Research Article

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Reproductive Toxicity Associated To *Elaeis Guineensis* Roots Extract In Wistar Rat.

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ABSTRACT

Elaeis guineensis roots have been traditionally used for the treatment reproductive troubles. This study aimed to investigate the reproductive toxicity of the aqueous extract of *Elaeis guineensis*' roots. Twentyfour males and 48 females (Two subgroups of 24each) aged 9 and 12 weeks were randomly separated into 4 groups of 6 rats each. The control received distilled water (1 mL/po), while the test groups received aqueous extract of *E. guineensis* at 100, 200 and 400 mg/kg doses per gavage during 54 days (males) and 10 days (females). Subsequently, males were mated in rapport 1:2 (females untreated and treated) during 10 days, while receiving extract. After mating, sexual behavioral parameters (mount latency and frequency, intromission latency and frequency, ejaculation latency and frequency, post ejaculation interval, Average interval of copulation) were recorded in males using ovariectomized female's rats (10). In females the percentages of fertility, fecundity and prolificacy were calculated. Consecutively, treated rats were serially sacrificed; Sperm count and histologic analysis of androgen and estrogen dependent organs and pituitary gland were evaluated. Organs section of treated groups presented normal architecture, and sexual behavioral parameters did not show any significant difference when compared to control group. Sperm count significantly increased at 200 mg/ kg dose. The percentage of fertility was 100% in all groups, but the percentages of fecundity and prolificacy significantly decreased at the dose of 400 mg/ kg. Aqueous extract of *E. guineensis* roots did not affect sexual behabior, improved fertility at traditional use dose, but could negatively affect spermatozoids' structure and fetus development at high doses.

Keywords : *Eleais guineensis* roots, male sexual behaviour, Male fertility parameters, Offspring, Reproductive toxicity.

INTRODUCTION

Approximately 80% of the world population use or depend on plant materials for their primary health care (Jiofack et al. 2010). Thus, numerous studies have highlighted the medicinal properties of plants (Roozbeth et al. 2021). In low-income settings, herbal medicine is very often the main, and sometimes the only source of health care, especially in rural and remote areas. Variety, flexibility, easy availability and/ or affordability, social/religious acceptability as well as less side effects and perecived lower costs, greatly contribute to growth of herbal medicine (Payyappallimana 2010; WHO 2014). Because of this widespread use, medicinal plants are considered as a vital source of new medicines (Dzobo 2022).

A sharp decline in fertility rates was observed over the world in virtually every country (Aitken 2022). A recent report by WHO found that between 1990 and 2021, nearly 17.5% of the population has experienced infertility worldwide at some stage in their lives (WHO, 2023). About 20 – 40% of Sub-Saharan Africa's population suffers from infertility (Emokpae and Uadia 2015). In Cameroon, the prevalence of infertility remains poorly known, however a 10 years' retrospective study carried out in the Dshang health District found that approximately 83.9% of patients attending urology services had abnormal spermogram (Momo et al. 2020). Infertility

affects both males and females; but scientific evidence shows that male factors are involved in 50-60% of overall infertility (Masoumi et al. 2015). This pathology devastates health and has societal consequences including psychological distress, stigma, marital discord, intimate partner violence and economic hardship especially in developing countries where the majority of the affected people live (Wang et al. 2022; Thoma et al. 2021). Because infertility has for a long time been considered as a femalederived problem in Africa, men most often resort to traditional medicine (Tsobou et al. 2016). The traditional Cameroonian pharmacopoeia is among the richest in Africa and in that context, a wide range of plants are traditionally used to alleviate infertility. Plants belonging to the Arecaceae family such as *Raphia vinifera*, *Elaeis guineensis* are used to treat sexual disorders (Watcho et al. 2019; Reddy et al. 2019). *Elaeis guineensis* also known as oil palm, is largely used in traditional medicine by African indigenous communities through preparations from its different parts (leaf, fruit, seed and root) (Reddy et al. 2019) for various ailments (Mohammed et al. 2010; Vijayarathna et al. 2012; Kalman et al. 2013; Sharif et al. 2015; Fongod et al. 2014; Owoyele and Owolabi 2014; Ikechukwu et al. 2017; Reddy et al. 2019). Moreover, Maduabuchukwu et al. (2014) reported profertility effects of *E. guineensis*. This scientific background reinforces the traditional usage of *E. guineensis* which continues to occur without scientific data to back up its safety. Many people still believe that because herbal medicines have been used by the ancestors for a long time without apparent side effects and are natural, their safety is guaranteed. However, a Chinese proverb says that, "*all medicines have their own side effects*". Therefore scientific evidence confirming or refuting the safety of these plants is necessary to maintain or guarantee the Indeed, reproductive toxicity refers to the potential risk from a given chemical, physical or biological agent to adversely affect both male and female fertility, and offspring development. The present study was designed and carried out with the aim to investigate the reproductive toxicity of the aqueous extract of the roots of *E. guineensis* in Wistar rat.

MATERIAL AND METHODS

Animals

Twenty-four healthy albino male rats (9-12 weeks old) and 48 females (12 weeks old) were used in this study. Additionally, 10 ovariectomized females were used for sexual behavior testing. All these animals were obtained from the animal house of the Research Unit of Animal Physiology of the Faculty of Science, University of Douala (Cameroon). Rats were housed under specified pathogen free conditions, with natural light/dark cycles and free access to food and water. Animals were managed in conformity with the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Technology Innovation.

