Asian Journal of Research in Biochemistry

3(3): 1-7, 2018; Article no.AJRB.46154

Effects of Methanolic Root Extract of *Holarrhena floribunda* on the Lipid Profile and Sex Hormones in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2018/v3i329829
Editor(s):
(1) Dr. Asmaa Fathi Moustafa Hamouda, Assistant Professor, Faculty of Applied Health Sciences, Jazan University, Saudi Arabia.
Reviewers:
(1) S. Murugesan, University of Madras, India.
(2) Javier Rodríguez Villanueva, University of Alcalá, Alcalá de Henares, Spain.
(3) Hassan Daniel Mhya, Abubakar Tafawa Balewa University Bauchi Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/28087

Original Research Article

Received 05 October 2018
Accepted 22 December 2018
Published 03 January 2019

ABSTRACT

Aim: This study aims to determine the effects of methanolic root extract of *Holarrehna floribunda* on the level of some serum sex hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estradiol, progesterone and lipid profile in Wistar rats.
Materials and Methods: Twenty- four male and female Wistar rats (150-250 g body weight) were randomly assigned into 4 groups of 6 rats each. Group 1 (control male) took normal rat chow and drinking water. Group 2 (control female) took normal rat chow and drinking water, Group 3(Male test group), was administered with 200 mg/kg of *Holarrehna floribunda* extract, Group 4(Female test group), was administered with 200 mg/kg of *Holarrehna floribunda*. The feeding regimens lasted for 5weeks.
Results: The values for the lipid profile shows CHOL in Group 1 and Group 3 were 4.02±0.41 mmol/l and 5.75±0.09 mmol/l, Group 2 and Group 4 were 3.87±0.22 mmol/l and 5.80±0.10 mmol/l respectively, TRIG for Group 1 and Group 3 were 1.30±0.05 mmol/l and 2.11±0.15 mmol/l for

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Groups 2 and 4 were 1.15±0.03 mmol/l and (2.10±0.12 mmol/l,HDL-C for Groups 1 and 3 were 30.01±0.82 mg/dL and 39.88± 1.24mg/dL,for Groups 2 and 4 were 29.98± 0.77 mg/dL and 38.20± 1.83 mg/dL, LDL-C for Groups 1 and 3 were 2.26±0.37 mmol/L and 4.17±0.35 mmol/L respectively, for Groups 2 and 4 were 3.27±0.47 mmol/L and 4.06±0.21 mmol/L respectively, shows significant increase($p<0.05$) in HDL-C (mg/dL) in Groups 1,2,3 and 4.The values obtained showed significant increase ($p<0.05$) in HDL-C (mg/dL) in Groups 1,2,3and 4The serum concentrations of FSH, Estradiol, LH and progesterone in the control Group 2 were 0.38 ±0.07mIU/mL, 7.83±0.35 mIU/mL, 15.50±0.15 Pg/mL and 0.86±0.03 ng/mL respectively, and in test Group 4 were 0.77±0.04mIU/mL, 8.75±0.17 mIU/mL, 21.09±0.79 Pg/Ml 0.33±0.05 ng/mL. Follicle stimulating hormone levels was significantly higher ($p<0.05$) in test group compared with control. While in Group 1 and 3, the serum concentrations of FSH, LH, and testosterone in control Group 1 were (0.51 ±0.06) mIU/mL, (16.44±0.31) mIU/mL and (8.41±0.50) ng/mL respectively and in test Group 3 were 0.88±0.06 mIU/mL19.88±1.46mIU/mL and10.68±0.64 ng/mL, respectively.

Conclusion: The extract improves the level of sex hormones in both the male and female rats. which could enhance reproductive functions in normal rats and those with loss of reproductive function.

Keywords: Sex hormones; follicle stimulating hormone (FSH); luteinizing hormone (LH); testosterone; Estradiol and progesterone; lipid.

