

EFFECT OF AQUEOUS EXTRACT OF *Rauvolfia vomitoria* (Apocynaceae) LEAVES ABOUT SEXUAL ACTIVITY OF MALE RATS

ABSTRACT

Objectif : The aim of this study is to determine the pharmacological effects of *Rauvolfia obscura* leaves on the sexual activity of animal model (male rat)

Methods: Acute toxicity was performed according to OECD Guideline 423, by a dose limit of 2000 mg /kg body weight. The observations (during 14 days) concerned the number of deaths, convulsions, sleep and coma. Study of sexual activity has been realized by using 20 male rats, distributed in 4 lots of 5 animals. Group 1 treated with distilled water, group 2 treated with 5 mg/kg B.W. of sildenafil citrate and the other two batches received respectively the doses 500 and 1000 mg/kg of the aqueous extract of *Rauvolfia obscura*. Females in estrus state (in heat) were introduced for a period of 30 minutes. During this period, parameters of sexual behavior were recorded. At the end of the 8-day treatment, penis testicles, seminal vesicles, prostate, epididymis, elevator muscle were removed, were studied for determine pharmacological effects of aqueous extract of *Rauvolfia obscura*.

Results: The aqueous extract of *Rauvolfia obscura* showed no evidence of single dose toxicity (2000 mg/kg) when studying acute toxicity. Ride latency time, coitus latency time and ejaculation latency time have significant decreased ($P < 0.001$) to regard to negative control (distilled water). Ride frequency, coitus frequency and ejaculation frequency have significant increased ($P < 0.001$) to regard to negative control (distilled water). No significant difference ($P > 0.05$) has been recorded on organ androgeno-dependant.

Conclusion: Aqueous extract of *R. obscura* has sexual stimulating activity or an aphrodisiac potential which could justify its traditional use. The aphrodisiac potential is higher at the dose of 1000 mg/kg b.w .

Keywords: *Rauvolfia vomitoria*, acute toxicity, aqueous extract, aphrodisiac potential

Introduction

Sexuality disorders are a major health problem, with many consequences. They mainly encompass the decrease in sexual desire, erectile dysfunction and ejaculation disorders^[1]. Although still taboo, erectile dysfunction (ED), sometimes called impotence, is a medical condition characterized by a repeated inability to initiate or maintain a rigid erection during^[2]. Of psychological origin, neurological, hormonal or vascular, ED is very widespread and affects men at all ages, but mainly 40 to 70 years old. This problem can also seriously hamper relationships within a couple, sometimes resulting in divorce^[3]. It is therefore very important to improve the quality of life of men who are affected as well as that of their sexual partner by means of adequate treatments.

Modern treatments for erectile dysfunction have seen advancements in recent decades. However, these treatments are still very expensive for most people with this pathology, especially in developing countries. This constraining situation, pushes the majority of people with erectile dysfunction, using herbal remedies as a means of treatment. The World Health Organization (WHO), estimates the population that uses medicinal plants at more than 80 %^[4].

Medicinal plants are used in the treatment of many pathologies such as malaria, diarrhea, infertility and diabetes^[5-10]. For the treatment of Erectile Dysfunction, several plant such as *Bridelia ferruginea*, *Rauvolfia obscura* et *Dactylorhiza hatagirea* have been studied and revealed interesting aphrodisiac effects^[11-13]. Some plants like *Rauvolfia vomitoria*, are very well known by traditional medicine, for the treatment of erectile dysfunction, however they received no special attention.

Rauvolfia vomitoria is a plant belonging to the Apocynaceae family, known for its anti diabetic, anti hyperglycemic and anticonvulsant properties.^[14-15]

The aim of this study is to determine the pharmacological effects of aqueous extract of *Rauvolfia vomitoria* leaves about sexual activity of rats.

Material et Methods

Plant material

The plant of *Rauvolfia vomitoria* Afzel were collected in the Abobo commune of the District of Abidjan (Ivory Coast) among herbalist. A sample of this plant was authenticated at the National Floristic Center of the University Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire), compared to the N°UCJ002178 sample.

Preparation of extract

The leaves of *Rauvolfia vomitoria* were washed and then dried in the shade (sheltered from the sun). Thereafter, the leaves are crushed using an electric blinder, brand RETSCH GM 300 to obtain a powder.

50 g of leaf powder were macerated in 1 L of distilled water in a blinder for 9 minutes, due to 3 minutes in a series of maceration. A minute's time, separates each series of maceration. The macerate is filtered 4 times on poplin fabric and then on Wattman No. 1 paper. The filtrate obtained was evaporated at 55 ° C in an oven. A dry extract was obtained^[16].

Phytochemical study

The qualitative phytochemical study was performed to identify the main phytoconstituents present in the leaves of *Rauvolfia vomitoria*

Animal material

Male rats (185-230g) and female rats (170-200g), from the vivarium of ENS (Ecole Normale Supérieure) of Abidjan, ont été utilisés. They were raised at ambient temperature of 22 ± 3 °C with 40 to 60% moisture and a photoperiod of 12 hours light and 12 hours darkness.

The animals were fed with diet of fish, bread, corn and water *ad libitum*.

Acute toxicity study

Acute toxicity study was conducted under the guideline OECD N° 423 ^[17]. Two groups (control, test group) of three healthy wistar female rats (170-190g) have received a limit dose of 2000 mg/kg of the AERV by oral administration. The observations focused on mortality, convulsions, salivations, sleep and coma each day for 14 days.

Female rat ovariectomy

Ovariectomy of females was performed according to the method used by **Cariton** ^[18].

Female rats, anesthetized with ether, underwent bilateral ovariectomy at the level of the dorsolateral abdominal walls. The ovaries were severed at the junction of the oviduct and the uterine body, then extracted from the abdominal cavity. The abdominal incision is then sewn with several stitches. Pain relievers have been used to prevent infections and to optimize postoperative healing. A 21-day post-operative period is necessary before starting the copulation tests.

Induction of estrus in ovariectomized female rats

Ten females (10) previously ovariectomized were induced in estrus (heat), by oral administration of 50 µg/kg ethinyl estradiol. Following administration of ethinyl estradiol, 48 hours later, females received a subcutaneous injection of 1 mg / kg of progesterone. Vaginal smears performed on these females confirmed successful estrus induction. Copulation tests began 6 hours after the induction of estrus.

