

Assessment Of Oestrus Cycle Regularity And Toxicity Markers In African Giant Rats (*Cricetomys gambianus*) Treated With Ethanolic Extract Of *Senecio biafrae*

Takam Mbogne Boris^{1*}, Fonou Tadiesse Lavoisier², Foda Fopa Constant¹,
Tsambou Astride Martine¹, Ndam Florence¹, Fonkem Severin¹,
Vemo Bertin Narcise², Kenfack Augustave¹

¹: Department of Animal Science, Faculty of Agronomy and Agricultural Science, University of Dschang, Po Box 188 Dschang-Cameroon.

²: Department of Animal Science, Faculty of Agriculture and Veterinary Medicine, University of Buea, Po Box 63 Buea-Cameroon

Abstract:

Background: *Senecio biafrae* (*S. biafrae*) is a leafy vegetable commonly used in the treatment of female fertility problems. This work evaluated the effects of ethanolic extract of *S. biafrae* on oestrus cycle regularity as well as its toxic effects in female African giant rats (AGR).

Materials and Methods: For this, thirty-two adult female rats were randomly assigned into 4 lots of 8 animals each based on their body weight. Animals were housed individually and fed ad libitum. Lots 2, 3 and 4 animals were orally treated daily with 50, 100 and 150 mg/Kg bw plant extract blended with 10 g of tendered avocado. Lot 1 animals received corresponding doses of avocado only and served as control lot. After 30 days treatment, 3 animals per lot were sacrificed for blood and organ sampling while the others were individually introduced in an untreated male cage and continuously treated till mating.

Results: Results showed significant decrease ($p < 0.05$) in oestrus cycle length in lots 3 and 4 animals. Pre-coitus Co-habitation length was significantly higher in lot 2 animals compared to control lot. Mating rate was significantly higher in lots 3 and 4 compared to control lot. The ethanolic extract of *S. biafrae* equally induced a significant increase ($p < 0.05$) in serum AST and urea.

Conclusion: In conclusion, treatment of female AGR with 100 and 150 mg/kg b.w. ethanolic extract of *S. biafrae* decreased oestrus cycle duration and improved on cycle regularity. Caution to its usage should be taken with respect to treatment duration so as to minimize possible side effects.

Key Word: African giant rat, *Senecio biafrae*, oestrus cycle, toxicity

Date of Submission: 12-05-2024

Date of Acceptance: 22-05-2024

I. Introduction

African giant rat is highly consumed as food and constitutes an income source for hunters in Africa (Asibey and Addo, 2002; Cooper, 2008). Due to its overexploitation, AGR started to be scarce in several localities. That is why the domestication of this species is needed to preserve it from extinction. Several studies have been carried out with the hope of becoming technically able to produce AGR in captivity. So far, Tsambou, (2020) showed that, an increase in dietary energy level improves growth but not reproductive performances. Fonou *et al.* (2021) observed a reduction in reproductive performances in AGR subjected to long light exposure. More recently, a positive correlation between the age at breeding and reproductive performances was established (Fonkem *et al.* 2022a). Moreover, a similar tendency was observe following treatment of pregnant AGR with varying doses of 17- α -hydroxyprogesterone caproate (Fonkem *et al.* 2022b).

In African countries, medicinal plants have long been used in the treatment of reproductive problems (Mbemya *et al.*, 2017). Indeed, adding to their low cost, medicinal plants induce the least secondary effects compared to synthetic products. *S. biafrae* is one of these plants that occurs naturally in Africa and has been proven to contain molecules acting on reproductive functions. Previous studies on the ethanolic and aqueous extracts of this plant in wistar rats revealed their inducing effect on precocious puberty attainment, increase ovarian and uterine weights, increase steroid hormones and protein levels, along with a decrease in ovarian cholesterol signaling their gonadotrophin effects (Lienou *et al.*, 2010, 2015). Meanwhile, in female AGR, its effects on reproduction characteristics in general and the oestrus cycle in particular remain unknown. Moreover, consumption of herbal products by various ethnic groups involves challenges and drawbacks, including several

adverse effects, sometimes life-threatening, hence putting into question the safety of herbal remedies (Elvin-Lewis, 2001).

