Anatomy & Physiology: Current Research
ISSN: 2161-0940

Nnama, Anat Physiol 2017, 7:4
DOI: 10.4172/2161-0940.1000269

Research article Open Access

Effect of *Erytherina senegalensis* on the oestrogen level and histology of ovary of an adult female albino wistar rats

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Received Date: June 11, 2017; **Accepted Date:** June 20, 2017; **Published Date:** June 27, 2017

Abstract

Erytherina senegalensis are used in soups administered after delivery, also in Nigeria; it is given to women during labour to ease pain, for treatment of malaria, gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhoea, jaundice, nose bleeding, pain, bronchial infection, coughs, throat inflammation and administered for venereal diseases. In view of its usage, the study sought to investigate the effect of *Erytherina senegalensis* leaf extract on the oestrogen level and the histology of the ovary of albino wistar rat. Fresh mature leaf samples of *E. senegalensis* were collected, ethanolic extract was prepared and the effect of sub-chronic oral administration of the leaf extract of *E. senegalensis* was studied using twenty-five female albino wistar rats. The oral administration of the leaf extract was done three times per week, for 60 days at doses of 2000 mg/kg for group 1, 4000 mg/kg for group 2, 6000 mg/kg for group 3, 8000 mg/kg body weight for group 4 and group 5 is the control group, received distilled water and food alone. The rats were anaesthetised by chloroform in a close jar and blood samples were for hormonal test, experimental rats were sacrificed by cervical dislocation and the ovary harvested. There was a dose dependent increase in the level of oestrogen across the groups and there was no significant alteration on the histology of the ovary across the groups. Oestrogen level and histology architecture reveals the leaf extract didn't show any sign of toxicity on the hormone and organ of the study, this may suggest that the extract may be used as female hormonal supplement and may not be toxic when administered orally.

Keywords: *Erytherina senegalensis*; Hormones; Ovary

Introduction

Plant-derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their likely or potential benefit as well as adverse effects [1]. *Erythrina senegalensis* is an example of a medicinal plant commonly known as Senegal coral which belongs to the family *Fabaceae* and is of the tribe *Phaseolae*. It is a thorny shrub or small tree with common names that include Senegal coral tree (English) [2].

The leaf and bark of *Erytherina Senegalensis* extract have been reported used in Nigerian studies for treatment of malaria, gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhoea, jaundice, nose bleeding, pain and administered for venereal diseases [3]. A decoction of the bark has been used for the treatment of bronchial infection, coughs and throat inflammation. The pounded bark and leaves of *Erytherina Senegalensis* are used in soups administered after delivery, also in Nigeria; it is given to women during labour to ease pain [4]. Some of these plants are known to possess anti-fertility effect through their action on hypothalamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs resulting in inhibition of ovarian steroidogenesis [5].

The ovaries are the female pelvic reproductive organs that house the ova and are also responsible for the production of sex hormones [6]. Sex hormones are known to regulate the reproductive function and characteritics in both male and female organism. Measurement of serum hormone profile is therefore very useful in assessing the reproductive integrity in both animals and humans [7]. In view of continual consumption of the *Erytherina senegalensis* in treatment of diseases and paucity of literatures on the leaf extract, the present study is aimed to evaluate the effect of ethanolic leaf extract of *E. Senegalensis* on the oestrogen level and histology of the ovary of albino wistar rats.

Materials and Methods

Ethical approval

Necessary approval was obtained from the Faculty of Basic Medical Science Ethics Committee, Nnamdi Azikiwe University, Nnewi Campus.

Collection and authentication of the plant material

Fresh mature leaf samples of *E. senegalensis* were collected in Nnewi, Anambra State, and botanical identification of the plant was done by Mr. Egboka Tochukwu of the Department of Botany, Nnamdi Azikiwe University, Awka, with reference no NAU/BOT/232.

Plant preparation

All the samples of *E. senegalensis* were thoroughly rinsed with running tap water and distilled water before being air-dried at room temperature for 30 days. Then, the plant sample was pulverized to dry powder using an electric grinder into minute pieces and the extract was soaked in absolute ethanol for 4 days with frequent agitation at room temperature. The extract was filtered with Whatman paper No.1 and the residue of fine powder was then re-soaked with a fresh portion of ethanol twice for four days each time at room temperature. The filtrate was concentrated under reduced pressure in vacuum at 45°C

and evaporated to dryness on a rotary evaporator (Model 342/7, Corning Ltd). The yield of the extract was 17.1% based on dry weight.