Plant collection

The roots of *Elaeis guineensis* were collected in Mbouda subdivision (West region Cameroon). The roots were harvested from an old palm tree (in production for several years) that grew naturally in the forest, free from any phytosanitary treatment. The sample was identified at the Cameroon National Herbarium, where a specimen has been deposited under the number 34163 HNC.

Extraction

The harvested roots were washed, air-dried under shade at room temperature and then crushed using electric grinder. The ground powder, 560 g, was suspended in 8L of distilled water, heated and boiled for 45 minutes. After filtration using whatman filter paper n° 1, the obtained decoction was evaporated in an oven at 45°C. The yield of the crude extract was 5% (~ 28 g).

Determination of study doses

According to ethnomedical use, two glasses of decoction are required daily, which is approximately 28.55 mg/kg for a 70 kg adult. This dose was readjusted to 200 mg/kg and then divided to 100 mg/kg and doubled to 400 mg/kg, giving rise to 3 doses tested in this study (100 mg/kg, 200 mg/kg and 400 mg/kg).

Chemicals and reagents

Progesterone and Estradiol benzoate were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Phytochemical screening

A qualitative phytochemical screening of the extract was performed out to identify the classes of secondary metabolites such as alkaloids, flavonoids, terpenoids, steroids, saponins, tannins, polyphenols, carotenoids and quinone. The analyses were performed according to some standard protocols (Joshi et al. 2013; Ayoola et al. 2008).

Study design

Twenty-four males and 24 females were randomly divided into four groups of six animals as follows. The remainings 24 females did not receive extract, and were used for the determination of fertility parameters in treated males.

Group I: The control group received 1 mL of distilled water orally per day;

Group II: experimental rats received 100 mg/kg body weight of aqueous extract of roots of *E. guineensis* (D100);

Group III: experimental rats received 200 mg/kg body weight of aqueous extract of roots of *E. guineensis* (D200);

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Group VI: experimental rats received 400 mg/kg body weight of aqueous extract of roots of *E. guineensis* (D400).

The treated male rats received aqueous extract of *E. guineensis* for 54 days for (duration of spermatogenesis cycle in rat), while females received the plant extract 10 days (duration of at least two estrous cycles). Males of each group were coupled with females in report 1:2 (one treated female at the same dose as the male and one non-treated female) during 10 days. The plant extract was administered to both sexes during the mating period, and thereafter only to females during pregnancy and for the duration of the nursing period. Sexual behavior tests were performed before sacrifice of male rats and sperm count parameters were evaluated after sacrifice. To evaluate of female fertility, the following parameters were assessed for pregnant, live and stillborn offspring as follow (Peneme 1998a, 2018b):

- % of true fertility= (number of pregnant females)/ (number of mated females) x 100
- % fecundity = (number of live offspring at birth)/ (number of mated females) x 100
- % of prolificacy = (number of live offspring at birth)/ (number of litter) x 100

Ovariectomy of female rats

The rats were ovariectomized according to protocol previously described by Cariton in 1986 with some modifications as reported by Watcho et al. (2014).

Oestrous induction in female rats

Oestrus was artificially induced in ovariectomised female by subcutaneous injections of estradiol benzoate (30 µg/kg) and progesterone (600 µg/kg) 48h and 6 h respectively before the experiment. Only females with a lordosis reflex and without darting with males were selected and used for the sexual behaviour test.

Male sexual behaviour test

Males were introduced to the mating chamber 30 minutes before beginning each mating for acclimatisation. Then, a sexually receptive stimulated female was introduced and the following copulatory parameters below were evaluated for sixty minutes with very slight light from 8 pm (Koloko *et al*. 2019; Nde *et al.* 2020):

- Mounting latency (ML): the time between the introduction of the female into the chamber and the first mount;
- Intromission latency (IL): the time between the introduction of the female into the chamber and the first intromission;
- Ejaculation latency (EL): the interval of time between the IL and the first characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity;
- Mounting frequency (MF): the number of mounts with or without intromission from the time when the female is introduced till ejaculation;
- Intromission frequency (IF): the number of intromissions counted during the 60 min observation;
- Ejaculation frequency (EF): the number of ejaculations counted during the 60 min observation;
- Average interval of copulation (AIC): the time between the first intromission of a series and ejaculation marking the end of these series;
- Post-ejaculatory interval (PEI): period of time from ejaculation to the first intromission of the next series.

The test was considered negative if the intromission and ejaculatory latencies were more than 20 min.

Sacrifice of rats

The animals receiving extract were sacrificed through terminal exsanguination under ketamine (80 mg/kg)-diapzepan (10mg/kg) anaesthesia. The testes seminal vesicles, epididymis, prostate, ovaries, uterine horns, cervix and brain were removed. These organs were cleared from attached fat and connective tissue, weighted and fixed in bouin's fluid and 10% neutral buffered formalin (brain) for histological analysis. One testis and one epididymis were used for sperm count.

Semen parameters

Daily sperm production (DSP)

The left testis of each rat was separated from the albuginea and homogenised as described by Massoma et al. (2014). The sample was placed in a Neubauer chamber and head spermatozoa were counted in 25 squares for 3 strokes. The number of spermatozoids was the average of three counts for each sample. The number of DSP/testis and DSP/gram of testis were calculated using testicular homogenates (Massoma et al. 2014).

Epididymal sperm count and transit

The left epididymis of each rat was cleaned of adjacent tissue and divided into two parts: caput/corpus and cauda. Assessment of sperm counts as well as sperm transit time in the epididymis was determined as described by Massoma et al. (2014) and Gonzales et al. (2004).