Study of the effect of aqueous extract of *R. vomitoria* on the sexual activity of male rats

For this study, 20 male rats were used and distributed in 4 lots of 5 animals. Different treatments were administered to each batch, arranged as follows:

- **Lot 1** : Negative control treated with distilled water
- **Lot 2** : Positive control treated with 5 mg/kg b.w. of sildenafil citrate (viagra)
- **Lot 3**: Aqueous extract of *Rauvolfia vomitoria* batch treated with 500 mg/kg b.w. (AERV₅₀₀)
- **Lot 4**: Aqueous extract of *Rauvolfia vomitoria* batch treated with 1000 mg/kg b.w. (AERV₁₀₀₀)

Evaluation of pharmacological properties of *R. vomitoria* on sexual behavior were realized by placing male rats in different cages, then females in estrus state (in heat) were introduced for a period of 30 minutes. During this period, parameters of sexual behavior were recorded, such as :

- **Ride latency time** : time between the introduction of a female into the cage and first ride;
- **Coitus latency time** : time between the introduction of a female into the cage and first coitus ;

- **ejaculation latency time:** time between first coitus and first ejaculation;
- **ride frequency :** number of ride during period of observation;
- **coitus frequency :** number of coitus during period of observation;
- **ejaculation frequency :** number of ejaculations during period of observation.

Animals treated during 8-days, taking care weighings every 2 days during the experiment in order to study the evolution of body weight. At the end of the 8-day treatment, the animals were sacrificed by rapid decapitation. Testicles, seminal vesicles, prostate, epididymis, elevator muscle and penis were removed and immediately weighed to note the fresh weight.

Analysis Statistical

All data are expressed on average \pm SEM. Graphical representation and data processing were performed using Graph Pad Prism and EXCEL software. The statistical analysis was performed by analyzing the variances (ANOVA One-Way). The differences between the averages were determined according to the Newman-Keuls test. $P < 0.05$ is considered significant, $P < 0.01$ very significant and $P < 0.001$ highly significant.

RESULTS

Phytochemical study

Results of phytochemical screening are presented in the table 1.

Acute toxicity test

The acute toxicity results showed no evidence of toxicity of the aqueous extract of *Rauvolfia vomitoria* in animals administered at the 2000 mg/kg dose limit. The acute toxicity test showed the normal behaviour of the treated rats.

Effect of *Rauvolfia vomitoria* on sexual behavior

- *Effects of Treatment on male Body Weight Variation*

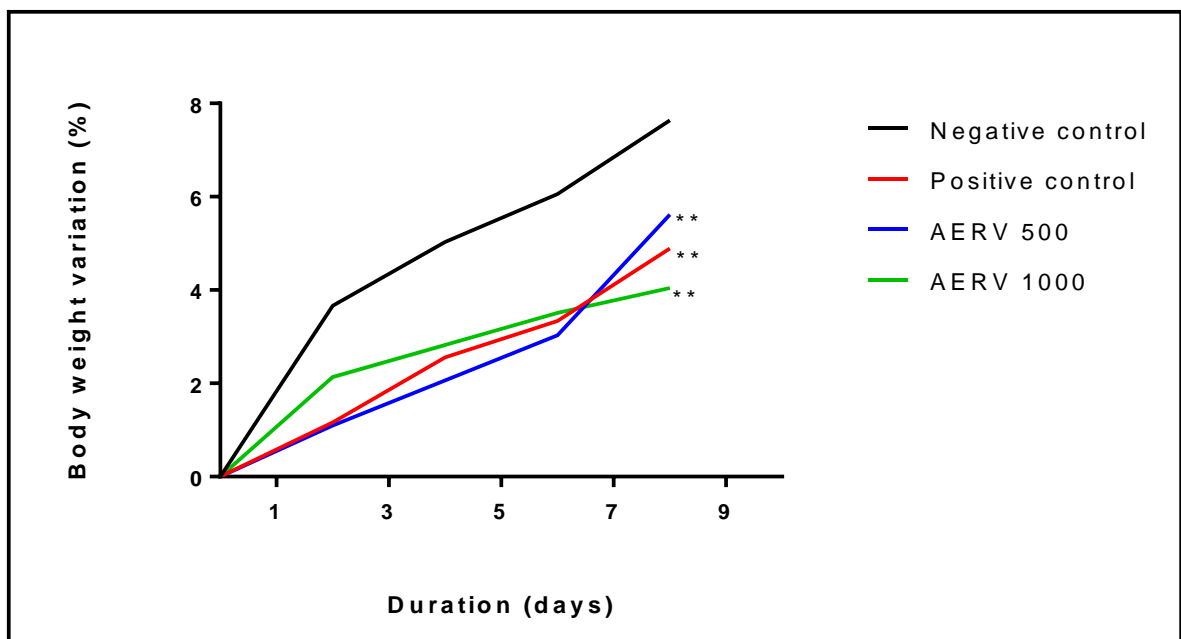
The results on body weight variation revealed very significant decreases ($P < 0.01$) of different treatments administered (positive control, AERV₅₀₀, AERV₁₀₀₀) compared to negative control. These decreases translate into 36.01 % ; 26.48 % et 47.11 % at the respective treatment of positive control, 500 and 1000 mg/kg b.w compared to negative control (**Figure 1**). However, no significant difference has been recorded at doses 500 and 1000 mg/kg b.w compared to positive control.

Table 1 : Phytochemical screening of extract of *Rauvolfia vomitoria* leaves

Phytochemical Compounds	Test used	AERV
Sterols et Polyterpenes	Acetic anhydride & Concentrated Sulfuric acid	+
Polyphenols	Ferric chloride FeCl ₃ (2%)	+
Flavonoids	Hydrochloric alcohol & Isoamyl alcohol	+
Tannins Catechin	Stiasny test (Formaldehyde & Hydrochloric acid concentrated)	+
Tannins Gallic	Sodium acetate & Ferric chloride	-
Quinones	Borntraeger test (Ammonia)	+
Alkaloids : Bouchardat	odured iodine reaction	-
Alkaloids : Dragendorff	Potassium iodobismuthate solution	-
Saponosides	Foam test	+

⊕ : Presence; ⊖ : Absence

AERV: Aqueous Extract of *Rauvolfia vomitoria*



** : $p < 0,01$; significant difference compared to negative control

Figure 1 : Effect of treatment with the aqueous extract of *Rauwolfia vomitoria* on body weight variation

- ***Effect on ride latency time***

The results on effect on ride latency time shown in **Figure 2**. The results revealed very significant differences ($P < 0.001$) of all treatments administered the 1st day and 8th day compared to negative control. These results translate into a significant decrease in 12,59 %, 23,05% and 19,46% the 1st day, to the respective treatments of Positive control, AERV₅₀₀ and AERV₁₀₀₀ compared negative control. The 8th day, the results also showed a significant decrease of 52.76 %, 70.59 %, 87.85 to the respective treatments of Positive control, AERV₅₀₀ et AERV₁₀₀₀ compared to negative control. Analysis of the results of the doses of the aqueous extract of *Rauvolfia vomitoria* (AERV₅₀₀ and AERV₁₀₀₀) compared to positive control revealed significant differences on day 1 and day 8. These differences translate into decreases, *ride latency time*, of 11.96 % and 7.86 % the 1st day of treatment at the respective doses of 500 and 1000 mg/kg de b.w compared to the positive control group. The 8th day of treatment, these decreases have been of 37.74 % and 74.29 % at the respective doses of AERV₅₀₀ and AERV₁₀₀₀ compared to positive control. The effect of AERV₅₀₀ significantly increased by 75.39 % ($P < 0.001$) from the 1st to the 8th day of treatment and that of AERV₁₀₀₀ decreased by 30.80% ($P > 0.05$) during this same period (from the 1st to the 8th day of treatment).

