International Journal of Biochemistry, Bioinformatics and Biotechnology Studies
Vol.5, No.1, pp.1-12, June 2020
Published by ECRTD-UK
Print ISSN: 2397-7728, Online ISSN: 2397-7736

EFFECTS OF ETHANOL LEAF EXTRACT OF *EREMOMASTAX SPECIOSA* (AFRICAN BLOOD TONIC) ON FEMALE REPRODUCTIVE HORMONES OF ALBINO WISTAR RATS

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ABSTRACT: *Eremomastax speciosa* is known to exhibit a wide range of biological activities because of the presence of tannins, saponins, alkaloids, flavonoids and cardiac glycosides. This work evaluates the effects of *Eremomastax speciosa* on reproductive hormones in female rats using standard analytical methods. The phytochemical screening and the antioxidant activities of *Eremomastax speciosa* leaves were also evaluated using standard methods. Twenty-five matured virgin female rats were divided into five groups of five rats each; groups 1 and 2 were the normal and positive controls and were given distilled water and the standard drug respectively while groups 3, 4 and 5 were administered the ethanol extract of the plant in graded dosages of 400, 200, and 100mg/kg respectively. The phytochemical screening reviewed the presence of alkaloids, tannins, saponins, terpenes and flavonoids while, the leaves showed a significant free scavenging activities with IC_{50} 90µg/ml. The hormonal result showed that the extract caused a dose dependent significant increase ($p<0.05$) in estrogen, prolactin, progesterone and FSH when compared with the normal control. However, there was a significant decrease ($p<0.05$) in serum LH levels also in comparison with the normal control. In conclusion, the ethanol leaf extract of *Eremomastax speciosa* suggested an increase in the reproductive enhancing hormones which might increase the reproductive activities of the female rats.
KEYWORDS: *eremomastax speciosa,* ethanol extract, phytochemical, antioxidant, reproductive hormones.

INTRODUCTION

The world health organization (WHO) defines a medicinal plant as a plant in which some or all of its parts can be used directly in the management of a disease (Acharya *et al*., 2008). Medicinal plants also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesise hundreds of chemicals compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effect of using a whole plant as medicine is uncertain. Further, the photochemical content and pharmalogical actions, if any, of many plants having medicinal potential remain unaccessed by vigorous scientific research to define efficacy and safety (Ahn, 2017).

Eremomastax speciosa (Hochst.) Cufod commonly known as "edem iduodut" or "ndadad edem" by Ibibio people and "African blood tonic" in Cameroon belong to the family Acanthaceae (Iba *et al*., 2015). It is a perennial herb found in Africa along the rainforest zone and occurs as weed. It is cultivated in Cameroon and Akwa-Ibom in Nigeria due to its medicinal values (Oben *et al*., 2006). It is a polymorphous herb that grows up to 2 m high with remarkable quadrangular stem (Amang *et al*., 2014). The leaf (decoction or infusion or maceration) is used by the natives in the treatment of dysentery, anaemia, menstrual pain, fracture, haemorrhoids and urinary tract infection (Oben *et al*., 2006; Kuete *et al*., 2013 and Iba *et al*., 2015). The aerial part of the plant has been reported in scientific literature to possess anti-anemic, antiulcer, anti-secretory, antimicrobial, antifungal and anti- diarrheal activities (Tan *et al*., 1996; Oben *et al*., 2006; Okokon *et al*., 2007; Amang *et al*., 2014; Iba *et al*., 2015; Mouokeu *et al*., 2015).

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne 1973; Okwu, 2004).

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxides (Csepregi *et al.*, 2016).

Reproduction is the biological process by which new individual organism "offspring" are produced from their "parents". Reproduction is a fundamental feature of all known life; it is the process by which organisms create descendants. This miracle is a characteristic that all living things have in common and sets them apart from nonliving things (Borst *et al*., 2014).

Female reproductive system is a system that produces the female egg cells necessary for production, called the ova or oocytes. The system is designed to transport the ova to the site of fertilization. The female reproductive system is regulated by hormonal interaction between the hypothalamus, anterior pituitary gland, and ovaries (Sharp and Corp, 2018).