The aim of this study was to investigate the effects of ethanolic extract of *S. bialfrae* on oestrus cycle regularity and toxicity markers.

II. Material And Methods

Study area

The study was carried out at the Teaching and Research Farm (TRF) of the University of Dschang (Cameroon) between June and August 2023. TRF is located in the western highlands at an altitude of 1420m, latitude 5-7 N and longitude 8-12 E. Precipitation is between 1500-2000mm/year and temperature fluctuates between 15 and 25°C.

Animal and housing

32 adult females AGR weighing 881 ± 27.3 g were used. They were obtained from hunters at a young age and bred at the TRF until the weight needed for the study. Animals were housed singly in superimposed concrete cages measuring 100 cm long, 80 cm wide, and 60 cm high, all equipped with feeder, water trough and litter. They were reared under a natural photoperiod (12:12 h light: dark).

Feeding

Throughout the study period, AGR had free access to potable water and food. Their diet was composed of foodstuffs they usually eat in the wild, mainly ripe bananas, potatoes and maize, to which was added provender with bromatological characteristics (Fonou *et al.*, 2021; Fonkem *et al.*, 2022a): energy (2700Kcal/Kg of DM), crude proteins (21.00%), lipids (3.50%), cellulose (6.00%), calcium (0.80%), phosphorus (0.80%).

Plant extract preparation

In March 2023, fresh leaves and stems of a plant were collected in the Moungo subdivision (latitude 5 N and longitude 9 E) and identified at the National Herbarium of Cameroon under voucher specimen code 32999/SRF as *S. bialfrae*.

Harvested parts were washed and dried in a ventilated oven at 45°C. Thereafter, plant ethanolic extract was prepared using the procedure described by Yakubu *et al.* (2005). Briefly, dried plant parts were crushed in a miller, and the powder obtained was macerated in ethanol 95° (500 g/5 L) for 72 h. Following this, a Whatman paper n°3 was used for extract filtration, and the filtrate obtained was concentrated by rotary evaporation of ethanol at 60°C. The ethanolic extract obtained was dried at 45°C in a ventilated oven.

Phytochemical screening

Chemical tests were carried out on *S. bialfrae* ethanolic extract, using standard procedures to identify its secondary chemical constituents as proposed by Tease and Evans, (1989).

Assay

Female AGR were randomly assigned to four lots of 8 animals each based on their body weight. The control lot received orally 10g of tendered avocado without ethanolic extract (EE), and the other lots (2, 3 and 4) were fed daily with 50, 100 and 150 mg/Kg bw plant extract blended with 10 g of tendered avocado for 30 days. Thereafter, 3 animals per group were sacrificed for blood and organ sampling while the others were continuously treated till mating.

Studied parameters and data collection

Reproductive parameters

Oestrus cycle monitoring

Cells present in the vaginal smear were identified and characterized daily between 7 and 8 a.m. following the method used by Marcondes *et al.*, 2002. This involved sampling the cells of the vaginal canal with 10 µL of sterile saline (NaCl 0.9 %) using a pipette. The recovered solution containing cells was placed on slides, stained with methylene blue, and examined under the light microscope (OLYMPUS BX51) at a magnification of 100. Cell type and proportions were used to identify the stage of the oestrus cycle (Aydin *et al.*, 2011). Overall cycle duration was considered as the time for a particular composition to re-occur.

Duration of Pre-coitus cohabitation

The duration of Pre-coitus cohabitation corresponds to the time that elapses from the introduction of a female rat into the male cage till the observation of sperm cells in its vaginal swab. The maximum time allocated for this excise was 21 days and above this period, a female rat was considered non receptive.

Mating rate

Females were considered mated when sperm cells were found in their vaginal smears. Mating rate was determined by evaluating the number of mated females and the number of females set in for reproduction.

$$\text{Mating rate} = \frac{\text{number of females mated}}{\text{number of females introduced for mating}} \times 100$$

Toxicity parameters

Organ weight and volume

The ovaries, uterus, liver and kidneys were weighed using a scale of capacity 160g and 10⁻³g precision. Volumes of the liver and kidneys were determined by immersing them in a 0.9% NaCL solution contained in a graduated cylinder and reading any net displacement.

