a blue-green color	+++
	Catechiques tannins	Appearance of a greenish-brown color	++
	Flavonoid	Appearance of a red color	_
	Coumarin	No Fluorescence	_
	Anthocyanin	Appearance of a blue-purplish color	_
N compounds	alkaloid	Appearance of a reddish-brown precipitate	++
Steroids and terpenoïdes	Sterol	Appearance of a green-bluish color	_
	Triterpenes	Appearance of a purplish red color	++
	Saponoside	Appearance of a persistence foam	++
Reducing compounds		Formation of a red-brick precipitate	++

+ + + = very concentrated Substance; + + = medium-concentrated substance; + = weakly concentrated substance; – = absence of the substance.

Study of the toxicity in young female rats

Effect de of aqueous extract in general appearances, general state and mortality

The study of the toxicity in young female rats shows that after gavage of the aqueous extract at doses ranging from 300 to 5000 mg /Kg, a decrease in the sensitivity to pain induced by pinching of the tail is observed. The dose of 5000 mg / kg resulted in a decrease in alertness for 3 min. Changes in the general appearance of the rats were not observed during the observation days. Behavioral changes were not observed, with the control group. Similarly, no deaths were recorded after 14 days of observation. The LD_{50} is therefore greater than 5000 mg/Kg of body weight (Table-2).

Table-2: General condition of the animals after administration of the aqueous extract of the bark of the trunk of *P. Africana*

	Treatments					
	Control	Group 1 (extract at 5 mg/Kg)	Group 2 (extract at 50 mg/Kg)	Group 3 (extract at 300 mg/Kg)	Group 4 (extract at 2000 mg/Kg)	Group 5 (extract at 5000 mg/Kg)
Number of Rats	03	03	03	03	03	03
Mobility	N	N	N	N	N	N
Aggression	A	A	A	A	A	A
Saddle states	N	N	N	N	N	N
Pain sensitivity	N	N	N	D	D	D
Vomiting	A	A	A	A	A	A
Vocalization	A	A	A	A	A	A
Pilot erection	A	A	A	A	A	A
Tail condition	N	N	N	N	N	N
Vigilance	+	+	+	+	D	-
Death	00	00	00	00	00	00

A: Absent; N: Normal; +: Yes; -: Very low; D: Decrease

Effect of aqueous extract on weight change

The effect of the aqueous extract of *P. africana* trunk bark on the body weight of young female rats was recorded on the day of administration of the extract (D0), up to the fourteenth day (D 14) in according to the doses administered to them (Figure-1). All the rats subjected to the experiment had a gain in weight, during all the duration of the study.

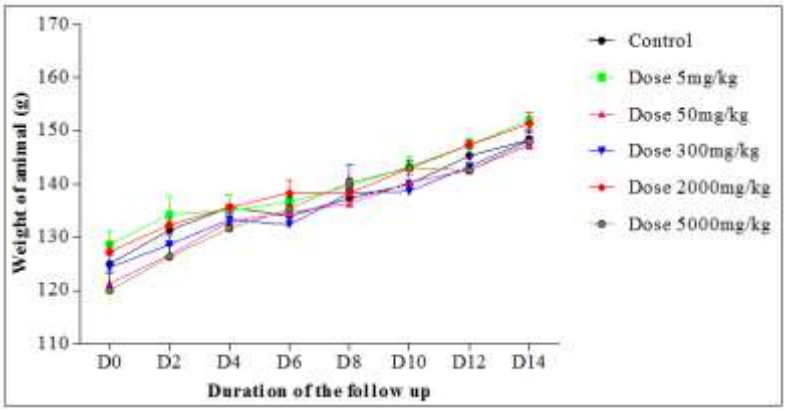

Fig-1: Effects of the aqueous extract of the barks of the trunk of P. Africana on the weight evolution of young female rats.

The values are expressed on average ± ESM, n = 3, * P < 0.05; P< 0.001.