- ***Effect on ride frequency***

The results relating to ride frequency are presented on **Figure 3**. Analysis of the results showed significant differences following the administration of the various treatments, on the 1st and 8th day of treatment when compared to negative control. The 1st day of treatment, the differences translate into very significant increases ($P < 0.001$) of 70.90%, 362,5% et 275% respectively to treatments of positive control, AERV₅₀₀ and AERV₁₀₀₀ compared to negative control. 8th day, significant increases in 220.58% ($P < 0.001$) et 108.82% ($P < 0.01$) were observed at the respective treatments of positive control and AERV₁₀₀₀ comparativement au negative control. However, an augmentation of 20.58% following the administration of the aqueous extract of *R. vomitoria* at the dose of 500 mg/kg de b.w compared to the negative control group has been recorded, but statistical analysis did not reveal any significant difference ($P > 0.05$). Comparatively, to positive control, statistical analysis of the results showed a significant increase ($P < 0.01$), the 1st day of treatment, following the administration of dose of AERV₅₀₀. Thus, an increase of 34.54%, at the dose of AERV₅₀₀ compared to positive control, was observed.

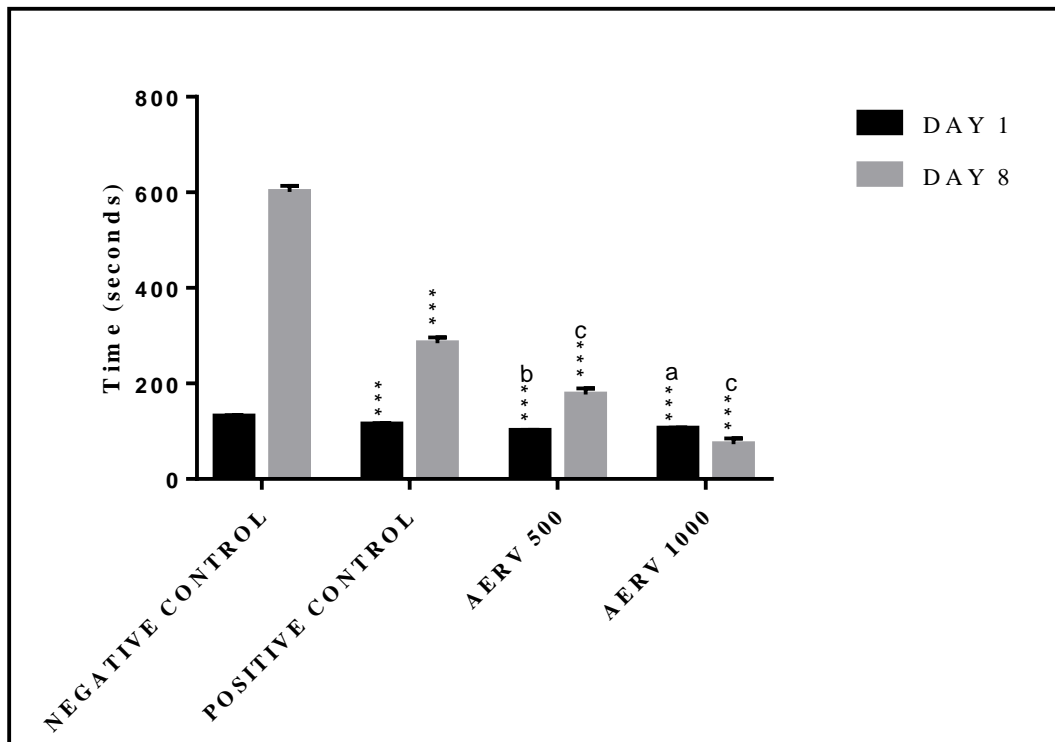


Figure 2 : Effect of treatment with the aqueous extract of *Rauwolfia vomitoria* on ride latency time

***: $P < 0,001$; significant difference compared to negative control (distilled water)

a : $P < 0,05$; b: $P < 0,01$; c: $P < 0,001$; significant difference compared to positive control (sildenafil citrate)

AERV₅₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 500mg/kg

AERV₁₀₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 1000 mg/kg

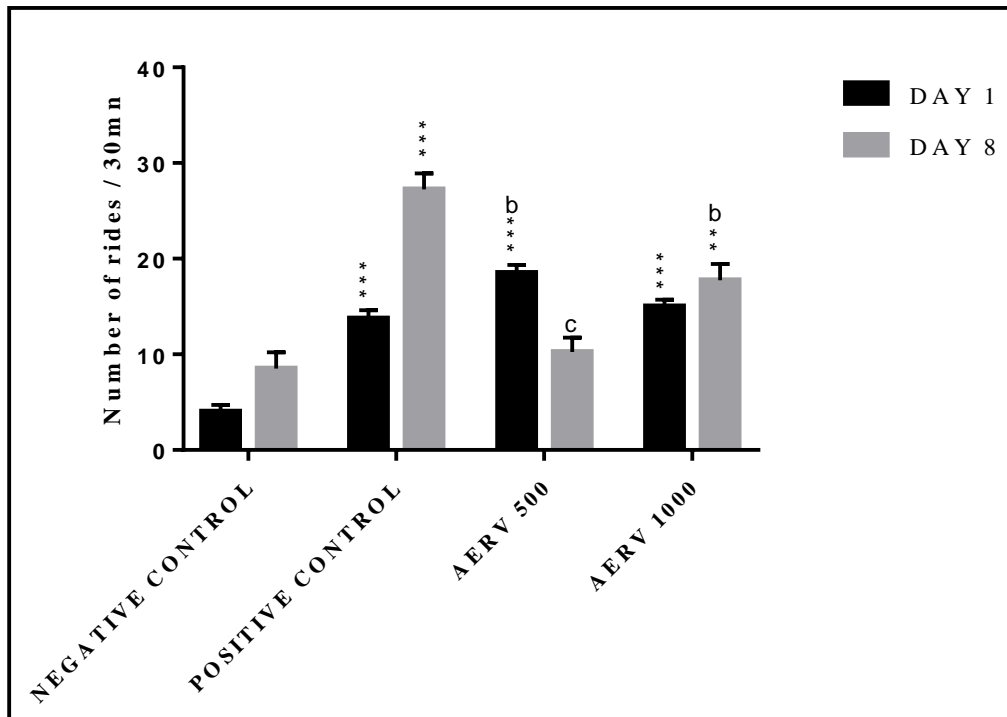


Figure 3 : Effect of treatment with the aqueous extract of *Rauwolfia vomitoria* on ride frequency

** : $P < 0,01$; *** : $P < 0,001$; significant difference compared to negative control (distilled water)

b : $P < 0,01$; c : $P < 0,001$; significant difference compared to positive control (sildenafil citrate)

AERV₅₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 500mg/kg

AERV₁₀₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 1000 mg/kg

The 8th day of treatment analysis of the results revealed significant decreases in 62.38% (P<0.001) and 34.86% (P<0.01) respectively at doses of 500 and 1000 mg/kg b.w compared to positive control. Effect of AERV₅₀₀ decreased in 44.59% (P<0.01) from the 1st to the 8th day of treatment, while that of AERV₁₀₀₀ increased of 18.33% (P>0.05).