Biochemical parameters concentrations

Some of the serum obtained was used for spectrophotometry of creatinine, urea, alanine amino-transferase (ALT) and aspartate amino-transferase (AST) following the notice instruction of the commercial CHRONOLAB kid (Barcelona, Spain).

Statistical analysis

Results were expressed as the mean ± standard deviation. The analysis was carried out using the statistical software SPSS. 21 (Statistical Package for Social Science). A one-way ANOVA was used to appreciate the effects of plant extract on the studied parameters, followed by Duncan’s post hoc test for mean separation. Significant differences were fixed at 5%.

III. Result

Phytochemical composition of ethanolic extract of *S. bialfrae*

Medicinal plants generally contain secondary metabolites responsible for their biological activities. Phytochemical testing of *S. bialfrae* leaves and stems showed presence of alcaloids, phenols, flavonoids, sterols, triterpenoids, tannins, saponinis, anthocyanins and anthraquinns (table 1).

Table 1: Chemical composition of ethanolic extract of *S. bialfrae*.

Compounds	Ethanolic extract
Alcaloids	+
Phenols	+
Flavonoids	+
Sterols	+
Triterpenoids	+
Tannins	+
Saponins	+
Anthocyanins	+
Anthraquinons	+

+ = Present

Oestrus cycle length (days)

Oestrus cycle length as well as durations of metestrus and diestrus (table 2) were significantly lesser (p < 0.05) in lots treated with maximal doses (100 and 150 mg/Kg bw) of ethanolic extract of *S. bialfrae* compared to that receiving 50 mg/Kg bw and control lot.

Table 2: Effects of ethanolic extract of *S. bialfrae* on the length (day) of the vairious phases in the oestrus cycle.

Phases of cycle	Ethanolic extract of <i>S. bialfrae</i> (mg/Kg BW)				P
	00	50	100	150	
Proestrus	1.90± 0.14	1.95± 0.11	1.85± 0.33	1.80± 0.45	0.86
Estrus	1.10± 0.14 ^a	1.05± 0.11 ^a	2.05± 0.10 ^b	2.10± 0.02 ^b	0.00
Metestrus	1.80± 0.33 ^b	1.70± 0.41 ^b	1.20± 0.45 ^a	1.20± 0.00 ^a	0.01
Diestrus	2.10± 0.20 ^b	2.12± 0.07 ^b	0.60± 0.55 ^a	0.60± 0.45 ^a	0.00
Cycle length	6.90± 0.45 ^b	6.82± 0.54 ^b	5.70± 0.80 ^a	5.40± 0.54 ^a	0.00

^{a, b}: In the same row, means with different letters are significantly different (p < 0.05).

Duration of Pre-coitus cohabitation

Cohabitation duration between the male and female (figure 1) was comparable among lots receiving ethanolic extract of *S. biafrae*. However, significant decrease ($P < 0.05$) in cohabitation duration was observed in control lot animals compared to lot given 50 mg/Kg bw plant extract.

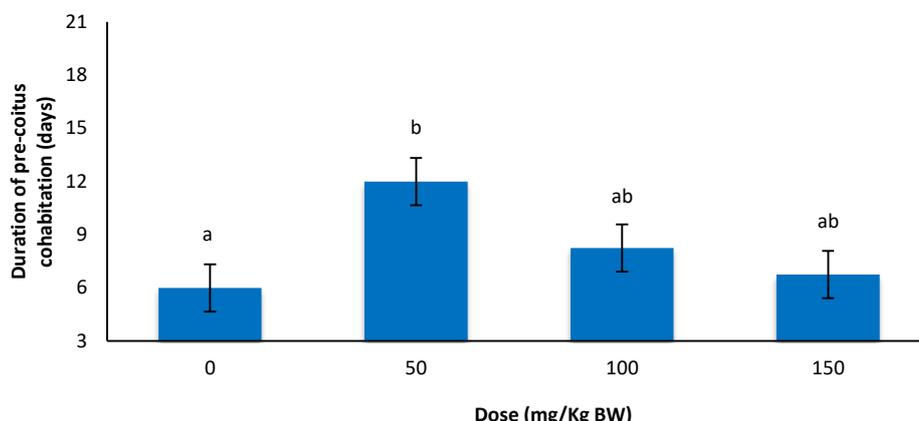


Figure 1: Effects of ethanolic extract of *S. biafrae* on duration of Pre-coitus cohabitation in female AGR. ^{a,b}: Means with different letters are significantly different ($p < 0.05$).