Sperm motility

This parameter was evaluated using spermatozoa collected from the cauda epididymis. Indeed, each cauda epididymis was separated, macerated and placed in a beaker containing 5ml of physiological saline solution (0.9% NaCl) at 37°C for 10 min. The seminal fluid was examined directly under a light microscope at 40x magnification (Massoma et al. 2014).

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Histological analysis

The organs were fixed, processed and stained with haematoxylin and eosin. Photomicrographs (100X) were taken and the histological changes analysed using a light microscope.

Statistical analysis

Stastistical analysis was performed with GraphPad Prism software version 5.1. Data were expressed as mean ± SEM (standard error of the mean) and subjected to one-way ANOVA followed by Turkey post-test. Results were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The use of plant extracts to improve male reproduction has been reported with various results (Reddy et al. 2019; Roozbeth et al. 2021). However, the reprotoxicity of some of them has not been investigated. This study conducted to investigate reprotoxicity of aqueous extract the roots of E. guineensis in rat. To evaluate of parental reproduction, weight gain, sexual behaviour, sperm count, rates of fecundity and fertility were assessed.

Phytochemical screening

Qualitative phytochemical screening of *E. guineensis* roots aqueous extract revealed the presence of bioactive compounds such as alkaloids, saponins, flavonoids (flavonols), cathechic tanins, polyphenols, and unsaturated sterols (**Table 1**). This result is similar to previous findings which showed the presence of these bioactive compounds in leaves extract of Elaeis guineensis (Yin et al. 2013; Che et al. 2020).

Table 1: Qualitative analysis of the phytochemical constituents of Elaeis guineensis roots' aqueous extract.

Bioactive compounds	Test	Results
Saponins	Frothing test	+++
Alcaloids	Wagner test	+++
Triterpens	Liebermann-Buchard test	-
Flavonoids (Flavonols)	Wilstater test	+++
Tanins	Fecl3 test	+++
Polyphenols	Gelatin test	+++
Unsaturated sterol	Salkowski test	+++
Tanins cathechic	Fecl3 test	+++
Free quinones	NaOH test	+
Carotenoids		-

"-"indicates absence, "+" indicates presence, number of "+ "indicate level of concentration

Effect of E. guineensis roots aqueous extract on body weight and relative weight of reproductive organs

After 64 days of experiment, rats (males and females) receiving the aqueous extract of Elaeis guineensis roots at all the tested doses gained less body weight than controls. Similar results were observed in females. In fact, some results reported antihyperlipidemic, hypocholesterolemic and hyperglycemic effects of different parts of *E. guineensis* (Ginjom et al. 2003, Owolabi et al. 2013, and Varatharajan et al. 2013). These studies indicated that *E. guineensis* could affect fat and carbohydrates metabolism, which can be attributed to alkaloids and polyphenols that can modify cell growth and act against fat deposition (Nakano et al. 2004; Amiot et al. 2009).

However, the relative weight of organs also increased at all doses, significantly in males (epididymis, seminal vesicle and prostate, ($p < 0.5$)) and not significantly in females (uterus, by 31.57%) (**Table 2**). This result may be attributed to alkaloids, phenol, flavonoids, saponins, cathechic tannins present in the E. guineensis roots extract. Indeed, these compounds have the ability to stimulate the hypothalamic-pituitary-gonadal axis (Yakubu et al. 2008). Saponins are reported to be able to influence testosterone secretion, by increasing LH levels, which helps to maintain testosterone levels (Peiris et al. 2015); as it is known that the administration of exogenous testosterone enhances androgen dependent organs' growth (Keel and Abney 1980). Besides, flavonoids can inhibit aromatase activity, enzyme responsible in the conversion of testosterone to estrogen (Parandin et al. 2011). These findings are consistent with the probability that our plant extract has androgenic activity (Keel and Abney 1980). In females, although not significant the relative weight of uterus increased by 31.57% when compared to

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control, consistent with some previous findings (Massoma et al. 2014, Ngaha et al. 2019).

Table 2: Body weight gain and relative weight of reproductive organs after administration of Elaeis guineensis roots' aqueous extract.

Males				
Organs	**Control**	**D100**	**D200**	**D400**
Body Weight gain (%)	122.6 ± 7.2	84,2 ± 9.9*	81.9 ± 5.9*	93.1 ± 4*
Testes (g)	0,65± 0.01	0.69 ± 0.059	0.65 ± 0.047	0.76 ± 0.019
Epididymis (g)	0.22 ± 0.01	0.23 ± 0.007	0.21 ± 0.009	0.24±0.01*
Seminal vesicle (g)	0.45 ± 0.064	0.52 ± 0.045	0.5 ± 0.025	0.65 ± 0.026*
Prostate (g)	0.16 ± 0.004	0.17 ± 0.011	0.15 ± 0.004	0.19 ± 0.012*
Females				
BWG J0 of gestation (%)	28.2± 5	12.3± 2.2*	11.5± 3.2*	6.6± 1.4*
BWG end of gestation	48.8± 3.2	29.9± 6*	35.2± 4.3	31.6± 6.8*
BWG after deliverance	40.7± 2.5	8.9± 2.1*	12.8± 3.5*	29.7± 5.3
BWG at the end	43.8± 3	15.3± 2.07*	14.4± 1.08*	21.9± 4.4*
Ovary (g)	0.025 ± 0.0004	0.026 ± 0.002	0.024 ± 0.003	0.24 ± 0.003
Uterus (g)	0.19 ± 0.026	0.21 ± 0.018	0.22 ± 0.018	0.25 ± 0.03