Hormones are special chemical messengers in the body that are created in the endocrine glands. These messengers control most major body functions, from simple basic needs like hunger to complex systems like reproduction, and even the emotions and mood. Understanding hormones and their functions will help patients take control of their health. Two hormones; follicle stimulating hormones and luteinizing hormones, are released from the brain and travel in the blood to the ovaries. The hormones stimulate the growth of about 15 to 20 eggs in the ovaries, each in its own shell called a follicle. These hormones also trigger an increase in the production of the female hormone; estrogen (Burger, 2012).

E. speciosa is cited for its various beneficial effects, which include stomach complaints, dysentery, hemorrhoids, urinary tract infection, painful menstruation, diarrhea, and male and female infertility (Adjanahoun et al., 1996; Oben et al., 2006; Okokon et al., 2007; Ndenecho, 2009; Focho et al., 2009; Dibong et al., 2011; Telefo et al., 2012; Erhabor, 2013) and is commonly referred to as "blood plant" since it is also widely used to treat cases of anemia. In addition to this investigation, little or no information on the reproductive activity is available. The present study was designed to study the female reproductive activity of ethanol extract of *E. speciosa* in female rat.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Eremomastax speciosa was acquired from Apkajo community in Eleme Local Government Area of Rivers State, Nigeria in May, 2018. The plant was identified and authenticated by Dr. Mrs Uduak Eshiet of the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Nigeria. It was given the Voucher Number UUPH 1 (b) and was deposited at the Department of Pharmacognosy and natural medicine Hebarium, Faculty of Pharmacy, University of Uyo, Nigeria.

Preparation and Extraction of Plant Material

The wet method of extraction was used for the extraction. The leaves were plucked from the plant stalk, thereafter the leaves were washed and drained to remove the debris, 500g weight of the leaves were cut into pieces and immersed in 2.5L of 70% ethanol and kept in an amber coloured bottle for 72hours. At the end of the three days the mixture was filtered using a cheese cloth and then with Whatman No.1 filter paper. The filtrate was then put in a beaker and kept in a water bath at 30-40^{O}C, it yielded 25g of extract and stored in a refrigerator at 4^{O}C until needed for analysis.

Antioxidant Activity of the Extract

The free radical scavenging activity of *Eremomastax speciosa* was determined using the modified method of Blois, 1985. The methanol extract, the chloroform and ethyl acetate fractions were evaluated for their free radical scavenging activity with 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay. Ascorbic acid was used as the positive control. 1ml of different concentration (500, 250, 125, 62.50, 31.25µg/ml), of extracts and standard ascorbic acid were added to 1ml of 0.3mm DPPH in methanol to bring the final concentrations to (250, 125, 62.50, 31.25 and 15.625µg/ml). The mixture was shaken and left to stand at a room temperature in the dark for 30 minutes and the absorbance read at 517nm against a blank (a DPPH control which contained 1ml of methanol). Assays were carried out in triplicate. The antioxidant activity of extract was expressed as IC_{50}, which is defined as the concentration (µg/ml) of extract which inhibits the formation of DPPH radicals by 50%. The IC_{50} values were obtained from graph of I% (inhibition percentage), versus

concentration of the sample in µg/ml. The percentage inhibition of DPPH (%) was calculated using the equation:

$I\% = [A_{blank} - A_{sample} / A_{blank}] \times 100$

Where:

A_{blank} = is the absorbance of blank solution [control]

A_{sample} = is the absorbance of the test sample.

Phytochemcial Screening

The qualitative phytochemical screening was carried out on the methanol extract of *Eremomastax speciosa* leaves according to standard methods to identify the classes of bioactive compounds present (Sofowora 1993, Evans, 2009).

Experimental Animals

A total of twenty five adult female Albino Wistar rats weighing 150-200g were used. They were obtained from the animal house of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Port-Harcourt, Nigeria and brought to the Department of Biochemistry, Faculty of Basic Medical Sciences, and University of Uyo, Nigeria where the study was done. The animals were randomly divided into five (5) groups of five (5) rats each. They were housed in a ventilated room in wooden cages with wire mesh top and maintained under standard conditions of humidity (50±5%) and temperature (28±2^{O}C) and 12hours light/12hours dark cycle and acclimatized for 14 days. The animals were fed with growers pellet feed (Grand, Bendel feeds, Edo state) and water *ad-libitum* throughout the experimental period of fourteen days. The experiment was done in accordance with the ethical approval by The Ethical Committee on Animal Usage of the College of Health Sciences, University of Uyo, Nigeria.