Mating rate and mating distribution over time

As shown in figure 2, mating rate was significantly ($p < 0.05$) higher in lots treated with maximal doses of plant extract (100 and 150 mg/Kg bw) followed by control lot then lot provided with 50 mg/Kg bw plant extract.

Figure 3 presents the distribution of matings with time following treatment of female giant rats with ethanolic extract of *Senecio biafrae*. It is observed that, female rats treated with 150 and 100 mg/ Kg bw plant extract registered the highest proportion of mated females during the first and second weeks respectively. However, no mating was noted during the third week irrespective of lot considered.

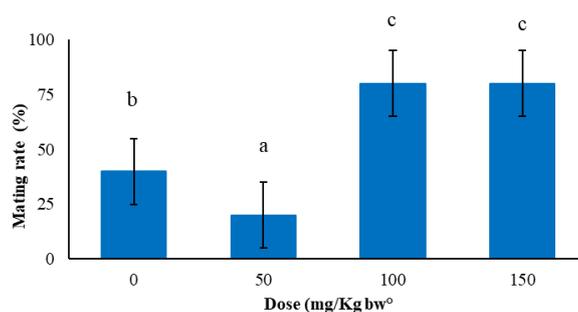


Figure 2: Effects of ethanolic extract of *S. biafrae* on mating rate in female AGR. ^{a,b,c}: Means with different letters are significantly different ($p < 0.05$).

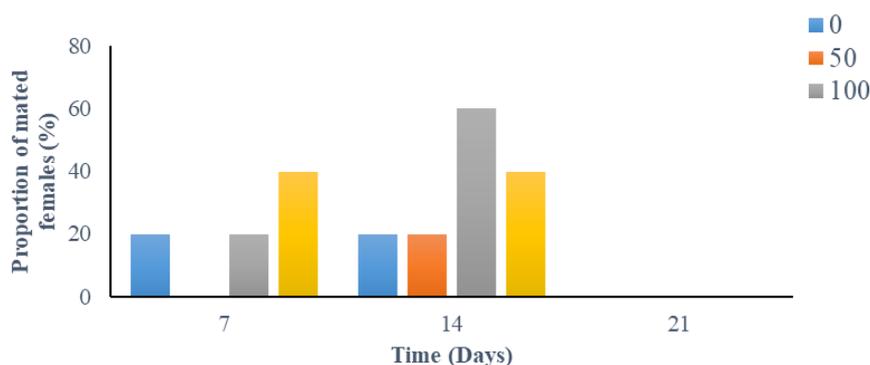


Figure 3: Effects of ethanolic extract of *S. biafrae* on mating distribution in female AGR.

Toxicity parameters**Weight and volume of detoxifying organs**

The effects of ethanolic extract of *S. bialfrae* on liver and kidney morphometric characteristics is presented on table 3. It is observed that morphometric characteristics increased dose dependently irrespective of trial considered. Nevertheless, no significant difference was observed between lots.

Table 3: Effect of the dose of ethanolic extract of *S. bialfrae* on the weight (g/Kg.bw) and volume of detoxifying organs in female giant rats

Toxicity parameters	Ethanolic extract of <i>S. bialfrae</i> (mg/Kg bw)				P
	00	50	100	150	
Weight of kidney (g)	1.85± 0.17	1.84± 0.09	1.89± 0.30	2.09± 0.33	0.58
Weight of liver (g)	18.79± 3.12	18.98± 2.72	19.68± 2.98	19.69± 2.83	0.69
Volume of kidney (mL)	1.88±0.03	1.77± 0.32	1.93± 0.06	1.98± 0.15	0.53
Volume of liver (mL)	19.33± 3.21	19.67± 3.78	20.33± 3.22	20.33± 2.51	0.94

Biochemical parameters

As shown on table 4, oral treatment of female AGR with ethanolic extract of *S. bialfrae* did not significantly affect serum content in ALT and creatinine compared to control lot. Contrarily, serum levels of AST and urea, significantly increased in lots receiving 100 and 150mg/Kg bw plant extract compared to those of control.