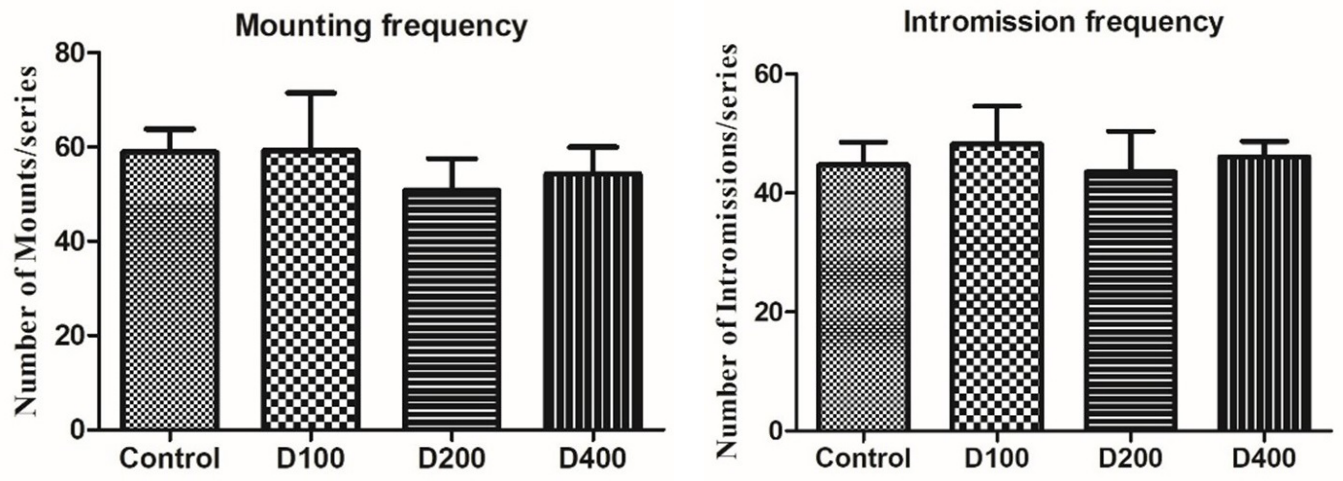
Control: Control group receiving distilled water; D100, D200 and D400: tests groups receiving the extract of roots of *Elaeis guineensis* at the doses of 100, 200 and 400 mg/ kg, respectively. BWG: Body weight gain. Each value represents the mean ± Standard error of the mean, n = 6. *= $p<0.05$: significant difference when compared to control.

Effect of E. guineensis roots aqueous extract on sexual behavioral parameters

In male of parental generation, there were no significant changes in sexual behavior parameters measured after 64 days of extract administration, neither in sexual performance (mounting frequency, intromission frequency, ejaculatory frequency and average interval of copulation) nor in sexual motivation (mounting latency, intromission latency, ejaculatory latency and post ejaculation interval) (**Figure 1**).

Figure 1: Sexual behaviour parameters further E. guineensis extract administration. A: sexual performance; B: sexual motivation. Control: Control group receiving distilled water; D100, D200 and D400: test groups receiving the extract of roots of Elaeis guineensis at the doses of 100, 200 and 400 mg/ kg, respectively. Each band represents the mean ± standard error of the mean.