Experimental Design

The animals were divided into five (5) groups of five (5) rats per group and labelled (1-5). The animals in group 1 serve as the Normal Control (NC) and were administered distilled water only. The animals in group 2 served as the Positive Control (PC) and were administered 17-β-estradiol subcutaneously at 400mg/kg twice daily for 14 days. Group 3, 4 and 5 were administered three graded dosage of the leaf extract of *Eremomastax speciosa* as follows 400, 200, 100mg/kg respectively, this was done for 14 days via oral route as shown on Table 3.

Table 1: Experimental Design

Groups	**No. of Animals**	**Treatment**	**Dosage**
I. (Normal Control)	5	Distilled water	5ml
II. (Positive Control)	5	Estradiol (E2)	400mg/kg
III. (High Dose)	5	*Extract of Eremomastax speciosa*	400mg/Kg
IV. (Medium Dose)	5	Extract of *Eremomastax speciosa*	200mg/Kg
V. (Low Dose)	5	Extract of *Eremomastax speciosa*	100mg/Kg

BIOCHEMICAL HORMONAL ASSAY:

Accu-Bind Elisa Microwells was the kits used for the hormonal assay and were obtained from Monobind Inc. Lake Forest, CA 92630, USA. The method of Abraham, (1981) was used to determine quantitatively the hormones; Progesterone, Follicle-stimulating Hormone [FSH], Luteinizing Hormone [LH], Prolactin and Estrogen concentrations by a microplate Enzyme Immunoassay, colorimetrically.

RESULTS

Phytochemical Screening

The phytochemical screening of ethanoic extract of *Eremomastax speciosa* plant as shown in Table 4.1, revealed the presence of the following secondary metabolites: Alkaloids, Tannins, Saponins, Terpenes, Flavonoids, Combined Anthroquinone. However, Cardiac Glycosides (Keller-Kiliani), Phlobatannins, and free anthroquinone were absent.

Table 2: Results of Phytochemical Screening of *Eremomastax speciosa* Leaf Extract

Tests	Observation	Inference
Alkaloids	Cream precipitate	++
Keller-Kiliani	No brown ring	-
Flavonoids	Yellow colouration	++
Saponins	Persistent foaming	+
Tannins	Dark green colour	++
Terpenes	Reddish-brown colour	+
Combined Anthraquinones	Pink colouration	+
Free Anthraquinones	No Pink colouration	-
Phlobatannins	No deposition of red precipitate	-

Key: - Absent, + Trace, ++ Positive.

Antioxidant Activity of Ethanol Leaf Extract of *Eremomastax speciosa*

The free radical scavenging activities of extract are as shown on Table 3. The result showed that the crude ethanol extract had a significant free radical scavenging activity, with IC_{50} of 90µg/ml, while the IC_{50} of ascorbic acid was 110µg/ml.

Table 3: Results of Antioxidant Screening of Extract

Group	Conc. (µg/ml)	Absorbance (517 nm)	% Inhibition	IC_{50}
	Control	1.167±0.003	0	
	15.625	1.128±0.002	23.34	
Ethanol	31.25	0.995±0.004	34.70	
Extract	62.50	0.676±0.003	42.10	90µg/ml
	125.00	0.317±0.002	72.80	
	250.00	0.139±0.001	88.10	
	Control	1.368±0.001	0	
	15.625	0.468±0.001	6.20	
Ascorbic	31.25	0.445±0.001	10.80	110µg/ml
Acid	62.50	0.353±0.001	29.30	
	125.00	0.208±0.002	58.30	
	250.00	0.092±0.001	81.60	

Effects of extract on sex hormones in females

The effects of extract on female sex hormones are as shown in Figs; 1, 2, 3, 4 and 5. The leaf extract caused a significant increases ($p<0.05$) in estrogen, prolatin, progesterone and FSH ($p< 0.05$) and a significant decrease ($p<0.05$) in serum LH levels.