Table 4: Effects of ethanolic extract of *S. bialfrae* on biomarkers of nephron-toxicity

Biomarker	Ethanolic extract of <i>S. bialfrae</i> (mg/Kg BW)				P
	00	50	100	150	
ALT (U/L)	26.77±5.59	29.17±4.75	30.67±6.35	34.92±5.37	0.38
AST (U/L)	1.73±0.02 ^a	4.07±0.79 ^b	6.57±0.87 ^c	9.89±0.76 ^d	0.00
Creatinine (mg/dl)	0.90±0.05	0.96±0.09	0.85±0.08	0.97±0.06	0.21
Urea (mg/dl)	35.16±4.64 ^a	77.35±7.26 ^b	67.97±6.44 ^b	91.47±7.60 ^c	0.00

^{a, b, c, d}: In the same row, means with different letters are significantly different (p < 0.05).

IV. Discussion

Data obtained in the present study showed decrease (p< 0.05) in overall oestrus cycle duration in animals treated with 100 and 150 mg/Kg bw ethanolic extract of *S. bialfrae* compared to those treated with minimal doses (50mg and 0mg/Kg bw). However, irrespective of lot considered, oestrus cycle duration was within the range proposed by previous works conducted on the same species (Ajayi, 1975; Malekani et al., 2002 and Fonou et al., 2021). Decreased oestrus cycle duration observed suggests for increase ovulatory frequency. Findings are similar to those proposed by Lienou *et al.* (2010) following treatment of albino wistar rats with 32 mg/Kg bw ethanolic extract of *S. bialfrae*.

Female AGR treated with 100 and 150 mg/Kg bw ethanolic extract of *S. bialfrae* presented and increase in the length of estrus. This condition favors chances for fertilization as this phase is characterized by sexual receptivity of the male by female. Contradictory findings were disclosed by Lienou et al. (2012) in wistar rats treated with 8, 32 and 64 mg/Kg bw aqueous extract of *S. bialfrae*. Contradiction with previous work could be due to difference in extract solvent. Indeed, changes in vaginal cytology after plant extract administration depends not only on the nature of active components in plants but also on the dosage (Malaivijitnond et al., 2006).

The regularity of the oestrus cycle is an important parameter in assessing the fertility of a specie. A cycle is said to be regular when its phases follow each other chronologically with regular intervals (Fonkem, 2022). As such, cohabitation length will be reduced in animals with regular cycles. In the present study, more females were mated within their first and second cycle in lots treated with doses of 100 and 150 mg/Kg bw ethanolic extract of *S. bialfrae* compared to control lot. This suggests for oestrus cycle regularity. In fact, plants contain secondary metabolites capable of interfering on the hypothalamic–pituitary–gonadal axis triggering the production of reproductive hormones implicated in the oestrus cycle (Afshan et al., 2020; Lienou et al., 2015)

Organ weight is essential in understanding its metabolic rate and possible intoxication following administration of plant extract (Oloyede et al., 2011). In the present work, treatment of female AGR with ethanolic extract of *S. bialfrae* induced a non-significant dose dependent increase in liver and kidney weights. These increases could be due to intensive metabolism performed by these organs (Kadota et al., 1976). In addition to organ weight, liver and kidney status may be monitored using Biochemical parameters (Kalender et al., 2005). In the present study, the dose dependent increase (p< 0.05) in the concentration of AST observed suggest for possible alteration in the secretory activity of the liver (Yousef et al., 1999). This increase may also be attributed to hepatic necrosis hence leading to the escape of tissue enzymes into plasma (El-Demerdash et al.,

2012). The significant increase in urea concentration observed in lots treated with plant extract could be due to the reduced ability of the kidney to remove this substance from blood.