Figure 1-A



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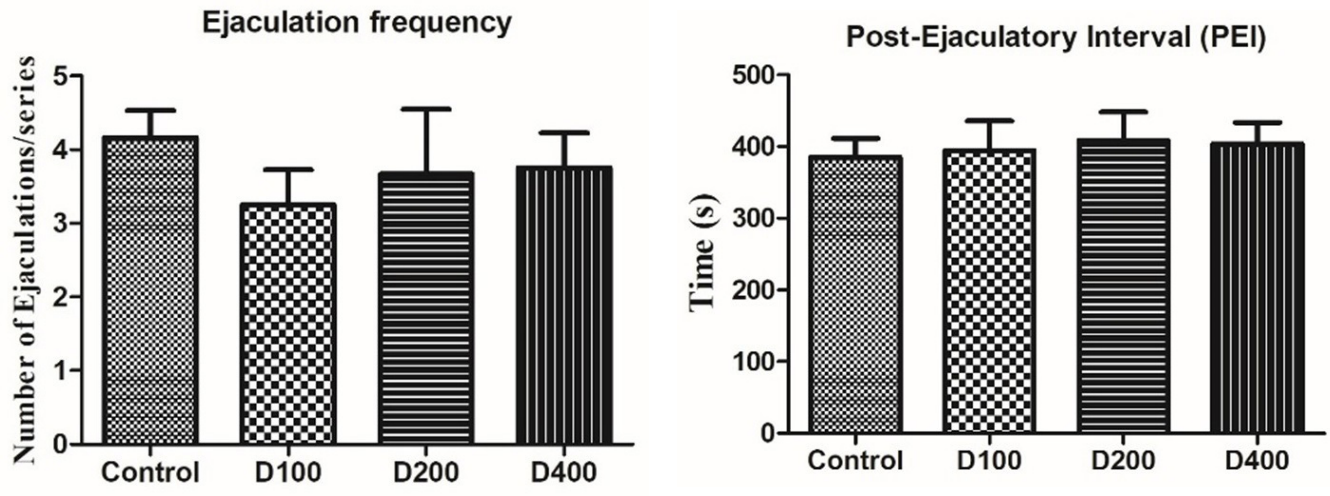
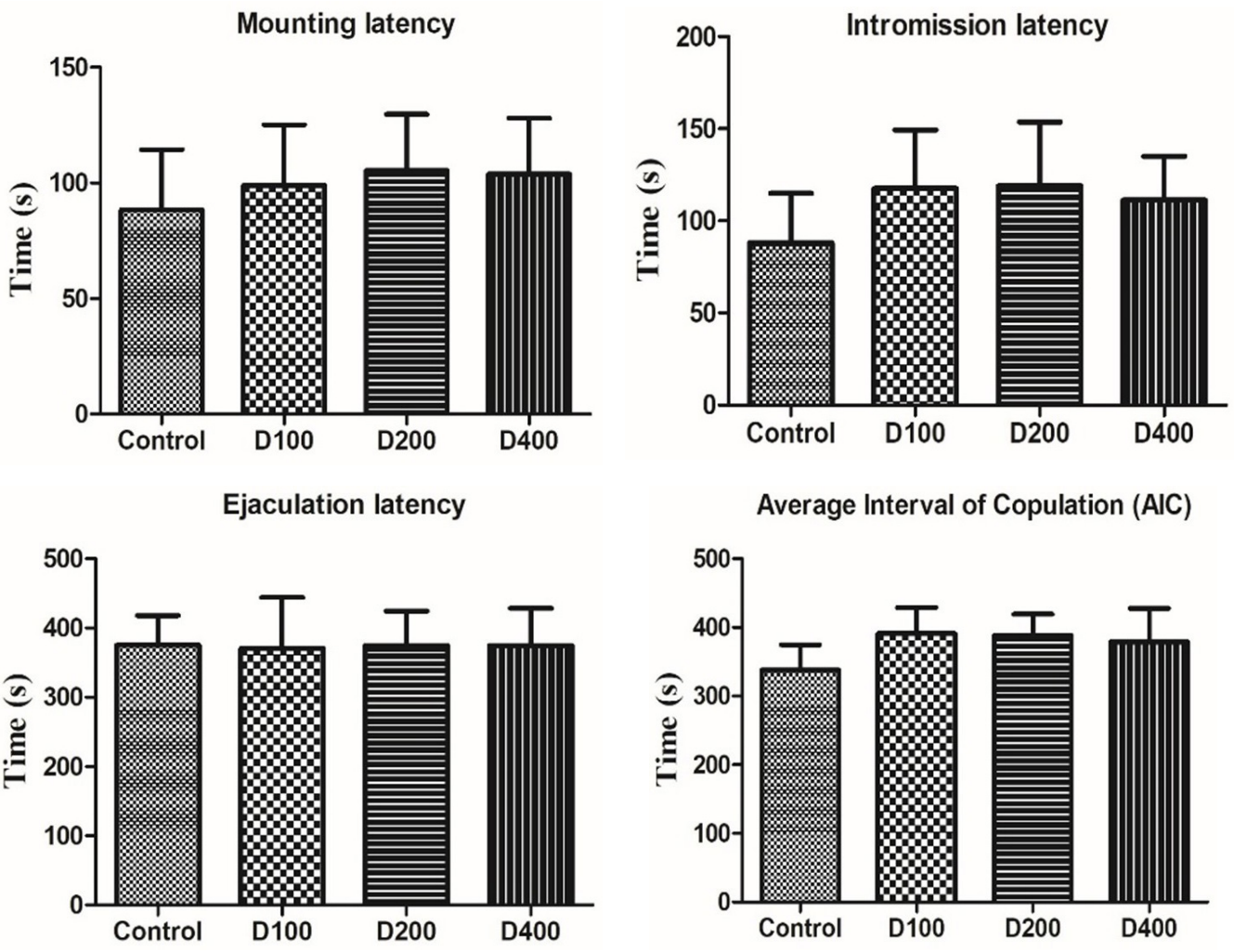


Figure 1-B



Effect of E. guineensis roots aqueous extract on male fertility parameters

Fertility parameters in parental male were evaluated by sperm count. For 64 days of administration of *E. guineensis* extract, daily sperm production, total epididymis count, DSP efficiency and total epididymis count per gram of epididymis significantly increased ($p < 0.05$) at the dose of 200 mg/kg compared to control and the other experimental groups. (**Table 3**). There was no significant difference in sperm motility and sperm transit in the epididymis between the test and control groups. These results

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can, on one hand, justify the traditional use of *E. guineensis* extract of 200mg per day, and may, be explained by the androgenic capacity of bioactive compounds such as alkaloids, saponins, sterols and flavonoids (Ngadjui et al. 2013, Watcho et al. 2017). Indeed, androgens play an important role in maturation, spermatogenesis and the maintenance of accessory sex organs (Dewan et al. 2000). These compounds may also be directly involved in the steroidogenesis as precursors (Dewan et al. 2000). Overall, fertility in the parental generation (males and females) was not adversely affected at any dose level or in the controls.

Table 3: Sperm parameters after the administration of Elaeis guineensis roots' extract.