Study of the aphrodisiac effect of the aqueous extract of *P. africana* in albino and normal male rats of Wistar strain

Effect of aqueous extract on the frequency of sexual uprisings

On the day 1, a significant increase in the frequency of sexual uprisings of rats treated at 100 mg/Kg (p <0.001) and at 200 mg/Kg (p <0.05) was observed compared with receiving distilled water (negative control) (Figure 2). Lot 1 treated with a dose of 100 mg/Kg of *P. africana* extract has a frequency of sexual mating very close to that of the positive control (lot treated with sildenafil citrate).

On the day 4, compared with the negative control, the incidence of sexual udders increased in all treated rats, although this increase was not significant at the 200 mg/Kg dose (lot 2).

On the day 8, a significant increase in the frequency of sexual uprisings was observed in the rats treated at doses of 100 mg/Kg (p <0.001) and 200 mg/Kg (p <0.01) compared to those treated at distilled water. This increase is of the order of 36.03% (22.66 ± 2.23 V 30.83 ± 1.4 sexual uprisings), for 200 mg/Kg and 51.46% (22.66 ± 2.23 V 36.33 ± 2.36 sexual uprisings), per 100 mg/Kg.

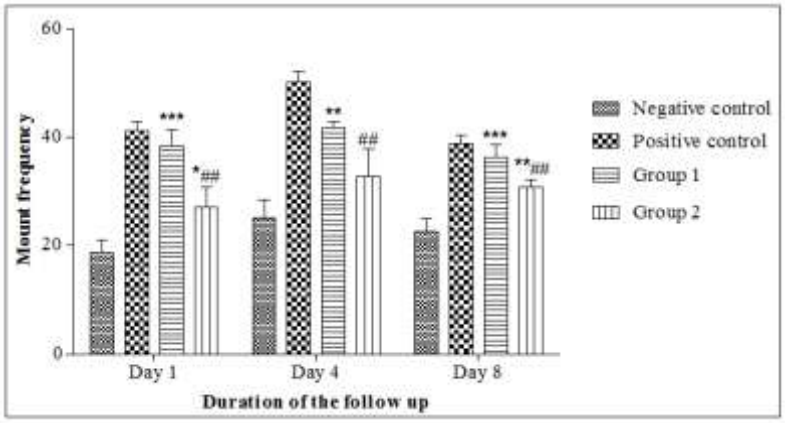

Fig-2: Effects of the aqueous extract of the barks of the trunk of *P. africana* on the frequency of sexual uprisings

The data are presented as mean ± standard deviation. Comparisons were made with negative (distilled water) (*) and positive (sildenafil citrate) controls (#).

* = Difference at p-value <0.05; **= Difference at p-value <0.01; *** = Difference at p-value <0.001

= Difference at p-value <0.05; ## = Difference at p-value <0.01; ### = Difference at p-value <0.001

Effect of the aqueous extract on the frequency of intromissions

On the day 1, the rats treated at doses 100 mg/Kg (lot 1) and 200 mg/Kg (lot 2) showed a non-significant increase in the frequency of intromissions compared to those treated with distilled water (Figure-3).

On the day 4, the aqueous extract of *P. africana* trunk bark at a dose of 100 mg/Kg caused a significant (p <0.05) increase in the frequency of intromissions for 30 min. This increase was 28 ± 1.183 intromissions versus 19.33 ± 1.83 intromissions in negative control rats. In contrast, the aqueous extract of

*P. africana*trunk bark showed no significant increase in the frequency of intromissions at a dose of 200 mg/Kg.

At the day 8, the dose of 100 mg/Kg of the aqueous extract causes a significant increase in the frequency of intromissions in the rats compared to the negative control. This increase was of the order of 25%. In addition, a significant increase ($p < 0.05$) in the frequency of intromissions was observed in rats treated at 100 mg/Kg compared with those receiving sildenafil citrate (positive control).