- *Effect on Coitus latency time*

On coitus latency time, the results showed very significant differences (P<0.001), following the different treatments administered when compared to negative control, the 1st and the 8th day of treatment. Thus, the 1st day of treatment, analysis of the results revealed significant decreases in 73.33 %, 27.25% and 34.50 % to respective treatments of positive control, AERV₅₀₀ et AERV₁₀₀₀ compared to negative control. 8th day, significant reductions in 30.59 %, 72.33 % and 72.21 % were also recorded respectively at the treatments of positive control, AERV₅₀₀ and AERV₁₀₀₀ compared to negative control. Compared to positive control, the analysis of the results on the 1st day of treatment, revealed significant increases (P<0.001) de 172.79 % and 145.58 % at the respective doses 500 and 1000 mg/kg b.w. of aqueous extract of *Rauvolfia vomitoria*. The 8th day of treatment, compared to positive control, significant decreases (P<0.001) of 60.14 % and 59.97 % have been recorded, at doses of AERV₅₀₀ et AERV₁₀₀₀. The effects of aqueous extract of *Rauvolfia vomitoria* have increased significantly (P<0.001) of 24.31 % and 38.68 % at the respective doses of 500 and 1000 mg/kg b.w during the experimental period, either from the 1st to the 8th day of treatment (**Figure 4**).

- *Effect on Coitus frequency*

The results on coitus frequency are presented at the **Figure 5**. Statistical analysis of the results showed significant increases (P<0.001) of 264.28 %, 235.71 % and 171.42 % to the respective treatments of positive control, AERV₅₀₀ et AERV₁₀₀₀ compared to negative control, the 1st day of treatment. The 8th day of treatment, compared to negative control, analysis of the results revealed significant increases in 342.85 % (P<0.001) and 135.71 % (P<0.05) to the respective treatments of positive control and AERV₁₀₀₀.

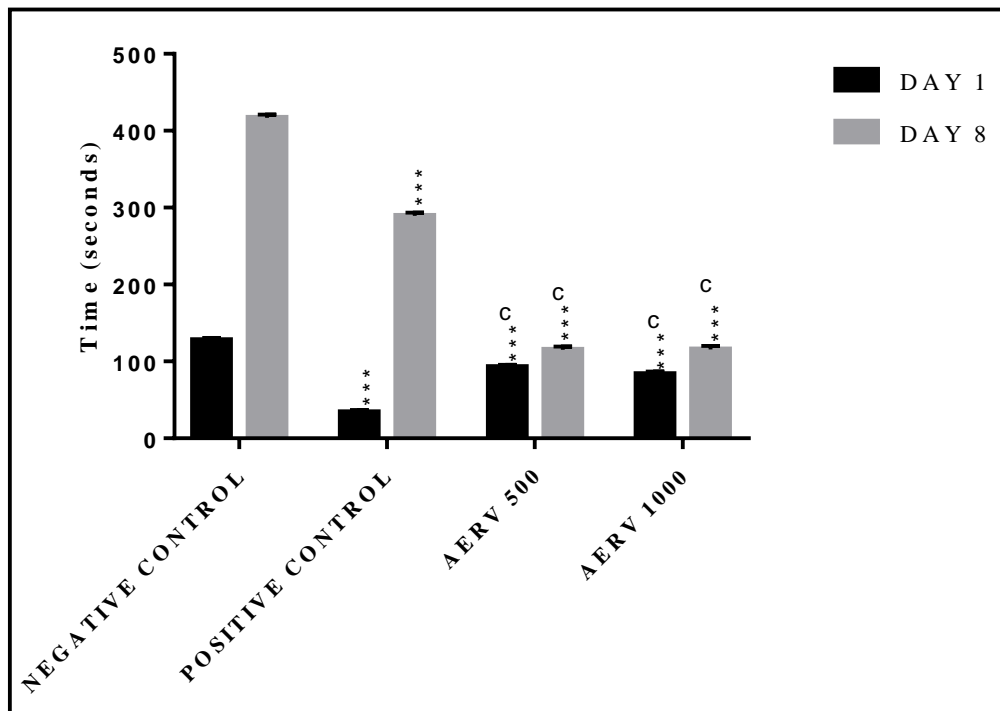


Figure 4 : Effect of treatment with the aqueous extract of *Rauvolfia vomitoria* on coitus latency time

*** : $P < 0,001$; significant difference compared to negative control (distilled water)

c: $P < 0,001$; significant difference compared to positive control (sildénafil citrate)