	Control	**D100**	**D200**	**D400**
DSP (× 10^6/ml)	3.2 ± 0.3	4.0 ± 0.4	6.0 ± 0.8^{abc}	3.7 ± 0.2
DSP efficiency (DSP/g testis)	2.3 ± 0.2	3.2 ± 0,3	4.6 ± 0.8 ^{ac}	2.7 ± 0.2
Epididymis caput/corpus (×10^6/ml)	52.9 ± 2.2	45.0 ± 2.7	55.0 ± 3	49.9 ±3.7
Cauda epididymis (× 10^6/ml)	62.2± 1.6	62.5± 5.8	74.4 ± 6,1	68 ± 5.2
Epididymis total (× 10^6/ml)	115.1 ± 6	107.5 ± 4.1	129.4 ± 4.3^{ac}	117.9 ± 4.8
Epididymis total (× 10^6/g)	226.1 ± 8.9	229.7 ± 4.7	321.1 ± 21^{abc}	211 ± 20.4
Epididymis sperm transit	18.4 ± 2.8	16.8 ± 3.3	16.3 ± 3.2	15 ±2.6
Sperm motility (%)	94 ± 4.3	94 ± 3.8	92.3± 7.2	97.11± 2.4

DSP: daily sperm production; Control: Control group receiving distilled water; D100, D200 and D400: tests groups receiving the extract of roots of *Elaeis guineensis* at the doses of 100, 200 and 400 mg/ kg, respectively. Each value represents the mean ± standard error of the mean. n = 6. a, b, c: significant difference when compared to control, D100 and D400 respectively. significance difference: $p<0.05$.

The integrity of animal fertility has been confirmed by histological structure of some reproductive organs. In males, the histologic structure of androgen-dependant organs (testes, epididymis, seminal vesicles, vas deferens, prostate) of was normal in all animals regardless of treatment (**Figure 2**). Cross-sections of rat testes showed seminiferous tubules with sex cells at various stages of development, from the periphery (spermatogonia) to the lumen (spermatozoa). A cross section of epididymis showed spermatozoa in the lumen. Histologic section of prostate shows glandular epithelium under a basal lamina and maintained by the fibromuscular stroma. The vas deferens section shows a pseudostratified cylindrical epithelium with stereocilia resting on a thin lamina propria composed of several concentric layers of myoid cells above the basement membrane. The seminal epithelium and the lumen of seminal vesicle contained eosinophilic secretions. In females, the uterus showed a normal structure, with the vaginal epithelium showing germinal, granular and horny layers in all groups. The ovary showed follicles at different stages of development, corpus luteum and atretic follicles, this in all groups. The pituitary has a normal structure with normal neurons and no changes in any group (**Figure 3**). Indeed, the integrity of the pituitary, which is enhanced by its secretions, is also involved in the regulation of reproductive function in both males and females (Seejore and Murray, 2020). These results confirm the fertility efficiency of parental generation.

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Figure 2: Histological cross section of androgen-dependent organs and pituitary gland (he, 100x) after administration of the root extract of *Elaeis guineensis*. Rats treated with Control: Control group receiving distilled water; D100, D200 and D400: tests groups receiving the extract of roots of *Elaeis guineensis* at the doses of 100, 200 and 400 mg/ kg, respectively Testis: Gc = Germinal cell, It = Interstitial tissue, Lst = Lumen of seminiferous tubule; Epididymis: Ee = Epididymal epithelium, Spz = Spermatozoids; seminal vesicle: Ct = Connective tissue; Es = Eosinophil secretion; Prostate: Vas deferent: L= Limen, E = Epithelium, Mu= Muscular.