Care of the animals and experimental design

All animal experiments in this study followed the principles of laboratory Animal Care [6] Daily, the animals were fed with the normal diet of feed grower's mash produced by Premier feed mills Co. limited (a subsidiary of Flour mills Nigeria PLC). The feed was provided in plastic plates while 20cl distilled water was provided in plastic bottles with stainless steel nozzles which were placed each at the top of the cages to allow the animals suck the water comfortably the animals were allowed access to feed and deionized water ad-libitum before the commencement of the experiment.

Twenty-five (25) apparently healthy female wistar rats (2-3 months old) weighing between 100 gm to 200 gm were used for this study. The rats were purchased from Animal Farm in Nnewi, Nnewi-North, Anambra State Nigeria and were transferred to the Animal House of the Department of Anatomy, College of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi. These animals were fed with the normal diet of feed grower's mash produced by Premier feed mills Co. limited (a subsidiary of Flour mills Nigeria PLC) and distilled water ad-libitum for the period of two weeks to help them acclimatize to the new environment under standard conditions (23 ± 2°C, humidity 60-70%, 12 hrs light and dark cycles) before commencement of the actual experiment.

The rats after acclimatization were randomly grouped into five groups for experimentation. The rats were weighed and the weight recorded. The rats were grouped and oral administration of the leaf extract of *Erytherina senegalensis* based on the acute toxicity study which was found to be greater than 5000 mg/kg body weight [8]. The rats were grouped and oral administration was done as below;

- GROUP 5 (control) received only water and feed for 8 weeks, weighed once per week.
- GROUP 4 (high dose) received 8000 mg/kg body weight, weighed once per week.
- GROUP 3 (medium dose) received 6000 mg/kg body weight, weighed once per week.
- GROUP 2 (moderate dose) received 4000 mg/kg body weight, weighed once per week.
- GROUP 1 (low dose) received 2000 mg/kg body weight, weighed once per week.

Observation of behavioural changes in the animals

Visual observations for mortality, behavioural pattern changes such as weakness, aggressiveness, food or water refusal, diarrhoea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotor activity, injury, pain or any signs of illness in each treated group were monitored carefully on daily basis throughout the experiment period.

Monitor of fluid, feed intake and body weight of animals

The fluid and feed intake of the animal were monitored and recorded daily while the weights of the animals were taken weekly.

Necropsy

After 60 days of exposure the final body weights of the Wistar albino rats were taken, and thereafter, sacrificed by painlessly anesthetization with chloroform using chloroform in a closed jar. After anesthetization blood samples were collected directly from the heart (at the thoracic region) using 5 ml syringes and indirectly from the eyes (ocular puncture) using cannula with catheter and placed in a specific sterilized plastic containers required collected for hormonal level evaluation. Thereafter the animals were sacrificed by cervical dislocation and the ovaries were harvested and weighed immediately. Thereafter, the organs were fixed in 10% buffered formal saline and processed for routine histopathological studies, and the slides analyzed.

Hormonal assay

The assay kit for oestrogen was supplied by Diagnostic Automation Inc., Calabasa, CA, USA. All other reagents used were of analytical grade and were prepared in volumetric flask using glass distilled water. The procedure described in the hormone assay kits was used according to the principle highlighted was used for the hormonal analysis that is oestrogen [9].

Histopathological assessment

Histopathological examinations were carried out on ovary of the rats. They were fixed in 10% formalin, dehydrated in gradual ethanol concentrations (50-100%), cleared in xylene and embedded in paraffin.

Sections (4-6 μM thick) were prepared and then stained with hematoxylin and Eosin (H-E) dye for photomicroscopic observation under light microscope at high power magnifications (x400 objective).

Statistical data analysis

The ovary weights (relative) and oestrogen level were evaluated using the statistical package of social sciences (SPSS) software version 21.0 (SPSS) Inc. Chicago and Microsoft.

Statistical analysis of variance was carried out using student T-test and one way ANOVA (SPSS 21.0). A value of $p<0.05$ was used as the level of significance.

Results

Physical observation and clinical signs

Daily oral administration of *Erytherina senegalensis* extract for 8 weeks did not induce any obvious symptom of toxicity in rats, including the highest dose tested at 8000 mg/kg body weight, deaths was not recorded and no obvious clinical signs were found in any groups throughout the experimental period.

Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioural changes, diarrhoea, tremors, salivation, sleep, and coma.