AERV₅₀₀ : Aqueous extract of *Rauvolfia vomitoria* to 500mg/kg

AERV₁₀₀₀ : Aqueous extract of *Rauvolfia vomitoria* to 1000 mg/kg

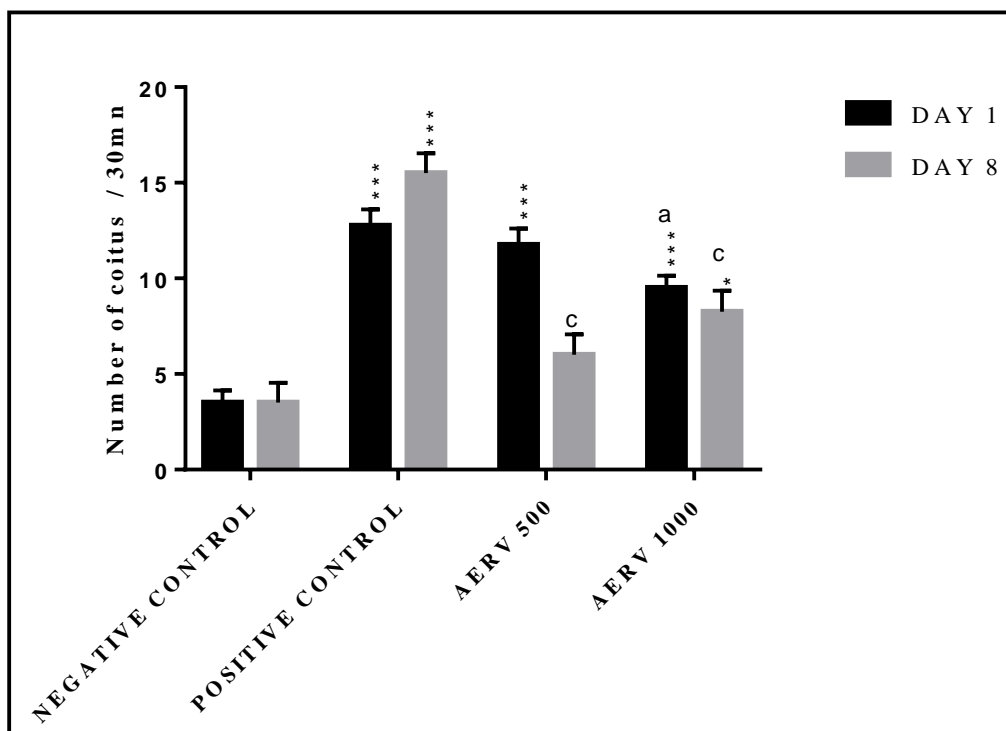


Figure 5 : Effect of treatment with the aqueous extract of *Rauwolfia vomitoria* on coitus frequency

*: $P < 0,05$; *** : $P < 0,001$; significant difference compared to negative control (distilled water)

a : $P < 0,05$; c: $P < 0,001$; significant difference compared to positive control (sildénafil citrate)

AERV₅₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 500mg/kg

AERV₁₀₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 1000 mg/kg

The administration of the aqueous extract of *Rauvolfia vomitoria* increased by 71.42% the 8th day of treatment, compared to negative control however no significant difference was recorded. Analysis of the results of the 1st day of treatment revealed no significant difference ($P>0.05$) between AERV₅₀₀ and positive control. However, a significant difference was recorded on the 1st day of treatment, translating in a decrease in 25.49% following treatment with the dose of 1000 mg/kg b.w. of aqueous extract of *R. vomitoria* compared to positive control. The 8th day of treatment, statistical analysis of the results revealed significant decreases in 61.29 % and 46.77 % at the respective doses of AERV₅₀₀ and AERV₁₀₀₀ compared to positive control. The effect of aqueous extract of *Rauvolfia vomitoria* decreased by 48.93% ($P<0.01$) and 13.15% ($P>0.05$) respectively at doses of 500 and 1000 mg/kg b.w., from the 1st to the 8th day of treatment.

- *Effect on ejaculation latency time*

Analysis of the results of ejaculation latency time showed significant differences ($P<0.001$) on the 1st day of treatment, following the administration of the different treatments compared to negative control. These differences are translating on the 1st day, by significant increases in 48 %, 38.35 % et 74.35 % respectively to the treatments of positive control, AERV₅₀₀ and AERV₁₀₀₀ compared to negative control. On the 8th day, the results revealed significant decreases in 42.38 %, 54.53 % and 42.13 % to the respective treatments of positive control, AERV₅₀₀ and AERV₁₀₀₀ compared to a negative control. Compared to positive control, the analysis of the results showed significant differences following treatments at different doses of the aqueous extract of *Rauvolfia vomitoria*. Thus, the 1st day of treatment, a significant increase was observed ($P<0.05$) of 17.81 % following the treatment of AERV₁₀₀₀ compared to positive control. No significant difference was observed in dose AERV₅₀₀ compared to positive control, the 1st day. The 8th day of treatment, statistical analyzes revealed a significant decrease in 21.08% at the dose of 500 mg/kg b.w of the aqueous extract of *Rauvolfia vomitoria* compared to positive control. Statistical analyzes relating to the administration of AERV₁₀₀₀, the 8th day of treatment, showed no significant difference compared to positive control. Effect of aqueous extract of *Rauvolfia vomitoria* from the 1st to the 8th day of treatment, increased by 9.69% and 8.41% at the respective doses of 500 and 1000 mg/kg b.w. However, statistical analyzes revealed no significant changes during this experimental period (**Figure 6**).

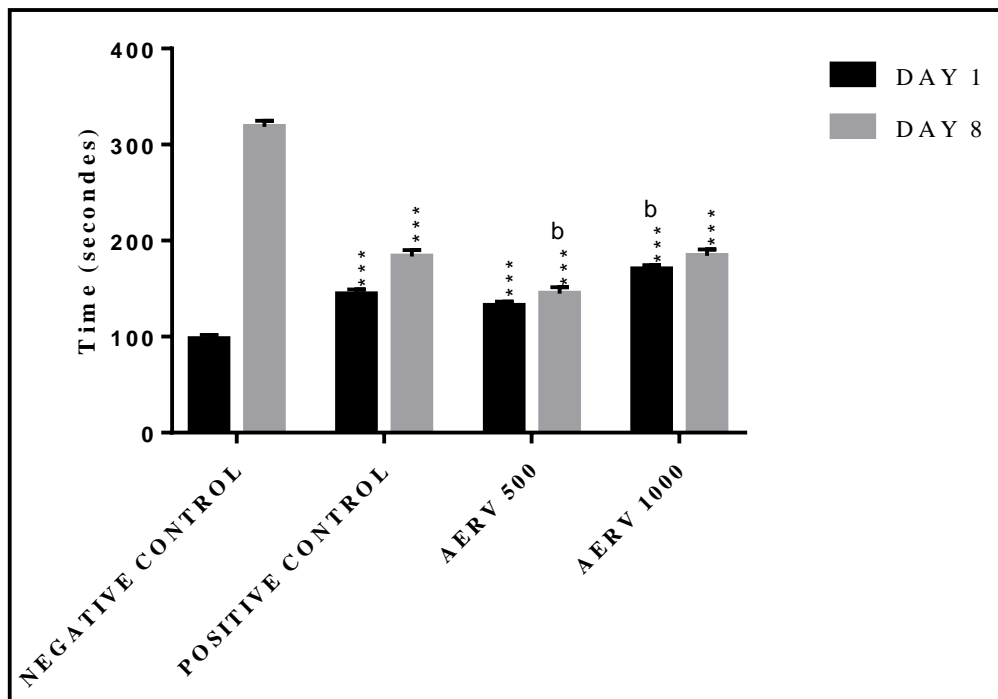


Figure 6 : Effect of treatment with the aqueous extract of *Rauwolfia vomitoria* on ejaculation latency time

***: $P < 0,001$; significant difference compared to negative control (distilled water)

b: $P < 0,01$; significant difference compared to positive control (sildénafil citrate)