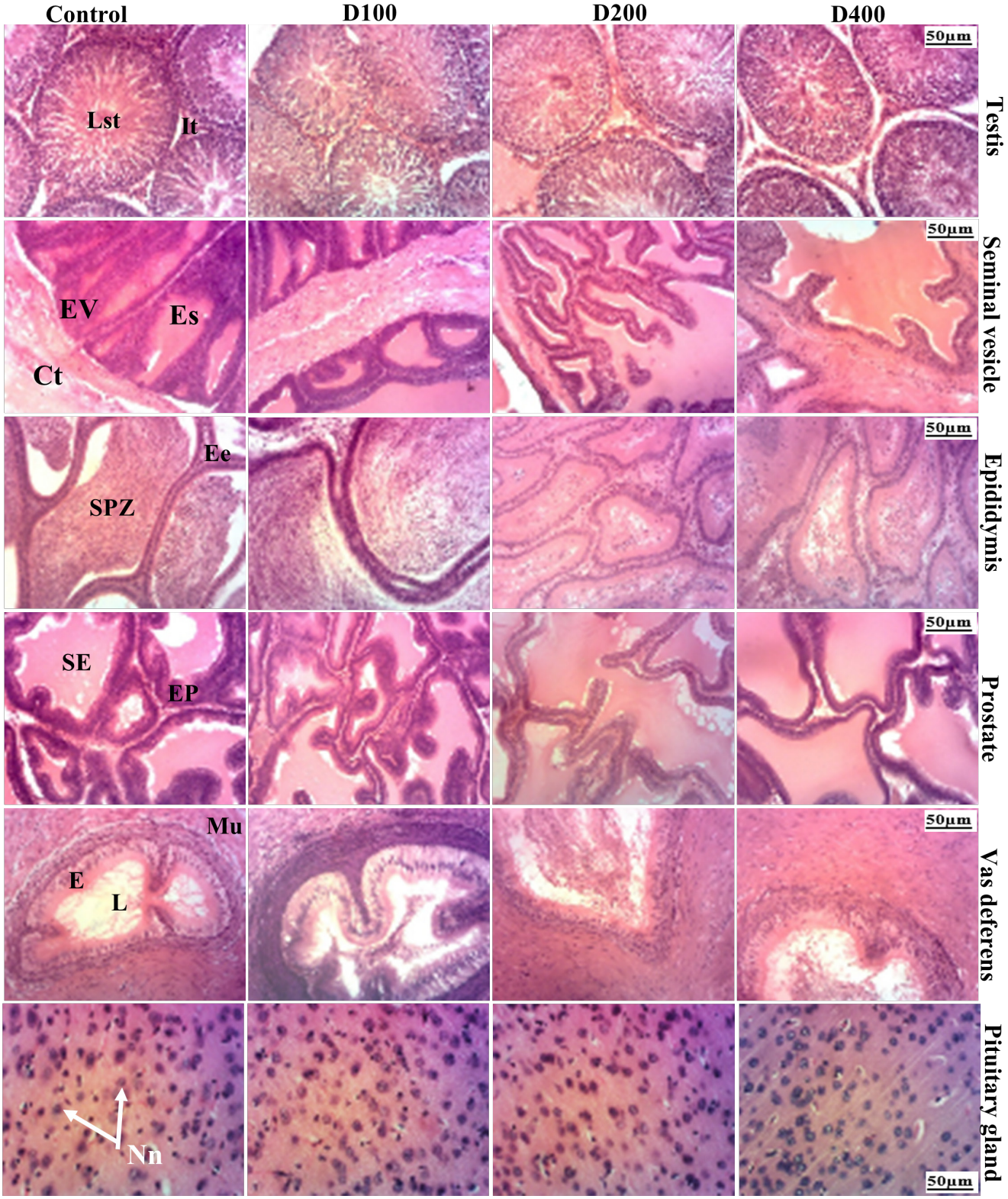

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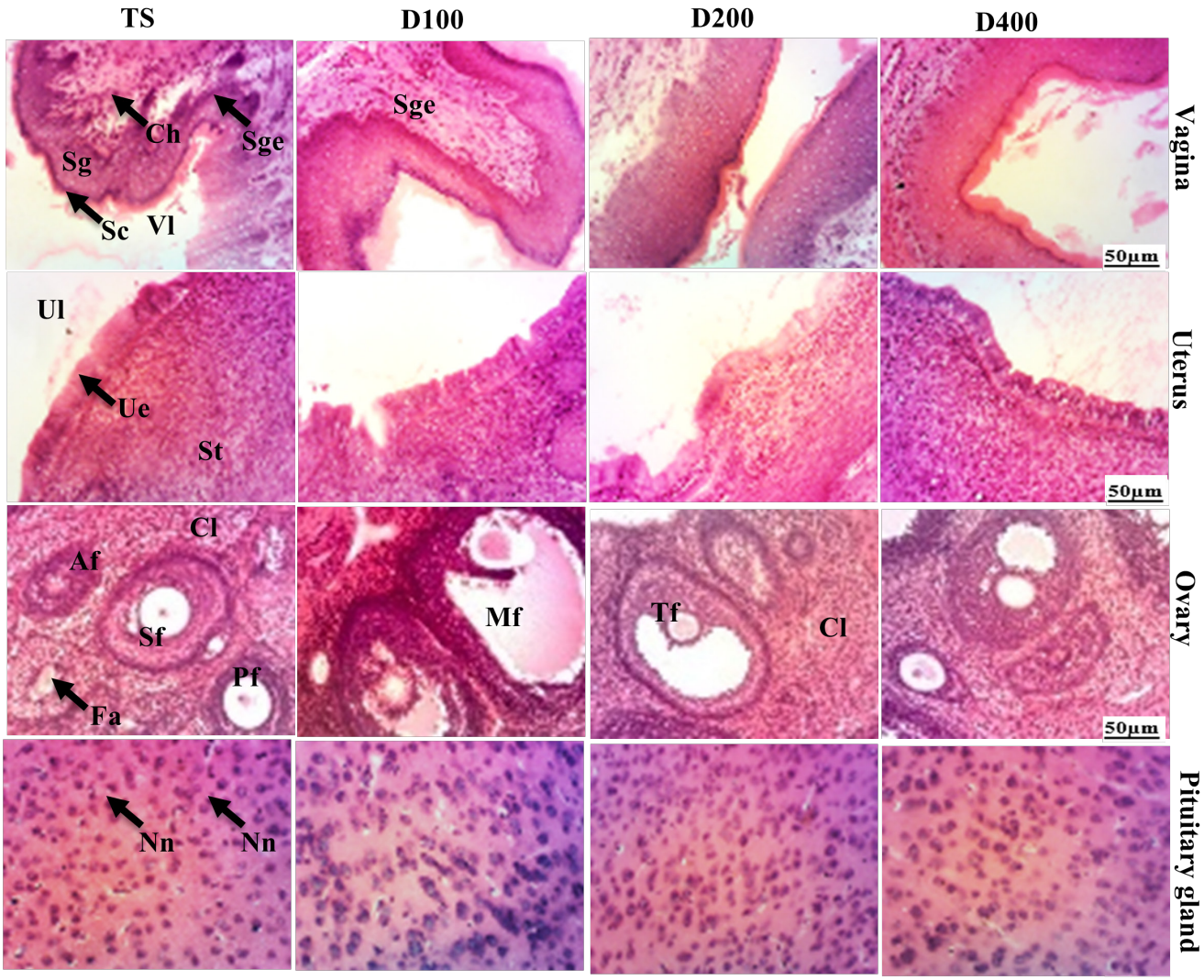
Figure 3: Histological cross section of female's reproductive organs and pituitary gland (HE, 100x) after administration of extract. Control: Control group receiving distilled water; D100, D200 and D400: tests groups receiving the extract of roots of *Elaeis guineensis* at the doses of 100, 200 and 400 mg/ kg, respectively. Uterus: Ue = uterine epithelium, Ul = uterine lumen, St = Stroma; Vagina: Sc = Stratum corneum, Sg = Stratum granulosum, Sge= Stratum germinativum Ch = Chorion, Vl = vaginal lumen; Ovary : Cl = Corpus luteum, Sf = Secondary follicle, Tf = Tertiary follicle, Af = Atretic follicle, Mf = Mature or Degraafian follicle, Pf = Primary follicle; Pituitary gland: Nn = Neuron.