Although rats in group 4 (high dose) showed relative weakness immediately after administration.

Gross observation of the organs showed no change in colour, hypertrophy and abnormal fat deposition.

Groups	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
I	87.71 ± 26.77	112.57 ± 20.59	77.86 ± 17.82	75.71 ± 12.12
II	79.86 ± 14.04	85.14 ± 29.05	72.29 ± 12.93	65.00 ± 15.62
III	87.29 ± 20.13	102.00 ± 24.47	67.29 ± 18.17	61.00 ± 18.81
IV	92.43 ± 23.17	99.43 ± 26.68	73.00 ± 16.46	60.71 ± 19.41
V (Control)	82.43 ± 13.55	105.14 ± 4.98	73.57 ± 12.49	76.43 ± 33.24
F-Ratio	0.414	1.364	0.401	0.949
Sig	0.797	0.27	0.807	0.45

Table 1: Food intake of the rats.

Table 1 showed the mean and standard deviation of the volume of weekly food intake in grams per group, in all the groups the food intake increased in the second week thereafter a progressive decline was observed. Analysis of variance (ANOVA) showed that there was no significant difference ($p>0.05$) in food intake between the test and control group during the 4 weeks of administration.

Group	Week 1 (ml)	Week 2 (ml)	Week 3 (ml)	Week 4 (ml)
I	81.43 ± 36.71	103.43 ± 43.52	82.29 ± 22.54	79.00 ± 43.82
II	85.83 ± 19.60	104.29 ± 47.12	86.71 ± 39.74	70.86 ± 37.61
II	93.00 ± 12.23	86.00 ± 42.97	79.43 ± 41.32	82.14 ± 38.06
IV	108.33 ± 21.13	106.29 ± 45.48	98.43 ± 14.11	64.86 ± 45.21
V(Control)	101.67 ± 9.83	123.71 ± 26.39	89.71 ± 22.37	105.14 ± 24.60
F-Ratio	1.533	0.717	0.423	1.118
Sig	0.221	0.587	0.791	0.366

Table 2: Fluid intake of the rats.

Table 2 showed the mean and standard deviation of weekly fluid intake in millilitres, groups I, II, IV, increased in the second week thereafter a progressive decline was observed. Data showed, Analysis of variance (ANOVA) showed that there was no significant difference ($p>0.05$) in fluid intake between the test and control group during the 4 weeks of administration.

	Group I (g)	Group ii (g)	Group iii (g)	Group iv (g)	V (control) (g)	SIG
Week 1	170.00 ± 4.47	180.40 ± 4.56	182.80 ± 9.96	167.00 ± 10.95	181.40 ± 18.81	0.112
Week 2	175.60 ± 5.18	184.80 ± 3.03	187.00 ± 10.27	171.80 ± 10.40	186.40 ± 16.82	0.098
Week 3	180.60 ± 4.45	190.40 ± 3.85	192.40 ± 7.37	180.00 ± 12.08	193.20 ± 17.98	0.152
Week 4	191.25 ± 9.67	194.80 ± 2.28	192.60 ± 6.07	179.20 ± 9.83*	193.60 ± 17.90	0.171
Week 5	191.50 ± 9.57	196.60 ± 1.95	194.25 ± 6.13	183.00 ± 9.17*	197.80 ± 19.03	0.267
Week 6	191.75 ± 9.18	197.00 ± 1.73	199.25 ± 2.99	185.40 ± 10.11*	200.60 ± 18.99	0.224
Week 7	197.33 ± 4.62*	203.25 ± 5.85	202.50 ± 4.65	194.00 ± 4.90*	209.00 ± 11.27	0.07
Week 8	203.33 ± 5.51	208.75 ± 5.06	204.00 ± 4.62	196.50 ± 4.43*	211.20 ± 12.46	0.102
*Significant difference compared to control ($p<0.05$).						

Table 3: Body weight changes throughout period of administration.

Table 3 showed the mean and standard deviation, Analysis of variance (ANOVA) showed that there was no significant difference in body weight between groups ($p>0.05$) during the 8 weeks of administration. Post-hoc analysis however, showed significant differences in body weight between group I and control at week 7 ($p<0.05$), and also between group IV and the control at weeks 4 ($p<0.05$), 5 ($p<0.05$), 6 ($p<0.05$), 7 ($p<0.05$) and 8 ($p<0.05$).

