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EVALUATION OF THE EFFECTS OF THE AQUEOUS EXTRACT OF EREMOMASTAX SPECIOSA ADMINISTERED DURING GAMETOGENESIS IN THE FEMALE RAT

Fisheries & Aquatic Ecosystems

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ABSTRACT

Background And Aim: Eremomastax speciosa (Hochst) Cufod (Acanthaceae) is a shrub used in traditional medicine to remedy several ailments. The aim of this work was thus to evaluate the reprotoxicity in female rats when the extract is administered during the period of gametogenesis. **Materials And Methods:** Thirty-six male albino rats were divided into four groups of nine rats each and given the extract at doses of 0, 250, 500 and 1000 mg/kg, respectively, for 60 days followed by a 10 days fertility study by pairing each treated male with an untreated female. Thirty-six other female rats were divided into four groups of nine rats each and given the extract at doses of 0, 250, 500 and 1000 mg/kg and were then crossed each with an untreated male. The effects on fertility parameters, body and organ weights were assessed, as well as biochemical, haematological and histological parameters. **Results And Conclusion:** In untreated female rats crossed with treated males during gametogenesis there was an increase in mating index (from 66.7 % to 88.9 %), increase in percentage of pregnant females (from 83.3% to 100%) and consequent increase in fertility rate from 55.6 % in controls to 66.6-77.8 % in females crossed with extract-treated males. When female rats were given *E. speciosa* extract during gametogenesis and crossed with untreated males, although copulation rates increased significantly but gravidity and fertility rates reduced significantly as evidenced by the significant decrease in numbers of implantations, corpora lutea and fetuses and live young.

KEYWORDS

Eremomastax speciosa, reprotoxicity, female rat, fertility

INTRODUCTION

Humans have used medicinal plants to treat illnesses for millennia. Despite the immense technological advancement in modern medicine, approximately 80 % of the African population still depends on traditional healing practices and herbal medicines for health care needs (Ojewole,2005). Natural products derived from plants and food supplementary compounds can have disruptive effects on the reproductive process (Nchegang and *al*., 2016), and exposure of the reproducing female to such chemical compounds can be deleterious to the achievement of pregnancy (Nchegang and *al*.,, 2020). Therefore, studies focusing the reproductive toxicology of chemical compounds are essential in order to provide reliable information on possible toxic effects (Nchegang and *al*., 2020). In the present experiment we studied the fertility parameters of female rats treated with the aqueous extract of *E. speciosa* and those who have no treatment and cohabited with untreated and treated males respectively.

MATERIAL AND METHODS

Animal

Male and female adult albino Wistar rats raised in the Animal house of the Animal Physiology laboratory, Faculty of Science, University of Yaoundé I, were used. The animals were maintained in a well-ventilated room with a 12:12 hour light/dark cycle at room temperature. They were fed with a standard laboratory diet and given tap water *ad libitum*.

Preparation Of Plant Extract

The aqueous extract of *E. speciosa* was prepared by infusing 560 g of the ground powder in 5 liters of boiled water for 15 minutes. After filtration through watman filter paper N° 3, the filtrate was evaporated at 40 °C using a *Raven* convection air oven (Jencons-PLS, UK). The brownish solid obtained (10 % yield) was stored at 4 °C.

Experimental Protocol

The protocol employed was the segment 1 study procedure (administration of test substance prior to and in the early stages of pregnancy) recommended for reproductive and development toxicity (WHO, 1993). Thirty-six adult female rats were randomly divided into four similar groups of nine rats each and given the extract (0; 250; 500; 1000 mg/kg of body weight) orally for 14 days. A fertility study was done by cohabiting an untreated male with a female from each group for 10 days. Administration of the extract to the female continued during mating and after successful copulation until the sixth day of pregnancy. Thirty-six immature male rats were divided into four groups of nine rats each. Group I served as the control and received distilled water (2.5 mL/kg of body weight) while groups II, III and IV were treated orally with 250; 500 and 1000 mg/kg of body weight of the aqueous extract of *E. speciosa*, respectively, for 60 days. A fertility study was done by cohabiting one treated male with one untreated female for 10 days. After successful copulation, the treated and untreated adult female rats were sacrificed and autopsied. The ovaries, uteri and placenta were removed and weighed, and samples were stored for subsequent histological analysis as described by Gornal and *al (1949)*, fertility parameters of maternal and offspring were examined. The analysis of total protein and total cholesterol, were estimated in serum using Commercial kits (Fortress and GCM). Enzymatic activities, atherogehernic index were calculated as described respectively by authors (Wroblewski and *al*., 1956; Youmbissi and *al*., 2001).