Effect of E. guineensis roots aqueous extract on fecundity, duration of gestation and selected offspring parameters in treated and untreated females up to weaning

The rate of gestation was 100% in treated and untreated females mated with treated males at all doses. There was no significant change in gestation length in treated females after administration of *E. guineensis* roots extract. In addition, gestation, parturition, and lactation were normal. No behavioral abnormalities were observed during the experiment (**Table 4**). However, there was a significant decrease ($p < 0,001$) in the number of offsprings at dose of 400 mg/kg even in untreated females mated with male treated at dose of 400mg/kg (D400U) (Table 4).. In addition, fertility and prolificacy percentages were significantly ($p < 0.001$) reduced at 400 mg/kg for both treated and untreated females compared with the control. At the same dose, many pups were stillborn compared with the control and others doses, and all surviving births in treated females died after a few days. (**Table 4**). These results suggest that high doses of *E. guineensis* roots extract may prevent normal fetal development and adversely affect sperm quality (Ugwah-Oguejiofor et al. 2020). Disruption of ovarian hormones may be responsible. In fact, it was observed that the increase in relative uterine weigth was non-significant, indicating a possible estrogenic effect of the extract (Hewitt et al. 2003). Previous studies have reported that phytoestrogens may cause loss of pregnancy and fertility (Burton and Wells 2002). In addition, the bioactive compounds in the extract have beneficial effects. However, some of them may have opposite effects at certain concentrations. For example, some alkaloids could have a toxic effect on spermatozoa and prevent normal fetal development (Yakubu and Bukoye 2009, Poole RK. and Poole DH, 2019, Sicong

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et al. 2019). Some saponins also have anti-implantation, abortifacient, and antifertility effects (Dande et al. 2014). These two compounds are thought to act individually or synergistically to exert toxic effects on fetuses. An increase in stillbirths and a decrease in fecondity and prolificacy percentages were also observed in untreated females mated with treated males at a dose of 400 mg/kg however, live offspring showed normal development with no difference in weight from controls, and no physical abnormabilities (**Figure 4**). These results suggest a possible alteration in spermatozoa, but further studies on sperm morphology are needed to confirm this hypothesis.

Table 4: Reproductive parameters of female rats after E. guineensis extract' administration.

	Control	D100 T	D100 U	D200 T	D200 U	D400 T	D400 U
Number of mated	6	6	6	6	6	6	6
% of fertility	100	100	100	100	100	100	100
gestation duration (day)	23.4 ± 1.3	22.6 ± 0.7	22 ± 0	22 ± 0.8	22.5 ± 0.3	24.2 ± 1	21 ± 0.3
% of fecundity	883.3	850	916,7	850	900	200*	433.3*
% of prolificacy	883.3	850	916,7	850	900	200*	433.3*
Number of offspring	53	51	55	51	54	26 *	27*
Stillborn	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	14*	4*

Control: Control group receiving distilled water; D100 T, D200 T and D400 T: groups of females receiving the aqueous extract of the roots of *E. guineensis* at dose of 100, 200 and 400 mg/kg, respecively and mated with males treated at doses of 100, 200 and 400 mg/kg respectively. D100U, D200U and D400U: untreated female rats untreated with the extract and mated with males treated at doses of 100, 200 and 400 mg/kg respectively. Each value represents the mean ± standard error of the mean. n = 6. *= p<0.05: significant difference when compared to control. Number of male crossed: 24; number le females crossed: 48; Male/female ratio: 1:2 (treated and untreated females.

Figure 4: Weigh evolution of offsprings during lactation. D100 T, D200 T AND D400 T: groups of females receiving the aqueous extract of the roots of E. guineensis at dose of 100, 200 and 400 mg/kg, respectively and mated with males treated at doses of 100, 200 and 400 mg/kg respectively. D100U, D200U and D400U: untreated female rats untreated with the extract and mated with males treated at doses of 100, 200 and 400 mg/kg respectively.

CONCLUSION

In conclusion, the use of *E. guineensis* roots aqueous extract presented weak reproductive toxic effect on parental generation at the tested doses, but improved sperm count at the therapeutic dose (200 mg/kg), proving its administration for the management of male reproductive disorders. However, the use of this extract must be monitored to avoid any adverse effects on the offsprings. In fact, at the highest dose (400 mg/kg), some of the offsprings were stillborn; and in couples (male and female) receiving extract, no children survived. Thus, high-dose extract could be lethal to offspring.

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Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

Ethics approval for animal study

All the rats were treated according to the Guide for the Care and Use of Laboratory Animals prepared by the European Union Council (CEE Council 86/609). The study was approved by the Institutional Ethics Committee of the University of Douala-Cameroon.

Declaration of data authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [AACN], upon reasonable request.

Declaration of interest

The authors report there are no competing interests to declare.

Author's contribution

Conceived and designed the work: AA-CN, ML-D, SN-Q; Experimental assess: SN-Q, TT-C, NTJ, E-NB; Wrote the article: SN-Q, A-SB, ML-D, M-SH; Final edit of paper: AA-CN.

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