Organ weight (G)	Group I (g)	Group II (g)	Group III (g)	Group IV (g)	Group V (Control)	SIG
Right Ovary	0.27 ± 0.06	0.25 ± 0.10	0.25 ± 0.13	0.33 ± 0.05	0.30 ± 0.14	0.783
Left Ovary	0.23 ± 0.06	0.18 ± 0.05	0.20 ± 0.08	0.28 ± 0.05	0.30 ± 0.14	0.229

Table 4: Showing organ weight.

Table 4 showed organ weight for test and control groups, data showed mean and standard deviation. Analysis of variance (ANOVA) between groups showed no significant difference in weight of the ovary ($p<0.05$).

% of Organ Weight in Relation to Body Weight	Group I	Group II	Group III	Group IV	Control Group	SIG
Right Ovary	0.12 ± 0.04	0.12 ± 0.05	0.12 ± 0.06	0.17 ± 0.03	0.14 ± 0.06	0.706
Left Ovary	0.10 ± 0.00	0.08 ± 0.02	0.10 ± 0.04	0.14 ± 0.03	0.14 ± 0.06	0.189

Table 5: Showed organ weight in relation to the body weight.

Table 5 showed relative organ weight for test and control group, data showed means and standard deviation. Analysis of variance (ANOVA)

between groups showed no significant difference in % relative weight of the ovary ($p<0.05$).

% of Organ Weight in Relation to Body Weight	Group I	Group II	Group III	Group IV	Control Group	SIG
Right Ovary	0.12 ± 0.04	0.12 ± 0.05	0.12 ± 0.06	0.17 ± 0.03	0.14 ± 0.06	0.706
Left Ovary	0.10 ± 0.00	0.08 ± 0.02	0.10 ± 0.04	0.14 ± 0.03	0.14 ± 0.06	0.189

Table 6: Showed organ weight in relation to the body weight.

Table 6 showed relative organ weight for test and control group, data showed means and standard deviation. Analysis of variance (ANOVA) between groups showed no significant difference in % relative weight of the ovary ($p<0.05$).

Oestrogen (pg/mls)					
Group 1	Group 2	Group 3	Group 4	Control Group	P-Value
1.20 ± 0.00	7.05 ± 0.07*	9.93 ± 0.12*	6.00 ± 0.00*	1.05 ± 0.07	0

Table 7: Showed the oestrogen level of test group and control group.

Table 7 showed oestrogen level of test group and control, data are means and standard deviations. Analysis of variance (ANOVA) showed that the control group had a significantly lower estrogen level ($p<0.001$) than the test groups.

Histopathological findings (Figures 1-5)

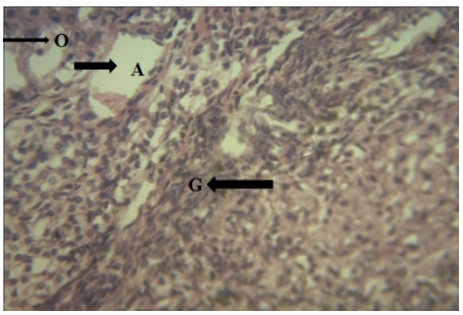

Figure 1: Photomicrograph of the ovary of group 5 (control group) showing the oocytes (O), Antrum (A) and the Granulosa layer (G) of normal histology with no visible lesion [x400] [H&E].

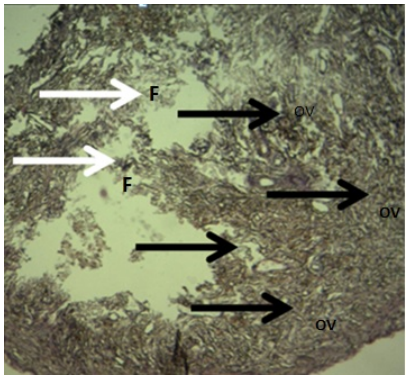

Figure 2: Photomicrograph of the ovary of group 1 (low dose) of albino rat, showing normal architectural features. Only the ovarian stroma seen, (OV)black arrows No follicle observed,(F) white arrows [H&E x400].

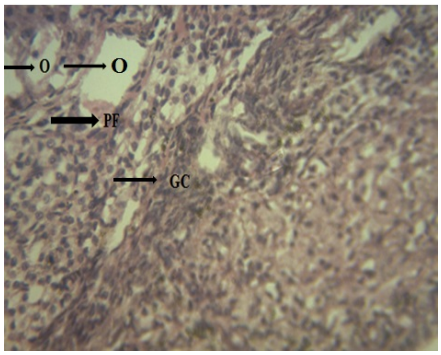

Figure 3: Photomicrograph of the ovary of group 2 (moderate dose), showing Oocytes (O), Granulosa cells, (GC) Primary follicles (PF), with all the follicles appearing to have a uniform stage of development [x400] [H&E].