V. Conclusion

The present study showed that, oral treatment of AGR with ethanolic extract of *S. bialfræe* at doses of 100 and 150 mg/kg b.w. decreased oestrus cycle duration and improved cycle regularity. Awareness has also been created on the side effects related to the prolonged administration of this plant at these doses.

References

- [1]. Afshan A., Rahila I., Faiza H., Sana S And Qurrat Ul A. 2020. Fennel Fortified Diet: New Perspective With Regard To Fertility And Sex Hormones. *Pakistan Journal Of Pharmaceutical Sciences*. 33:2595-2600.
- [2]. Ajayi S. 1974. The Biology And Domestication Of The African Giant Rat (*Cricetomys Gambianus* Waterhouse) Ph.D. Thesis, University Of Ibadan Nigeria.
- [3]. Ashafa T., Orekoya O. And Yakubu T. 2012. Toxicity Profile Of Ethanolic Extract Of *Azadirachta Indica* Stem Bark In Male Wistar Rats. *Asian Pacific Journal Of Tropical Biomedicine*. 2: 811-817.
- [4]. Asibey E And Addo P. 2000. The Glasscutter, A Promising Animal For Meat Production. In: *African Perspective Practice And Policies Supporting Sustainable Development*, Turnhan D (Ed). Waever Press, Zimbabwe, Pp: 251-263.
- [5]. Aydin I., Sur E., Ozaydin T. And Dinc A. (2011). Determination Of The Stages Of The Sexual Cycle Of The Bitch By Direct Examination. *Journal Of Animal And Veterinary Advances*, 10: 1962-1967.
- [6]. Cooper R. 2008. Care, Husbandry And Diseases Of The African Giant Rat (*Cricetomys Gambianus*). *Journal Of The South African El-Demerdash F., Attia A. And Elmazoudy R. (2012). Biochemical And Histopathological Changes Induced By Different Time Interval Of Methomyl Treatment In Mice Liver. Journal Of Environmental Science And Health*. 47:1948-1954.
- [7]. Elvin-Lewis M. 2001. Should We Be Concerned About Herbal Remedies? *Journal Of Ethnopharmacol*. 75(2-3):141-164.
- [8]. Fonkem S., Kenfack A., Kouamo J., Atsamo D., Nyamsi A., Tsambou M., Fonou L., Vemo B., Foda F. And Takam M. 2022a. Effect Of Age Of Breeding On Female African Giant Rat (*Cricetomys Gambianus*) Fertility In Captivity. *International Journal Of Veterinary Science*. 11:443-447. Doi: Y In Captivity. *International Journal Of Veterinary Science* 11(4): 443-447.
- [9]. Fonkem S., Tsambou M., Fonou L., Foda F. Vemo B., Takam M., Kouamo J. And Kenfack A. 2022b. 17-A- Hydroxyprogesterone Caproate Improves Female African Giant Rat (*Cricetomys Gambianus*) Fertility In Captivity. *Journal Of A Griculture And Veterinary Science*. Pp15-21.
- [10]. Fonou T., Fopa F., Vemo B., Tsambou A., Fonkem S., Takam M. And Kenfack A. 2021. Effects Of Photoperiod On The Estrus And Reproductive Organs In Female African Giant Rat (*Cricetomys Gambianus* Water House). *International Journal Of Agricultural Research*. 8:87-92.
- [11]. Kalender S., Oguteu A., Uzunhisarcikly M., Acikgoz F., Durak D., Ulusoy Y. And Kalender Y. (2005). Diazinon Induced Hepatotoxicity And Protective Effects Of Vitamin E On Some Biochemical Indices And Ultrastructural Changes. *Toxicology*, 211: 197-206.
- [12]. Kodata T., Okuna K And Mtyamoto J. 1976. Mammalians And Toxicological Study Of Permethrin, 3 Phenoxy Benzyl (\pm) Atrans-2,2 Dimethyl-3-(2,2 Dichlorovinyl)- Cyclopropane-1-Carboxylate. *Botyukgoku*, 41: 143-151.