Statistical Analysis

The results were analyzed using the one-way ANOVA followed by the Student-Newman-Keuls post test for comparison of treatment means. $p < 0.05$ was considered significant. Values in tables are given as means + standard error for the mean (SEM).

RESULTS AND DISCUSSION

RESULTS

Effects Of Aqueous Extract Of *E. Speciosa* On Fertility Parameters In Untreated Female Rats

An increase ($p < 0.05$) in the number of implantations in untreated rats crossed with males treated with the extract was noted compared to

those crossed with males who received distilled water. The mating index increased from 66.7 % in females cohabited with males receiving distilled water to 88.9 % in females crossed with treated males. The percentage of pregnant females was 83.3%, 100%, 100 %, and 87.5 %, respectively, at doses 0, 250, 500 and 1000 mg/kg. Consequently, the percentage of fertility increased from 55.6 % in rats crossed with control males to 66.6-77.8 % in females crossed with treated males (Table 1).

Table 1: Effects Of Aqueous Extract Of *E. speciosa* On Fertility Parameters In Untreated Female Rats Crossed With Males Receiving Aqueous Extract

Fertility parameters	Dose (0 mg/kg of bw) G1	G2	G3	G4
Number of implantations	5.11±1.70	6.54±1.36	7.11±1.37	6.66±1.34
Number of fetuses (day 12)	4.77±1.64	6.33±1.29	6.66±1.28	6.44±1.31
Number of corpora lutea (day12)	3.22±0.95	3.55±1.05	3.55±1.05	3.00±0.62
Number of resorptions	0.33±0.33	0.22±0.22	0.44±0.24	0.22±0.22
Total resorptions per group	03	02	04	02
Number of live young	4.77±1.64	6.33±1.29	6.66±1.28	6.44±1.31
Copulation rate (%)	66.66	77.77	77.77	88.88
Gravidity rate (%)	83.33	100.00	100.00	87.50
Fertility rate (%)	55.55	66.66	77.77	77.77

N = 9 rats per group
G1 = females crossed with males receiving distilled water
G2= females crossed with males receiving aqueous extract of *E. speciosa* (250 mg/kg)
G3= females crossed with males receiving aqueous extract of *E. speciosa* (500 mg/kg)
G4= females crossed with males receiving aqueous extract of *E. speciosa* (1000 mg/kg)

Effects Of Aqueous Extract Of *E. speciosa* Administered During Gametogenesis In Female Rats Crossed With Untreated Males

Effects Of *Eremomastax Speciosa* On Relative Weight Of Organs (g/100 G Of bw) Of Female Rats

Internal organs such as ovaries, uteri, and placenta of females treated with the extract at different doses experienced significant ($p<0.05$ - $p<0.001$) decreases in their weights compared to those of rats given distilled water (Table 2).

Table 2: Relative Organ Weights (g/100 g of bw) Of Female Rats Treated (14 Days) With Aqueous Extract Of *E. speciosa*

Organs	Doses (mg/kg) 0	250	500	1000
Left Ovary	0.04±0.005	0.01±0.002***	0.01±0.001***	0.02±0.002***
Right Ovary	0.04±0.005	0.01±0.002***	0.01±0.001***	0.02±0.002***
Uterus	0.51±0.11	0.20±0.02**	0.19±0.02**	0.22±0.03**
Placenta	0.07±0.02 (5)	0.02±0.02* (1)	0.002±0.002* (1)	0.008±0.006* (2)

Values are expressed as Mean ± SEM, N= 9 rats per group; on the same line, statistically significant relative to the controls, *$p<0.05$; **$p<0.01$, ***$p<0.001$, ( ) = number of placenta

-Effects of aqueous extract of *E. speciosa* on fertility parameters

A significant decrease ($p < 0.05$) in the number of corpora lutea on the twelfth day of gestation in treated rats [at doses of 250 (1.17±1.03), 500 (0.88±0.61) and 1000 mg/kg (2.11±1.29)] compared to the control [6.00±2.06] was noted; this was followed by a decrease in the number of fetuses, resorptions, live and dead fetuses from the treated animals compared to those from the control group. The mating index increased by 55.6% in females receiving distilled water and by 100% in test groups. The percentage of fertility decreased from 55.6% in control rats to 11.1-22.2% in treated females. The number of resorptions and implantations decreased in all test groups (Table 3).