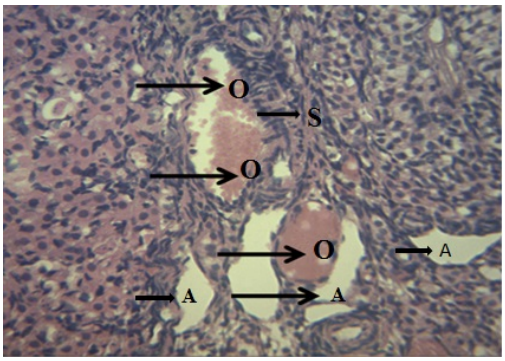

Figure 4: Photomicrograph of the ovary of group 2 (medium) showing the Oocytes (O) the antrum (A) ovarian stroma (O) no visible lesion seen [×400] [H&E].

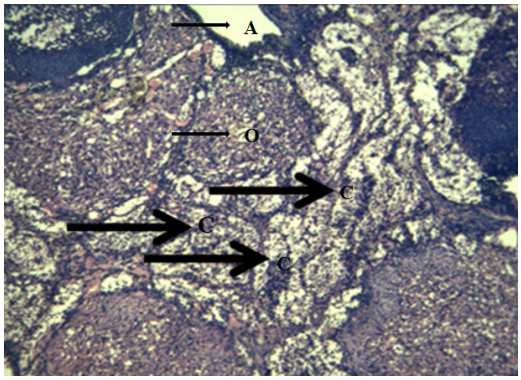

Figure 5: Photomicrograph of the ovary of group 4 (high dose) showing normal histology of the corpus luteum(C) the oocytes (O) and the antrum (A) with no visible lesion [x400] [H&E].

Discussion

General toxicity can be accessed through organ weight measurements, in which changes in the body weight and organ weight is a sensitive indicator of toxicity in their respective studies reported that toxicity indication can be seen in organ weights rather than absolute weight of rats [10-12]. The study reveals that following the oral administration of leaf extract of *Erytherina senegalensis* that there was a significant difference in the body weight of the albino rats at doses of 8000 mg/kg (Group iv) from the 4th week to 8th week (Table 3), this may show that the extract may not be toxic, although there was a decline in the fluid and food intakes from week 2 (Tables 1 and 2), The weights and relative organ weights of the ovaries of experimental rats showed no significant difference when compared with the control, which signifies that the leaf extract of *Erytherina senegalensis* may be nutritional and not toxic on the albino rats and the research agreed with that there were no significant changes in the relative weights of organs between the control and treated rats [13].

Hypogonadism is a clinical condition in which low level of serum sex hormones, including testosterone in males, as well as estradiol and progesterone in females. These signs and symptoms may include diminished libido and sense of vitality, erectile Dysfunction, dysmenorrhoea, reduced muscle mass and bone density, depression and anemia. By restoring these serum sex hormones level to the normal range using such agents as hormone supplement therapy, many of these symptoms can be relieved [14]. From the study, there was a dose dependent increase in the level of oestrogen across the groups (Table 7) this may suggest that the leaf extract may be used as hormone supplement therapy and the effect of the *Erytherina senegalensis* leaf extract increased oestrogen level and caused no distortion on the architecture of the ovary, though there is paucity of literatures to support the effect of leaf extract of *Erytherina senegalensis* on the hormone level, this can serve as a base line for further study.

High oestrogen levels are important for the luteinizing hormone surge that induces ovulation while a decline in oestrogen prevents ovulation [15-17]. Photomicrograph of the haematoxylin and eosin staining (H & E) of the ovary, revealed no visible lesion across the groups, which signifies that the presence of some biological molecules attempt to justify some of the ethno-medicinal applications of *E. senegalensis* [18-22].

In conclusion, the effect of *Erytherina Senegalensis* on the oestrogen level and histological architecture reveals the leaf extract didn't show any sign of toxicity on the hormone and organ of the study, which suggests that it may be used as a female hormonal supplement. Also with these result, it is possible that the functions of the ovary may not be hampered when the extract is consumed. However we recommended that further studies be carried out to authenticate these findings.

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