AERV₅₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 500mg/kg

AERV₁₀₀₀ : Aqueous extract of *Rauwolfia vomitoria* to 1000 mg/kg

- ***Effect on ejaculation frequency***

The results relating to ejaculation frequency are shown on la **Figure 7**. the 1st day of treatment, the results showed significant differences ($P < 0.001$) following the various treatments administered compared to negative control. Thus, these differences translating by significant increases of 130%, 230% and 155% respectively to the treatments of positive control, AERV₅₀₀ and AERV₁₀₀₀ compared to negative control. 8th day, analysis of the results revealed significant increases in 186.66 % ($P < 0.001$) et 106.66 % ($P < 0.05$) to the respective treatments of positive control and AERV₁₀₀₀ compared to negative control. Compared to positive control, the analysis of the results showed significant differences. Thus, the 1st day of treatment, a significant increase in 43.47 % ($P < 0.01$) has been observed, at dose of 500 mg/kg b.w of aqueous extract of *Rauvolfia vomitoria* compared to positive control. The 8th day of treatment, analysis of the results revealed, a significant decrease in 58.13 % ($P < 0.01$) and 27.90 % ($P < 0.05$) at the respective doses of AERV₅₀₀ and AERV₁₀₀₀ compared to positive control. Effect of aqueous extract of *Rauvolfia vomitoria*, from the 1st to the 8th day of treatment, decreased in 72.72 % ($P < 0.001$) and 39.21 % ($P < 0.01$) respectively at doses of 500 and 1000 mg/Kg b.w (Body Weight).

- ***Effect on relative weight of some organs***

The results relating to the effect of aqueous extract of *Rauvolfia vomitoria* on the weight of the organs, are recorded in the **table 2**.

On prostate, the analysis of the results revealed a significant difference ($P < 0.05$) following the treatment of positive control compared to negative control. This difference translates into a significant increase in 57.55 %, following the treatment of positive control compared to negative control. In addition, still on this organ (Prostate), the results revealed significant decreases in 48.88 % et 56.45 % respectively at doses of 500 and 1000 mg/kg b.w of aqueous extract of *Rauvolfia vomitoria* compared to positive control.

On other organs (penis, seminal vesicle, cowper's gland, elevator muscle, testicle and epididym), analysis of the results did not reveal any significant difference ($P > 0.05$) following the administration of AERV₅₀₀ and AERV₁₀₀₀ compared to negative control and positive control.

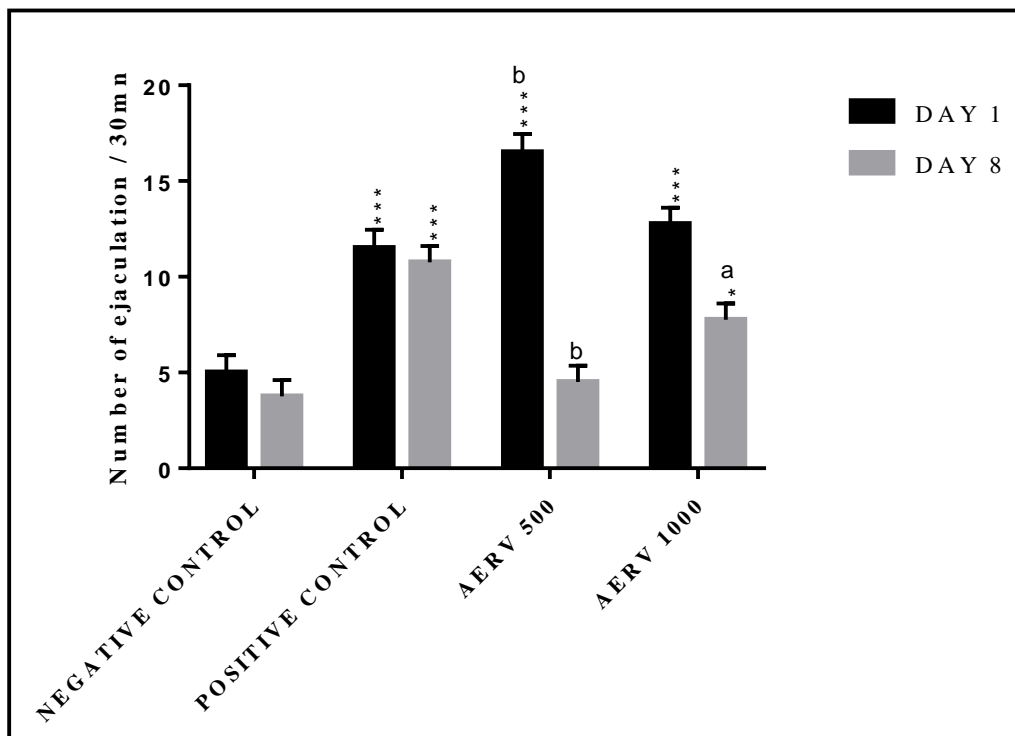


Figure 7 : Effect of treatment with the aqueous extract of *Rauvolfia vomitoria* on ejaculation frequency

*: $P < 0,05$; ***: $P < 0,001$; significant difference compared to negative control (distilled water)

a : $P < 0,05$; b: $P < 0,01$; significant difference compared to positive control (sildénafil citrate)

AERV₅₀₀ : Aqueous extract of *Rauvolfia vomitoria* to 500mg/kg

AERV₁₀₀₀ : Aqueous extract of *Rauvolfia vomitoria* to 1000 mg/kg

Table 2 : Effect of aqueous extract of *Rauvolfia vomitoria* on organ weights rats

ORGAN (g/100g b.w)	NEGATIVE CONTROL	POSITIVE CONTROL	AERV 500	AERV 1000
PENIS	0,09075±0,010	0,1511±0,022	0,1170±0,013	0,1061±0,004
SEMINAL VESICLE	0,3607±0,100	0,3598±0,108	0,3931±0,079	0,3573±0,044
PROSTATE	0,1819±0,222	0,2866±0,036*	0,1465±0,018 ^b	0,1248±0,017 ^b
COWPER'S GLAND	0,02822±0,004	0,02787±0,006	0,03132±0,0009	0,02835±0,003
ELEVATOR MUSCLE	0,3027±0,047	0,3772±0,066	0,3082±0,020	0,2526±0,019
TESTICULE	1,165±0,098	1,234±0,071	1,327±0,079	1,186±0,030
EPIDIDYM	0,3694±0,031	0,3763±0,058	0,4565±0,039	0,4830±0,078

*: P<0,05; significant difference compared to negative control (distilled water)

b: P<0,01; significant difference compared to positive control (sildénafil citrate)

AERV₅₀₀ : Aqueous extract of *Rauvolfia vomitoria* to 500mg/kg

AERV₁₀₀₀ : Aqueous extract of *Rauvolfia vomitoria* to 1000 mg/kg

DISCUSSION

Phytochemical results revealed the presence of several secondary metabolites described in Table 1. The results obtained are similar to those obtained by **Olajumoke et al.** but different to those obtained by **N'Guessan et al.** [19-20]. Indeed, the triphytochemical tests carried out on the leaves of *Rauvolfia vomitoria* by **N'Guessan et al.** did not highlight the presence of Polyphenols, flavonoids, tannins and quinone compounds. This difference could be explained by the geographical location, the harvest period and storage conditions of the plant. These results show that *Rauvolfia vomitoria* possess some molecules with known biological activity. Indeed, flavonoids and Saponin have anti inflammatory, antioxidant, bone properties and hepatoprotective effect [21-24]. Polyphenols have cardiovascular properties and fight against degenerative diseases [25-26].