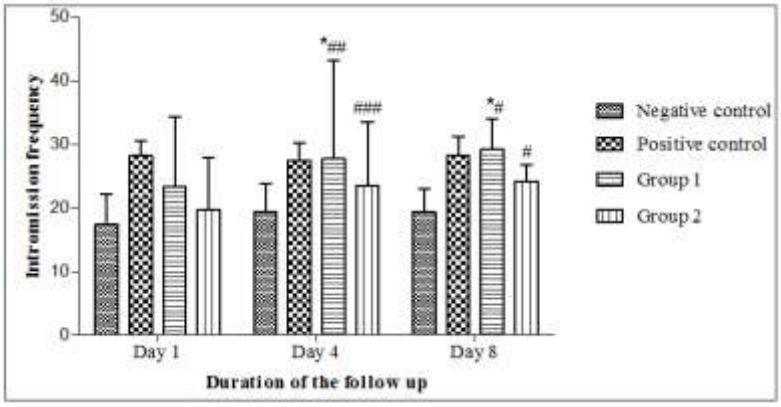

Fig-3: Effects of the aqueous extract of the barks of the trunk of *P. Africana* on the frequency of intromissions

The data are presented in the form of a standard mean ± deviation. Comparisons were made against negative (distilled water) (*) and positive (Sildenafil Citrate) (#) controls.

* = difference to P-value < 0.05; * * = difference to P-value < 0.01; = difference to P-value < 0.001, # = difference to P-value < 0.05; # # = difference to P-value < 0.01; # # # = difference to P-value < 0.001

Effect of the extract on the number of erections

On the day 1, a significant decrease ($p < 0.05$) and ($p < 0.01$) in the number of erections, respectively for Lots 1 and 2, was observed compared to the positive control. The number of erections increased non-significantly in rats treated at different doses of 100 and 200 mg/Kg of extract compared to the negative control (Figure-4).

On The day 4, the dose of 100 mg/Kg resulted in a significant ($p < 0.01$) increase in the number of erections in rats compared to those treated with distilled water. On the day 8, the number of erections increased significantly ($p < 0.001$) and ($p < 0.05$) in rats treated at 100 and 200 mg/Kg of extract compared to that of the negative control. In comparison with the positive control, only the lot that received the 100 mg/Kg extract showed a significant increase ($p < 0.05$) in the number of erections. This increase is of the order of 20.19% (42.68 ± 2.95 V 35.5 ± 1.41 erections).

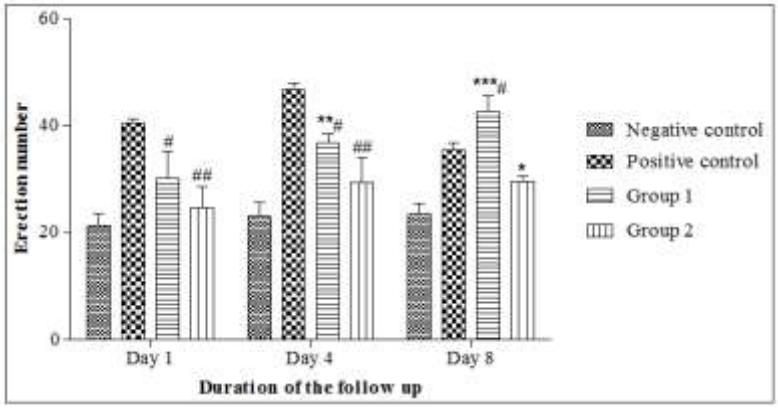

Fig-4: Effects of the aqueous extract of the barks of the trunk of *P. Africana* on the number of erections

The data are presented in the form of a standard mean ± deviation. Comparisons were made against negative (distilled water) (*) and positive (Sildenafil Citrate) (#) controls.

* = difference to P-value < 0.05; * * = difference to P-value < 0.01; = difference to P-value < 0.001, # = difference to P-value < 0.05; # # = difference to P-value < 0.01; # # # = difference to P-value < 0.001

Effect of the aqueous extract on the frequency of ejaculations

On the day 1, the frequency of ejaculations in rats treated at 100 mg/Kg (lot 1) and 200 mg/Kg (lot 2) extract increased compared with the negative control group, although this increase was only significant ($p < 0.05$) in rats receiving the 100mg/Kg extract (Figure-5).