Table 3: Effects Of Aqueous Extract Of *E. speciosa* On Fertility Parameters in Female Rats

Fertility parameters	Dose (mg/kg of bw) 0	250	500	1000
Number of implantations	6.00±2.06	1.17±1.03*	0.88±0.61*	2.11±1.29*
Number of fetuses (day 12)	4.11±1.34	0.77±0.77*	0.55±0.55*	1.66±1.13*
Number of corpora lutea (day12)	6.00±2.06	1.17±1.03*	0.88±0.61*	2.11±1.29*
Number of resorptions	1.88±0.88	1.00±0.52	0.33±0.33	0.44±0.29
Total resorptions per group	17	0 9	03	04
Number of live young	4.11±1.34	0.77±0.77*	0.55±0.55*	1.66±1.13*
Copulation rate (%)	55.60	88.90	100.00	77.80
Gravidity rate (%)	100.00	12.50	11.10	28.60
Fertility rate (%)	55.60	11.10	11.10	22.20

Values are expressed as mean ± SEM, on the same line, statistically significant relative to the controls, *$p<0.05$ N= 9 rats per group

- Effects Of Aqueous Extract Of *E. speciosa* On Biochemical Parameters

Total tissue (ovarian) cholesterol showed little increase ($p > 0.05$) in females treated at doses of 250 (99.59±9.36 mg/dL), 500 (118.34±15.01 mg/ dL) and 1000 mg/kg (100.22±6.51 mg/dL), compared to the control group (94.00 ± 1.64 mg/dL). Only slight decreases in serum total cholesterol levels in the 250 [157.89±10.83 mg/dL (6.2%)]; 500 [147.72±6.47 mg/dL (12.3%)] and 1000 mh/kg dose groups [158.16±13.31 mg/dL (6.0%)] compared to control [168.34± 9.80 mg/dL] were noted (Table 4).

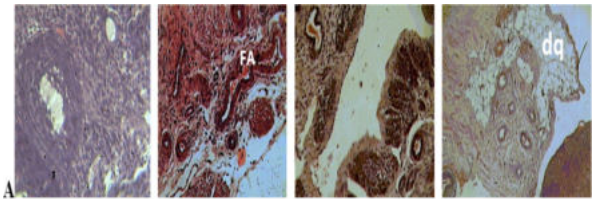
Significant increases in serum total protein levels were observed in animals treated with doses of 250 mg/kg [5.68±0.37 mg/mL (24.3 %)] ($p < 0.05$) and 500 mg/kg [6.12±0.34 mg/mL (33.9 %)] ($p < 0.01$) compared to the control. Differences in total tissue (ovarian) protein levels between test and control groups remained unchanged. (Table 4).

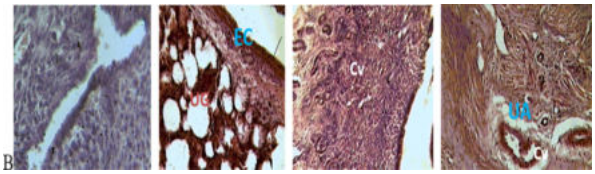
Table 4: Effects Of The Aqueous Extract Of *E. Speciosa* Administered During Gametogenesis Period On The Cholesterol Levels And Serum And Tissue Proteins In Female Rat

Biochemical parameters	Tissue/ Organ	Dose (mg/kg) 0	250	500	1000
Total cholesterol (mg/dL)	Serum	168.34± 9.80 (9)	157.89±1 0.83 (9)	147.72±6.4 7 (9)	158.16±13.31 (9)
	Ovary	94.00±1 .64 (9)	99.59±9. 36 (9)	118,34±15, 01 (9)	100.22±4.51 (9)
Total protein (/mL)	Serum	4.57±0. 10 (9)	5.68±0.3 7* (9)	5.17±0.14 (9)	6.12±0.34** (9)
	Ovary	3.66±0. 04 (9)	3.72±0.0 4 (9)	3.83±0.10 (9)	3.66±0.03 (9)
	Uterus	3.75±0. 12 (9)	3.69±0.0 5 (9)	3.64±0.04 (9)	3.73±0.04 (9)

Values are presented as mean ± SEM; in the same line, line, statistically significant compared to the controls, *$p<0.05$;**$p<0.01$ the values between ( ) = number of animals per batch.