- [13]. Lienou L., Telefo B., Bale B., Yemele M., Lemfack C., Mouokeu C., Goka S., Tagne R., Moundipa P. (2010). Effect Of Ethanolic Extract Of *S. Bialfræe* On Puberty Onset And Fertility In Immature Female Rat. *Cameroon Journal Of Experimental Biology* 2010 Vol. 6: 101-109.
- [14]. Lienou L., Telefo B., Bale B., Yemele D., Richard S., Stephanie C., Lemfack M., Mouokeu C. And Moundipa F. 2012. Effect Of The Aqueous Extract Of *Senecio Bialfræe* (Oliv. & Hiern) J. Moore On Sexual Maturation Of Immature Female Rat. *Bmc Complementary And Alternative Medicine*. 12:36
- [15]. Lienou L., Telefo B., Njimou J., Nangue C., Bayala B., Goka S., Biapa P., Yemele M., Donfack N., Mbemya J., Tagne S., Rodrigues A. 2015. Effect Of The Aqueous Sextract Of *S. Bialfræe*(Oliv.&Hiern)J.Moore On Some Fertility Parameters In Immature Female Rat. *Journal Of Ethnopharmacology*. 161-156-162.
- [16]. Malaivijitmond S, Chansri K, Kijkuokul P, Urasopon N, Cherdshewasart W. 2006. Using Vaginal Cytology To Assess The Estrogenic Activity Of Phytoestrogen-Rich Herb. *Journal Of Ethnopharmacology*. 107 :354-60
- [17]. Malekani, M., Westlin, L., Paulus, J., & Potgieter, H. (2002). Oestrous Occurrence In Captive Female *Cricetomys Gambianus*(Rodentia: Cricetidae). *Journal Of Zoology*, 257(3), 295-301.
- [18]. Marcondes F., Bianchi J., Tanno P. 2002. Determination Of The Oestrus Cycle Phases Of Rats: Some Helpful Considerations. *Brazilian Journal Of Biology*, 62(4a): 609-614.
- [19]. Mbemya G., Luis Alberto V., Francisca G., Otilia D., Ana Paula R. 2017. Reports On In Vivo And In Vitro Contribution Of Medicinal Plants To Improve The Female Reproductive Function. *Reprodução & Climatério*. No. Of Pages 11:
- [20]. Oloyede A., Okpuzor J., Omidiji O., Odeigah P. 2011. Evaluation Of Sub-Chronic Oral Toxicity Of Joloo: A Traditional Medicinal Decoction. *Pharma Biol*. 49: 936-941.
- [21]. Trease, G.E And Evans, W.C (1989a): A Text-Book Of Pharmacognosy. Bailliere Tindall Ltd, London; Pp.53
- [22]. Tsambou M. 2020. Effets Du Niveau D'énergie De La Ration Sur La Croissance Et Les Performances De Reproduction Du Rat De Gambie (*Cricetomys Gambianus*) En Captivité. Thèse De Doctorat/Phd En Biotechnologie Et Productions Animales. Université De Dschang. Faculté D'agronomie Et Des Sciences Agricoles, P135.
- [23]. Vemo B. 2018. Effets Thérapeutiques Des Extraits Aqueux Et Ethanoïque De Feuilles De *Bersama Engleriana* (Melianthaceae) Sur La Toxicité Reproductive De La Cyperméthrine (Insecticide) Chez Le Cobaye (*Cavia Porcellus*) Male. Thèse De Doctorat /Phd En Biotechnologie Et Production Animales. Faculté D'agronomie Et Des Science Agricoles. Université De Dschang. 157pp
- [24]. Yakubu Mt, Akanji Ma, And Oladiji At (2005). Aphrodisiac Potentials Of The Aqueous Extract Of *Fadogia Agrestis* (Schweinf. Ex Hiern) Stem In Male Albino Rats. *Asian Journal Andrology*, 7: 399-404.
- [25]. Yousef M., Abbassi M. And Yacout M. (1999). Assessment Of Cypermethrin And Dimethoatotoxicity In Barkysheep: Biochemical And Historical Changes Of Tissue Residues. *Egyptian Journal Of Animal Production*. 36: 25-41.