On day 4, lot 1 was the only one to show a significantly positive difference compared to the two controls ($p < 0.001$: negative control, $p < 0.05$: positive control). This increase was of the order of 324.74% and 54.55% respectively for the negative control and positive control groups.

On the day 8, a significant increase ($p < 0.001$) in ejaculation frequency of ejaculations in rats of lot 1 compared to those treated with distilled water was observed. Similarly, lot 1 had a higher frequency of ejaculations than the positive control, although this difference is not significant.

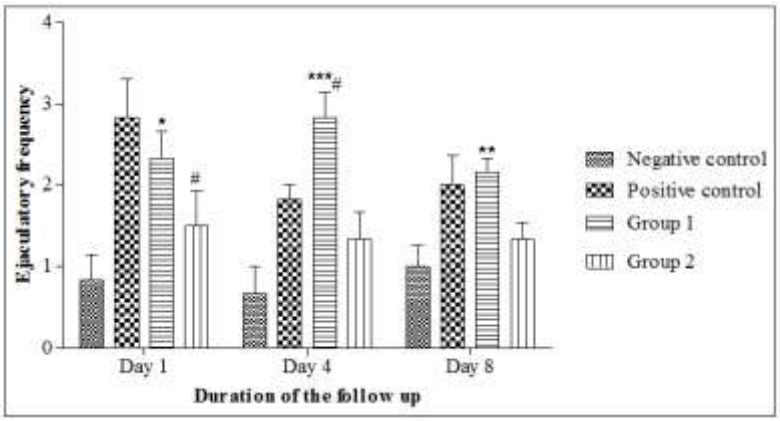

Fig-5: Effects of the aqueous extract of the barks of the trunk of *P. africana* on the frequency of ejaculations

The data are presented in the form of a standard mean ± deviation. Comparisons were made against negative (distilled water) (*) and positive (Sildenafil Citrate) (#) controls.

* = difference to P-value < 0.05; * * = difference to P-value < 0.01; = difference to P-value < 0.001, # = difference to P-value < 0.05; # # = difference to P-value < 0.01; # # # = difference to P-value < 0.001

Effect of the extract on the latency of sexual uprisings

On the day 1, a significant decrease ($p < 0.05$) in latency of sexual uprings was observed in rats of lot 1 and the positive control group ($p < 0.01$) compared with the control group negative (Figure-6).

On the day 4, doses of 100 and 200 mg/Kg resulted in a significant ($p < 0.05$) decrease in rats' latency of sexual uprings compared with the negative control. A significant decrease with a p-value less than 0.001 of the latency was observed in the rats of the positive control group compared to the negative control group.

On the day 8, a significant decrease ($p < 0.05$) in latent latency was observed in lots 1 and 2 compared to the negative control. In comparison with the negative control group, no significant difference was observed with lot 1.

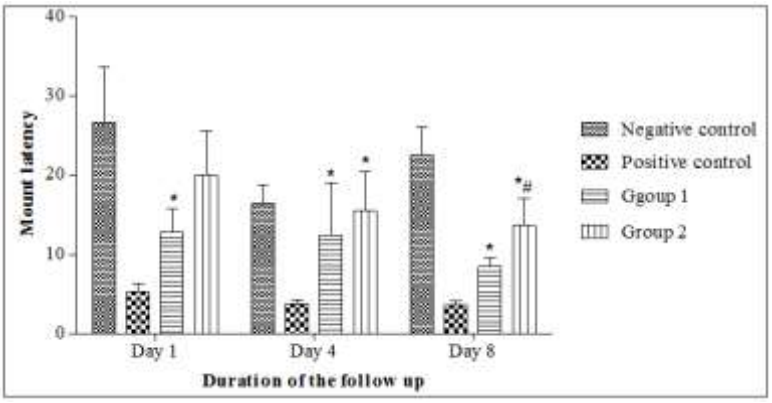

Fig-6: Effects of aqueous extract of *P. africana* trunk bark on the latency of sexual uprisings

The data are presented in the form of a standard mean ± deviation. Comparisons were made against negative (distilled water) (*) and positive (Sildenafil Citrate) (#) controls.