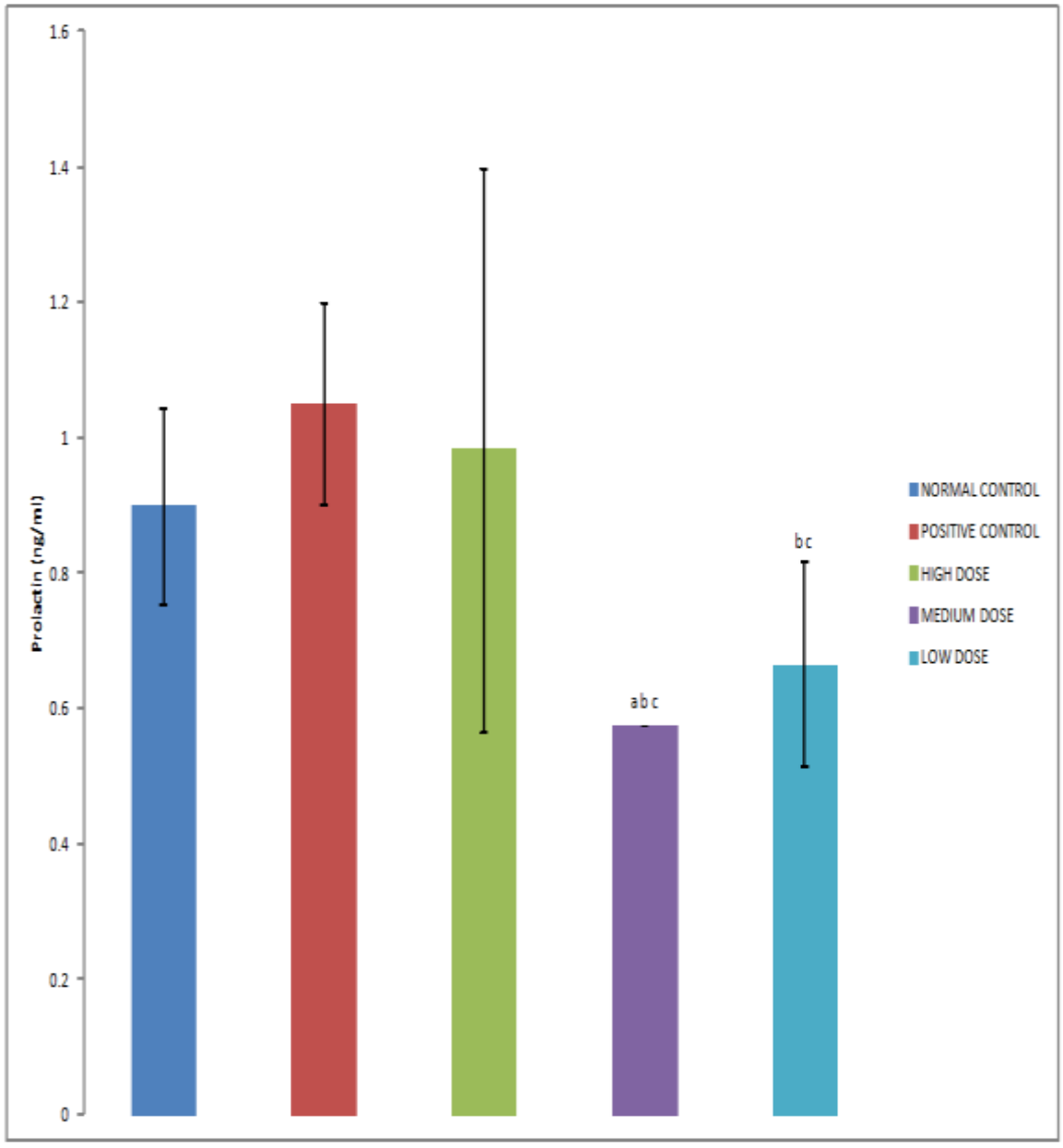

Fig. 1: Effects of *Eremomastax speciosa* Leaf extract on prolactin

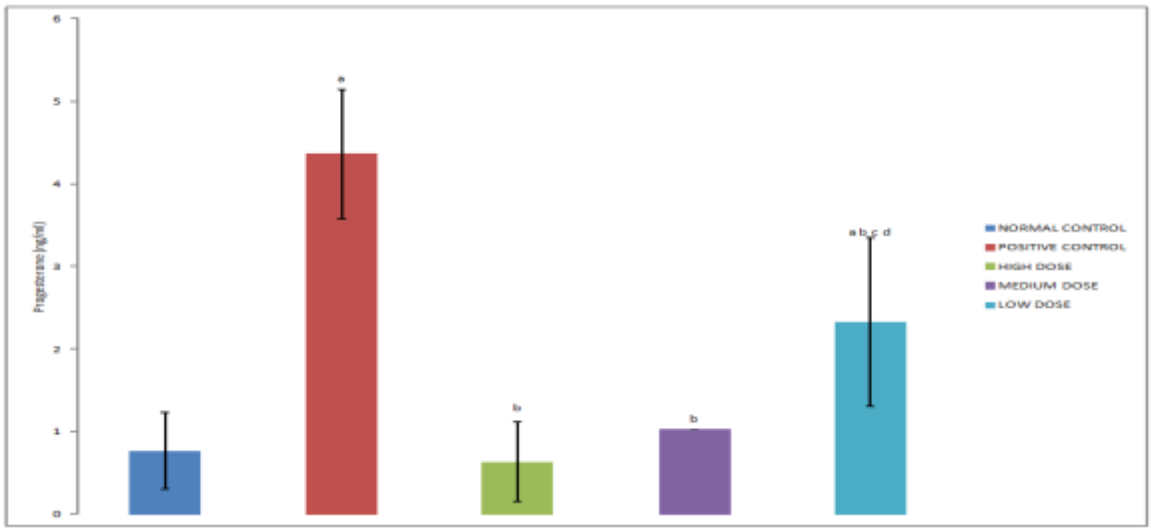

Fig. 2: Efects of *Eremomastax speciosa* Leaf extract on progesterone

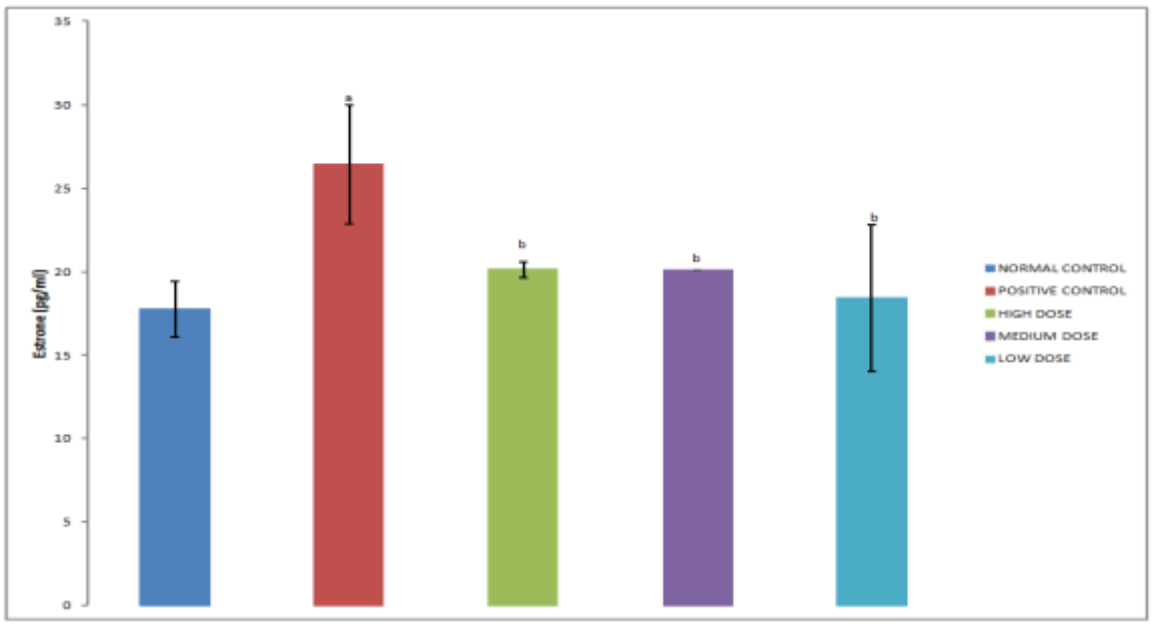

Fig. 3: Effects of *Eremomastax speciosa* Leaf extract on Estrogen

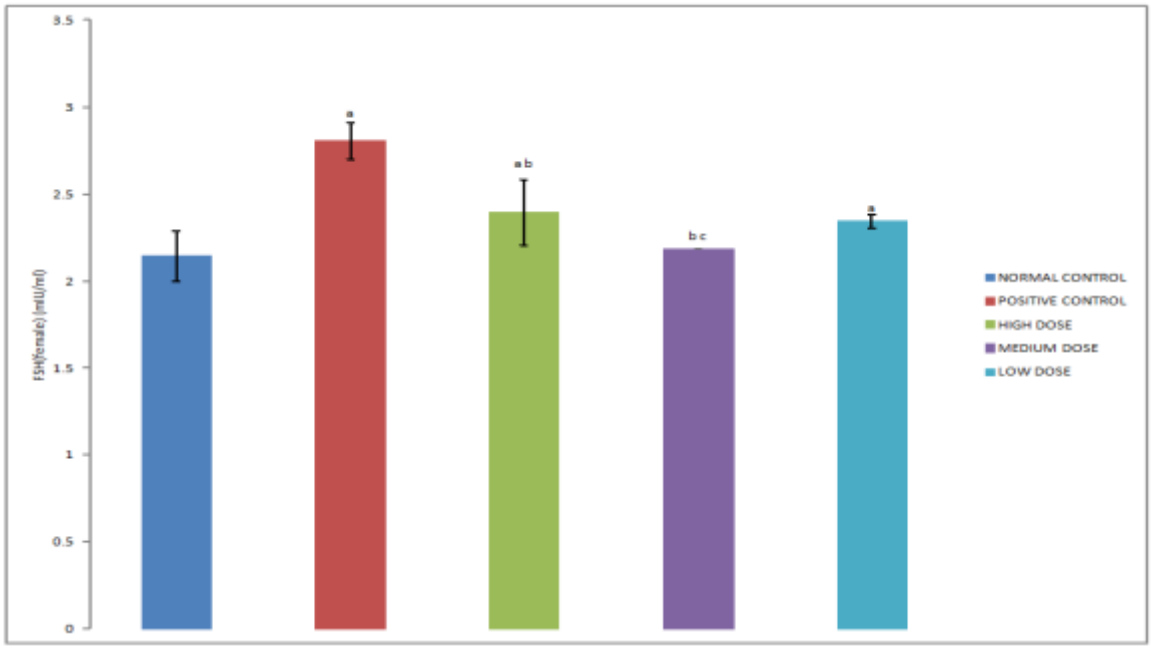

Fig. 4: Effects of *Eremomastax speciosa* Leaf extract on FSH

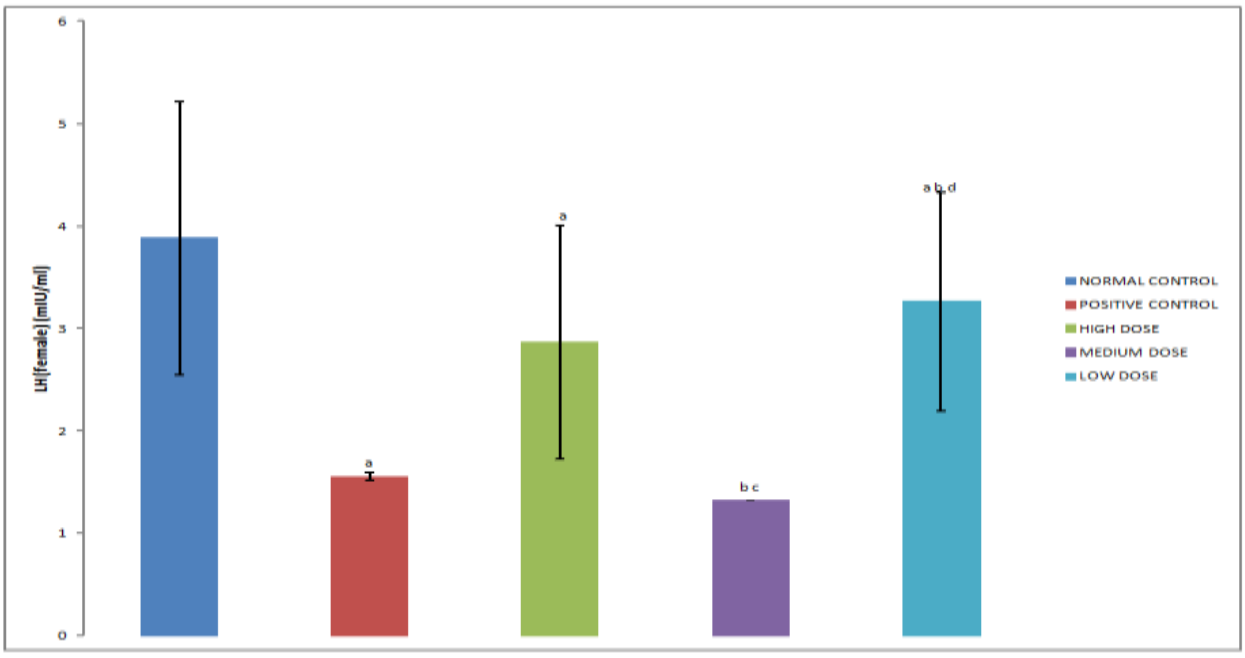

Fig. 5: Effects of *Eremomastax speciosa* Leaf extract on LH

DISCUSSION

The phytochemical screening of the extract, revealed the presence of tannins, saponins, alkaloids, flavonoids and cardiac glycosides. These phytochemical compounds are known to play important roles in bioactivity of medicinal plants and their medicinal effects lie in these phytochemical compounds. Flavonoids, alkaloids and tannins which were the predominant compounds in *Eremomastax speciosa* are known to exhibit a wide range of biological activities. This result is in tandem with the report of (Trease and Evans 2002) and (Mboso *et al.,* 2013).

The results of the antioxidant activities of the ethanol extract of *Eremomastax speciosa* leaf via the DPPH free scavenging activity showed that the crude ethanol extract possessed significant free scavenging activity, with an IC_{50} value of 90 µg/ml. Comparing the obtained IC_{50} value of