No mortality and signs of toxicity were observed after administration of the dose limit (2000 mg/kg). These results on the acute toxicity of the aqueous extract of *Rauvolfia vomitoria* have shown that the lethal dose of this plant is greater than the 2000 mg/kg limit dose. These results confirm the work of **Amole et al.** which locates the toxicity of the extract of this plant beyond 5000 mg/kg [27].

8 days of treatment with the aqueous extract of *Rauvolfia vomitoria*, has shown significant difference in body weight compared to the negative control. This difference, which resulted in significant reductions in body weight at different doses of the aqueous extract of *Rauvolfia vomitoria* could be explained by the stress caused by the administration of the extract. Also, the decrease in body weight could be attributed to the effect of the saponins present in the extract. Indeed saponins have anti-inflammatory, antioxidant properties, and help in weight loss [28].

Ride time latency, coitus time latency and ejaculation time latency significantly decreased following administration of the aqueous extract of *Rauvolfia vomitoria*. The effects observed are similar to those of **Ondele et al.** [12]. Indeed, **Ondele et al.** observed a decrease ride time latency in rats treated with the aqueous extract of *Rauvolfia obscura*. Reduction of ride time latency, coitus and ejaculation are indicators of an aphrodisiac action. This decrease also reflects virility, because a long time causes nervous fatigue.

Ride frequency, coitus frequency and ejaculation frequency, following the administration of the various doses of the aqueous extract of *Rauvolfia vomitoria* (500 and 1000 mg/kg), significantly increased on the 1st and 8th day of treatment compared to negative

control. These results are similar to those of **watcho et al.** who observed increases of coitus frequency and ejaculation in the evaluation of the prosexual effects of extracts of *Bridelia ferruginea*^[11]. These observed effects could be attributed to the existence of flavonoids and sterols revealed by phytochemical tests in the aqueous extract of de *Rauvolfia vomitoria*. Indeed, these bioactive substances could induce changes in the level of neurotransmitters involved in erectile function, could modulate the action of neurotransmitters, at the level of their target cells or could raise androgen levels^[29].

On the relative weight of androgen-dependent organs, no significant difference was observed between the different doses of the aqueous extract of *Rauvolfia vomitoria* and negative control (distilled water). These observed results could suggest that the aqueous extract of the leaves does not have detectable androgenic properties over the period of treatment studied. Indeed, in erectile function, androgens have been shown to stimulate the expression of the neuronal isoform of nitric oxide synthase (NO_s) and modulate the activity of phosphodiesterase type 5^[30-31]. In penile tissue, NO represents the major neurotransmitter, responsible for smoothmuscle relaxation during the erectile response^[32]. The aqueous extract of the leaves of *R. vomitoria* could be a non-androgenic aphrodisiac which could act directly on the erection.

The results of this study showed that the doses of the aqueous extract of *R. vomitoria*, especially the dose of 1000 mg/kg b.w with a higher aphrodisiac potential than that of dose 500 mg/kg could have activities similar to those of the positive control (sildénafil citrate or viagra). Indeed, viagra (sildénafil citrate), reference molecule used in this study, is a non-androgenic aphrodisiac which acts directly on the penile cavernous tissue. They work by blocking an enzyme called phosphodiesterase type 5. This enzyme breaks down a molecule called cyclic guanosine monophosphate (cGMP). Blocking the enzyme releases more cGMP, which promotes relaxation of smooth muscles (involuntary) of penis; this allows a greater blood supply and facilitates the erection^[33].

CONCLUSION

It result from this study that the aqueous extract of the leaves of *Rauwolfia vomitoria* show no signs of toxicity when administered in a single dose. The aqueous extract of *Rauwolfia vomitoria* possesses, sexual stimulating activity or a potential aphrodisiac which could confirm its interest in traditional use. The aphrodisiac potential is higher at the dose of 1000 mg/kg aqueous extract of *Rauwolfia obscura*. However, additional studies are required in order to valorize again this plant.

UNDR PEER REVIEW

REFERENCES

- [1] **McMahon M., FACSHB B., Carmita A. Luca I., Michael P., David R., Marcel W. and Zhong C.** Disorders of Orgasm and Ejaculation in Men. *Journal of Sexual Medicine*. 2004 ; 1(1) : 58-65
- [2] **Ridwan S. and Aristotelis G.** Erectile Dysfunction. *Annual Review of Medicine*. 2003 ; 54: 153–168
- [3] **Chevret M., Jaudinot E., Kate S., Marrel A. and Annesolesse G.** Impact of Erectile Dysfunction (ED) on Sexual Life of Female Partners: Assessment with the Index of Sexual Life (ISL) Questionnaire. *Journal of Sex & Marital Therapy*. 2004 ; 30:157–172,
- [4] **W.H.O.** World Health Organization (WHO) strategy for traditional medicine for 2002-2005. WHO/EDM / TRM. 2002; 1:1-63
- [5] **Mahdi M., Rozhin N. and Fariba H.** Screening of Different Extracts from *Artemisia* Species for Their Potential Antimalarial Activity. *Iranian Journal of Pharmaceutical Research*. 2015 ; 14 (2): 603-608
- [6] **Belay M., Assefa B. A. and Zewdu B.** Antidiarrheal Activity of 80% Methanolic Leaf Extract of *Justicia schimperiana*. *Evidence- Based Complementary and Alternative Medicine*. 2018: 10 p
- [7] **Trabi F., Guy M., N’gaman K. and Clejesson M.** Études de quelques plantes thérapeutiques utilisées dans le traitement de l’hypertension artérielle et du diabète : deux maladies émergentes en Côte d’Ivoire. *Sciences & Nature*. 2008 ; 5 (1): 39- 48
- [8] **Blahi A. N. M., Zougrou N. E., Gnahoué G. and Kouakou K.** Mechanism of Action of the Aqueous Leaves Extract of *Sarcocephalus latifolius* (Smith) on the Reproductive System of Female Rat. *Journal of Physiology and Pharmacology Advances*. 2016 ; 6(12): 950-959
- [9] **Zougrou N., Blahi A., Kouassi K. and Kouakou K.** Effects of the Aqueous Extract of *Cnestis ferruginea* on the Histological Structure of Female Rat *Ovary and Uterine Horns*. *Biomed J Sci & Tech Res*. 2018 ; 2(1) : 2073-2078
- [10] **Affy M., Tovi W., Zougrou N. And Kouakou K.** Effect of methanolic extract of *Amaranthus viridis* leaves on reproductive functions in wistar female rats. *Journal of Drug Delivery & Therapeutics*. 2019; 9(6-s):119-126
- [11] **Watcho P., Nchegang B. Nguelefack T. and Kamanyi A.** Evaluation of pro-sexual effects of *Bridelia ferruginea* extracts in sexually naive male rat. *Basic and Clinical Andrology*. 2010 ; 20: 209–215
- [12] **Ondele R., Ossibi A., Bassoueka J., Peneme B., Itou R., Massengo B. And Abena A.** Toxicité aigüe et effet aphrodisiaque de l’extrait aqueux de *Rauvolfia obscura* k. Schum(apocynaceae). *Afrique SCIENCE*. 2015 ; 11(3) : 172-180
- [13] **Thakur M. and Dixit V.** Aphrodisiac Activity of *Dactylorhiza hatagirea* (D.Don) Soo in Male Albino Rats. *Evidence Based Complementary and Alternative Medicine*. 2007; 4(Suppl 1): 29–31