Figures 1 represent the histological sections of the ovaries and uterus of the rats treated with doses of 0, 250, 500 and 1000 mg/kg for 20 days and crossed with untreated males. In the treated rats, desquamation (dq) and atresia (Atr) of a few follicular cells were noted (Figure 1 A). A decrease of epithelial cells (EC) thickness in treated rats was observed (Figure 1B).





Control Extract 250 mg/kg Extract 500 mg/kg Extract 1000 mg/kg

Figure 1: Effects of the extract administered during gametogenesis period on the histology of the ovary (A) and uterus (B in the female rat (H.E.x 200)

Dq: Desquamation; Atr: Follicular atresia; EC: Epithelial Cell; UG: Uterine Gland; UA: Uterine Artery

- Effects of E. speciosa on the histology of the Ovary and Uterus in female rats

DISCUSSION

The results showed that in male rats treated with *E. speciosa* extract and crossed with untreated females, there was a significant increase in fertility rates (litter size, mating index and pregnancy rate) at all extract doses. These results confirm the earlier reported pro-sexual and androgenic effects of the aqueous extract of *E. speciosa* in male rats (Nchegang and *al*., 2020). Biochemical analysis showed that serum cholesterol levels tended to drop in the extract-treated female rats, contrary to an increase in ovarian cholesterol levels. Serum and ovarian total protein levels also increased but the uterine total protein levels remained unchanged (no real decrease) in treated female rats. The structural alterations of the ovaries and uterus in the treated rats in comparison with the controls would be due to the impact of the extract which could induce the degeneration of the follicular layer leading to a drop in serum cholesterol levels (hormonal precursor). This would cause an increase in the probability of the occurrence of anovulatory cycles and consequently a decrease in the litter size and the weight of the ovaries. This is confirmed by a significant drop in pregnancy and fertility rates, number of implantations, corpora lutea and live pups suggesting that steroidogenesis is influenced by the active development of oocytes (Lucidi and *al*., 2003). The desquamation of follicular cells observed in the histological sections can lead to a reduction of estrogen levels. Thus, the increase in ovarian cholesterol levels could mark a low synthesis of estrogen in animals treated with the extract. Ovarian proteins are one of the constituents that ensure the maturation of reproductive cells. In addition, a low concentration of ovarian protein would indicate poor ovarian growth, just as FSH is also essential for protein synthesis in the gonads (follicle development) (Oakberg, 1996). The small increase in protein level observed following the extract treatment could be the result of the action of estrogen. Indeed, such an increase in the concentration of ovarian proteins could well be the basis for the observed decrease in fertility rates which are revealing aspects of androgenicity. Body weight is well known to play an important role in regulating gonadotropin secretion and for regular cyclic function (Knuth and al., 1977). The organs removed at the end of the treatment (ovaries, uteri and placenta) showed differences in their relative weights between the control groups and the test groups.

CONCLUSION

When *E speciosa* extract was administered to male rats but not to the females during gametogenesis, fertility rate increase. However, when female rats were given *E. speciosa* extract during gametogenesis and then crossed with untreated males, although fertility rates reduced significantly. These results suggest that *E. speciosa* extract which is widely used for the management of various disease conditions may be contraindicated in reproducing females during the period prior to conception.

Authors Contributions

This work was carried out in collaboration among some authors. Authors Nchegang wrote the protocol, did the literature search, analysis of histological sections, and statistical analysis. Nchegang, Mezui, Kuissu and Lamou made microscopic observations on spermatozoa, Enow-Orock and Nchegang did the histological preparations, observations and analysis Author Nchegang managed the biochemical analysis and wrote the first draft. Tan did the final revision of the manuscript.

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