ascorbic acid (110 µg/ml) with the IC_{50} of ethanol extract of *Eremomastax speciosa*, it indicates that the crude extract exhibited a higher antioxidant activities than the standard Vitamin C (ascorbic acid); this agrees with the study conducted by (Neto, 2007).

Female reproductive hormones were assayed biochemically; the results of the effect of ethanol leaf extract of *Eremomastax speciosa* on the serum levels of the reproductive hormones were dose dependently significant. However, the changes in progesterone level were not significant relative to control except the low dose which was higher than the normal control but not as much as the positive control.

Prolactin: The leaf extract of *Eremomastax spiciosa* showed a significant increase in high and low dose of the extract groups when compared to the normal control and a significant decrease when compared to the positive control. This is in contrast to the studies conducted by (Mboso *et al*., 2013).

Progesterone: the level of progesterone was reduced in medium dose but there were significant increase in high and low dose when compared to normal control and a significant decrease when compared to positive control. This is in contrast to the previous studies conducted by (Adebayo, 2015). The reason being that the phytochemicals present in *Eremomastax speciosa* may have been in higher concentration than the ones present in the decoction, probably due to the season the leaves were harvested.

Estrogen: the leaf extract caused a dose dependent increase in estrogen when compared to the normal control but a significant decrease when compared to positive control. This is in tandem to the report of (Daramola *et al*., 2016) and in contrast to the research conducted by (Adebayo, 2015), which could be due to increased concentration presence of phytochemicals at that season.

FSH: the leaf extract caused a significant increase in the high and low dose when compared to the normal control and a significant decrease when compared to the positive control. This is similar to the report of (Daramola *et al*., 2016) and in contrast to (Adebayo, 2015). The result showed that the extract may have the potential to cause hormonal balance.

Luteinizing hormone: the leaf extract caused a significant increase in the high and low dose relative to the positive control and a significant decrease when compared to normal control. This is in tandem to the research conducted by (Damarola *et al*., 2016) but contrast to previous studies conducted by (Adebayo, 2015). Rise in luteinizing hormone triggers ovulation and development of corpus luteum, this implies that the extract could be effective when administered in high and low dose.

However the increase in various extract groups of each hormone may be as a result of the presence of alkaloids, flavonoids, terpenes and tannins as these phytochemical compounds are known to play important roles in bioactivity. FSH is required for the progressive growth of the ovarian follicles at various stages from the primary to the mature graffian follicular, this findings suggests the nourishing effect of the plant extract on the endocrine system by maintaining the FSH level thus, allowing the proper functioning of the endocrine system in the pubertal rats. Flavonoids are plants secondary metabolites that are widespread throughout the plant kingdom, flavonoid play important role in reproduction and fertility as a result of the inhibition of flavonoids production in plants through antisense suppression of the gene encoding chalcone synthase (Mboso *et al*., 2013). Terpenes play an important role in cellular membrane fluidity, as a result of the triterpenes which serve as a precursor molecule for the cholesterol. Cholesterol is a precursor for steroid hormones like progesterone and estrogen. Estogen and progesterone play a pivotal role in the functions of the female reproductive cycle, like ovulation, implantation and maintenance of pregnancy (Mboso *et al*., 2013). This therefore suggests that the plant extract investigated in this study could serve as a potential drug source for the management of female fertility.

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