- [14] **N'doua L., Kouakou J., Aoussi S., Kouakou K. and Ehile E.** Aqueous Extract of *Rauwolfia Vomitoria* Afzel (Apocynaceae) Roots Effect on Blood Glucose Level of Normoglycemic and Hyperglycemic Rats. *American Scientific Research Journal for Engineering, Technology, and Sciences*. 2016 ; 20(1) : 66-77pp
- [15] **Olatokunboh A., Kayode Y. and Oshikoya K.** Anticonvulsant activity of *Rauwolfia Vomitoria* (Afzel). *African Journal of Pharmacy and Pharmacology*. 2009 ; 3(6) : 319-322pp
- [16] **Yapo Y., Guédé K., Tra Bi O., Zirihi G. and Guessennd N.** Antibacterial Activity Of Leaves' Aqueous Crude Extract (Eta) Of *Mallotus oppositifolius* (Geisel.) Müll-Arg (Euphorbiaceae) On Methicillin-Resistant *Staphylococcus aureus* (Mrsa) And Phytochemical Screening. *Revue Bio-Africa*. 2020; 23: 38-48pp
- [17]-**OECD.** OECD guideline for testing of chemicals. Test N°423: Acute Oral Toxicity – Acute Toxic Class Method. OECD Publishing 2001, 14p
- [18] **Cariton E.** Experimental surgery of the genital system. In: William IG, James EH (eds) *Methods of animal experimentation: research surgery and care of the research animal; Part B Surgical approaches to organ systems*. *Academic Press, Inc*. 1986: 191p
- [19] **N'Guéssan K., Beugré K., Guédé N., Zirihi , Traoré, D. and AKÉ-ASSI L.** Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Kroubo (Agboville, Côte-d'Ivoire). *Sciences & Nature*. 2009; 6(1): 1 - 15
- [20] **Olajumoke O., Soretiwa S. and Lawrence O.** Phytochemical screening, anti-nutrient composition, proximate analyses and the antimicrobial activities of the aqueous and organic extracts of bark of *Rauwolfia vomitoria* and leaves of *Peperomia pellucida*. *International Research Journal of Biochemistry and Bioinformatics*. 2012 ; 2(6) : 127-134
- [21] **Nijveldt R., Van N., Van H., Boelens P., Van N. and Van L.** Flavonoids: a review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition*. 2001; 74:418-425
- [22] **Alaoui K, Lagorce J., Cherrah Y., Hassar M., Amarouch H. and Roquebert J.** Activité analgésique et anti-inflammatoire des saponines d'*Argania spinosa*. In: *Annales pharmaceutiques françaises*. 1998: 220-228p
- [23] **Soundarya S., Sanjay V., Haritha A., Dhivya S. and Selvamurugan N.** Effects of Flavonoids Incorporated Biological Macromolecules Based Scaffolds in Bone Tissue Engineering. *International Journal of Biological Macromolecules*. 2017 : 48p
- [24] **Lin Wan and Jian-Guo J.** Protective effects of plant-derived flavonoids on hepatic injury. *Journal of Functional Foods*. 2018 ; 284-291
- [25] **Min-Ho O., Cyril A., Eugenia B., Park S., Hyunho L., Valérie B. and Schini-Kerth.** Potential mechanisms underlying cardiovascular protection by polyphenols: Role of the endothelium. *Free Radical Biology and Medicine*. 2018 : 1-35.
- [26] **-Sakib Md. H, Yousuf A. Md., Jahurul M., Mohamed M., Siew H. and Khalil I.** Beneficial roles of honey polyphenols against some human degenerative diseases: a review. *Pharmacological reports*. 2017 : 1-36.

- [27] **Amole O., Yemitan O. and Oshikoya K.** Anticonvulsant activity of *Rauvolfia Vomitoria* (Afzel). *African Journal of Pharmacy and Pharmacology*. 2009; 3(6) : 319-322 pp
- [28] **Adjatin A., Dansi A., Badoussi E., Loko Y., Dansi M., Azokpota P., Gbaguidi F., Ahissou H., Akoègninou A., Akpagana K. and Sanni A.** Phytochemical screening and toxicity studies of *Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *Crassocephalum crepidioides* (Benth.) S. Moore consumed as vegetable in Benin. *International Journal of Current Microbiology and Applied Sciences*. 2013 ; 2(8): 1-13
- [29] **Suresh K., Subramoniam A. and Pushpangadan P.** Aphrodisiac activity of *Vanda tessellata* (Roxb.) Hook. Ex Don extract in male mice. *Indian Journal of Pharmacology*. 2000; 32(5) :300–304
- [30] **Mills T., Stopper V. and Wiedmeier V.** Effects of castration and androgen replacement on the hemodynamics of penile erection in the rat. *Biology of Reproduction*. 1994; 51 : 234-238
- [31] **Annamaria M., Sandra F., Rosa M., Michaela L., Linda V., Mirca M., Claudio O., Gabriella B., Antonio A., Alessandro N., Gianni F., Mauro G., Emmanuele A., Fabrizio L. and Andmario M.** Androgens regulate phosphodiesterase type 5 expression and functional activity in corpora cavernosa. *Endocrinology*. 2004 ; 145(5) :2253-2263
- [32] **Andersson K.** Pharmacology of penile erection. *Pharmacological Reviews*. 2001; 53(3): 417-450
- [33] **Haroldo A., Fernanda B., Saiprasad M., Edson A., Cleber T. and Clinton W.** Effect of the phosphodiesterase 5 inhibitors: sildenafil, tadalafil and vardenafil on rat anococcygeus muscle: functional and biochemical. *Clinical and Experimental Pharmacology and Physiology*. 2009 ; 36 : 358– 366