* = difference to P-value < 0.05; * * = difference to P-value < 0.01; = difference to P-value < 0.001, # = difference to P-value < 0.05; # # = difference to P-value < 0.01; # # # = difference to P-value < 0.001

Effect of the aqueous extract on latency of intromissions

On the day 1, a significant ($p < 0.05$) decrease in latency of intromissions for rats of lot 1 was observed and was not significant for lot 2 compared to the negative control (Figure-7).

On day 4, the comparison with the negative control group showed a significant decrease ($p < 0.05$) in the latency of intromissions for the rats of lots 1 and 2. This decrease was respectively of the order of 56.16%. (46 ± 7.72 s V 20.167 ± 6.31 s) and 40.94% (46 ± 7.72 s V 27.167 ± 9.638 s).

On At day 8, a significant ($p < 0.05$) decrease in latency of intromissions was observed in rats of lots 1 and 2 compared to the negative control.

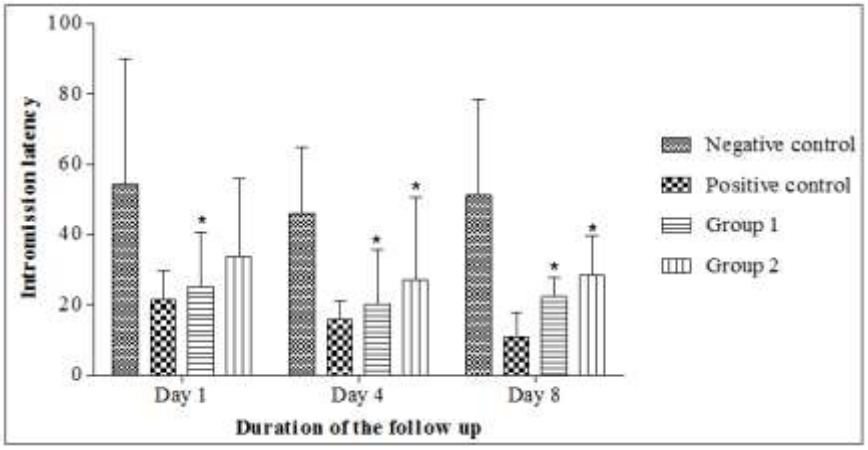

Fig-7: Effects of aqueous extract of *P. africana* Trunk Bark on intromission Latency of intromissions

The data are presented in the form of a standard mean ± deviation. Comparisons were made against negative (distilled water) (*) and positive (Sildenafil Citrate) (#) controls.

* = difference to P-value < 0.05; * * = difference to P-value < 0.01; = difference to P-value < 0.001, # = difference to P-value < 0.05; # # = difference to P-value < 0.01; # # # = difference to P-value < 0.001

DISCUSSION

The phytochemical screening revealed the presence of secondary metabolites (tannins, flavonoids, alkaloids, tri terpenes) in the aqueous extract of the bark of the *Prunusafricana* trunk. Indeed, the potential of a medicinal plant is attributed to the action of its phytochemical constituents. According to Mohammedi [15], secondary metabolites are produced in response to environmental stress or to provide a defense mechanism for plant-causing disease. The overall results of the phytochemical screening justify the craze of traditional healers to use medicinal plants, especially *P. africana*.

The acute toxicity study of the aqueous extract of *Prunusafricana* trunk bark showed that the oral extract produced a decrease in pain sensitivity at doses of 300, 2000 and 5000 mg/Kg. This decrease is due to the presence of secondary metabolites such as alkaloids in the plant, which confer an analgesic effect [16]. No deaths were found during the study. The LD_{50} is therefore greater than 5000 mg/ kg [17]. After Clarke and Clarke [18], a plant with an LD_{50} greater than 5000 mg/Kg body weight is considered non-toxic.

Oral administration of the aqueous extract of *P. africana* trunk bark stimulated the copulatory activity of the treated rats relative to the negative control. Specifically, the 100 mg/Kg extract significantly ($p < 0.05$) increased the number of erections, the frequency of sexual uprisings, intromisions, and ejaculations of rats. The latency as well as that of intromissions were reduced for the same dose. The significant increase in sexual erection and sexual uplift rates in treated rats suggests a lasting erection, hence an increase in the libido index. In addition, the decrease in the latency of sexual uprisings and intromissions are indicators of an aphrodisiac action [19]. The latency of sexuel uprisings and intromissions are inversely proportional to sexual motivation. Therefore, the significant decrease in latency and latency of intromissions observed in rats from lot 1 to day 1 may suggest stimulation of sexual motivation and arousal: the aqueous extract of the trunk bark *P. africana* induces an increase in motivation and sexual desire. The aphrodisiac effect of *P. africana* could be attributed to the existence of secondary metabolites revealed during the phytochemical screening of this plant. Indeed, the steroidal nature of saponins could provide an intermediary role in the androgen production pathway. Saponins may also bind to steroid hormone receptors, resulting in conformational changes and contributing to an increase in the function of these hormones [20]. Saponins also have a peripheral action by stimulating the release of nitric oxide (NO), vascular smooth muscle; NO being a mediator involved in the relaxation of vascular smooth muscle tissue [21].Similarly, the increasing number of sexual uprisings is due to the presence of sterols and flavonoids [8]. Indeed, these two chemical families

induce changes in the level of neurotransmitters involved in erectile function and modulate the action of neurotransmitters in their target cells or raise the androgen levels [22]. Furthermore, alkaloids known for their ergogenic properties, can act by inducing vasodilatation of blood vessels through the production of NO and eventually lead to erection or by stimulating steroidogenesis in testicles of animals. It is well documented that in erectile function, androgens stimulate the expression of the neuronal isoform of nitric oxide synthase (nNOS) and modulate the activity of phosphodiesterase type 5 [23].

An aphrodisiac is a substance that increases desire and/or sexual pleasure or that can arouse sexual desire or libido [24]. If we stick to this definition, it is possible to conclude that the aqueous extract of the trunk bark of *P. africana* has a pro-ejaculatory aphrodisiac effect. This aphrodisiac effect would be completely different from that of sildenafil citrate, the reference molecule used in this study. Indeed, sildenafil citrate is a non-androgenic aphrodisiac that is to say that it acts directly on penile erectile tissues inhibiting the activity of phosphodiesterase type 5, an enzyme involved in the specific degradation of cGMP, second messenger involved in the mechanism of erection. The prolonged action of cGMP is at the base of the aphrodisiac effect of sildenafil citrate [25].

CONCLUSION

The present work was undertaken to evaluate the aphrodisiac property of the aqueous extract of *P. africana* trunk bark in male albino and normal rats of Wistar strain. The study showed that *Prunusafricana* trunk bark contains tannins, flavonoids, alkaloids and sterols. Acute toxicity has shown that the aqueous extract of *P. africana* trunk bark has an LD_{50} greater than 5000 mg/Kg. The aphrodisiac test showed that *P. africana* trunk bark at doses of 100 and 200 mg/Kg body weight influenced the copulatory activity of male albino and normal male rats. Compared to rats in the negative control group, the effect of the aqueous extract of *P. africana* trunk bark on sexual behavior of the rats were materialized by the stimulation of sexual pleasure. The latter results in an increase in the frequency of ejaculations. The stimulation of the sexual performance is confirmed by the increase of the frequency of intromissions and the decrease of the latency of intromissions. The stimulation of sexual motivation is evidenced by an increase in the frequency of erections and sexual uprisings as well as the decrease in latency of sexual uprisings : the empirical use of *Prunusafricana* for the treatment of erectile impotence would be justified.

ACKNOWLEGEMENTS

We thank Dr ETAME LOE Gisèle (Email: giseloetame@yahoo.fr ; P.O. Box 15515 Douala Cameroon; Tel: 237 699 833 818), General Manager of Industrial Laboratory, GENEMARK for their grant support.

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