1. INTRODUCTION

In developing countries, 80% of the population continue to use medicinal plants and plant products in handling primary medical problems due to their accessibility, availability and affordability. In these countries, a variety of plants are acclaimed to have fertility regulating properties and a few have been tested for such effect [1,2,3]. *Holarrhena floribunda* belongs to the Family Apocynaceae, the common name is Conessi, Holarrhena. The Hausas call it *Bakin mutum*, Yorubas call it *Irene* and Fulani call it *Niwahin*, Efiks and Ibibios call it *Okpo ikot* or *Mba enin*. *Holarrhena floribunda* grows as a shrub or tree up to 25 metres (82 ft) tall, with a stem diameter of up to 30 centimetres (12 in). Its fragrant flowers feature a white corolla. Fruit is pale grey to dark brown with paired follicles, each up to 60 centimetres (24 in) long. Vernacular names for the plant include "false rubber tree" and "kurchi bark". *Holarrhena floribunda* is found in a variety of habitats from sea-level to 1,000 metres (3,300 ft) altitude. The plant's numerous local medicinal uses include as a treatment for dysentry, diarrhea, fever, snakebite, infertility, venereal disease, diabetes and malaria. *Holarrhena floribunda* has been used as arrow poison. The plant is found in Senegal the Gambia, Guinea Bissau, Guinea, Mali, Burkina Faso, SierraLeone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Chad, Cameroon,Central Africa Republic, Gabon,the Republic of Congo and the Democratic Republic of Congo. *Holarrhena floribunda* is a shrub of 15 m high and 1 m in girth. The bark is gray, smooth and pale brown and if slashed yield copious white latex. The roots are used to relieve pains, malaria and treat female infertility. The bark is dispensed as a febrifuge, as a tonic, as remedy for snake bite and for the treatment of venereal diseases, dysentery and fever. In Nigeria, Burkina Fasso and Cameroon the bark is used to treat abdominal pains, nausea, indigestion and diarrhea. It also shows inhibitory activity against drug resistant strains of *Plasmodium falciparum* and contains conessine that is used for the destruction of amoeba without emetic effect [4]. In developing countries (including Nigeria) most people consume herbal medications without adequate knowledge of the pharmacologic actions and side effects of such medications, It is therefore important to evaluate the effect of *Holarrhena floribunda* on reproductive hormones in order to advice patients especially infertile couples of herbal medications. Fertility and reproductive health are regulated and maintained by the levels of serum sex hormones. With paucity in literature on the effect of *Holarrhena floribunda* on serum sex hormones, it is therefore the aim of the present study to investigate scientifically the effects of administration of the methanol extract of *Holarrhena floribunda* root on some reproductive hormones and lipid profile .Although there is no scientific evidence to support the ethnopharmacological property of *Holarrhena floribunda* on female reproduction, many tribes continue to use it in the management of cases of sterility/infertility in women. The present work will therefore be undertaken with an aim to scientifically validate this claim.

Picture of *Holarrhena floribunda*

2. MATERIALS AND METHODS

2.1 Materials

Chemicals and reagents: Several chemicals and reagents of analytical grade were purchased and used for this research which include: Methanol (98%), which was purchased from Globus Chemical, Mayne Avenue, Calabar, Cross River State, Nigeria. Kits for high density lipoprotein, total cholesterol, triacylglycerol were obtained form Dialab Production and laborinstriementen Gessellschaft M.b.H. A – 1160 Wien-panikengasse, Austria,Needles and other syringes used were obtained from Karmel Pharmacy, Goldie Street, Calabar while plain tubes were obtained form Meditec Laboratory, 91 White House, Calabar.

2.2 Methods

Identification and preparation of plant materials: Fresh roots of *Holarrhena floribunda* were collected from local garden at the University of Uyo, Uyo, Akwa Ibom State, Nigeria. The sample of the plant specimen was identified and authenticated by a Botanist from the botanical garden, and the voucher specimen with identification number (PES/Herb/uc/129) was deposited in the Herbarium of the University of Calabar. The roots were sorted to eliminate any dead matter and other unwanted particlesThe roots of *Holarrhena floribunda* were washed with clean water, cut into pieces and dried under shade at a temperature of 25±0.5^{0}C. Mortar and pestle were used to pulverize the root until it formed a coarse powder. The methanol extracts were obtained by soxhlet extraction of the powder. The extracts were concentrated to dryness in vacuo at 40ºC. The dried extracts were weighed, stored in specimen bottles and kept in the refrigerator at -4ºC until used. Appropriate concentration of 200mg/kg of the extract was subsequently made by dilution with distilled water and administered to the animals by oral gavage.

Acute toxicity testing: The median Lethal dose (LD_{50}) of the plant was carried out using eighteen (18) albino mice, According to Lorke [5]. This test is normally done to certain the dose of the plant extract that is safe or toxic to the mice. The LD50 of the methanol extract was 367.42mg/kg.

Handling and treatment of animals: Twenty-four (24) adult male and female albino rats weighing between 150-250g obtained from the disease free stock of the animal house, Biochemistry Department, College of Medical Sciences University of Calabar, Calabar Nigeria were used for the study. The rats were divided into four (4) groups of six (6) rats each as shown in Table 1. The rats were acclimatized in the experimental Animal House for one week before the commencement of the experiment. The animals were housed in stainless steel cages under standard conditions (ambient temperature, 28.0±2.0ºC and humidity, 46%, with a 12 hr light/dark cycle), fed with the normal rat pellets. All the rats in both test and control groups are allowed free access to feed and water *ad libitum*, throughout the experimental period. Good hygiene maintained by constant cleaning and removal of faeces and spilled feed from cages daily.The extract was administered for 40 days.

Collection of blood and tissues for analyses: All the animals were anaesthetized in chloroform vapour, twenty-four 24 hrs after the last day of extract administration, and dissected for blood collection. Blood was collected by cardiac puncture into a set of plain sample bottles, allowed to clot for 2 hrs after which serum was obtained by centrifugation at 3000 rpm. Serum was used for biochemical estimations.

Biochemical estimations: Estimations of lipid profile was done using assay kit, While estimations of serum progesterone and estradiol was done by the method of Lilaram and Nazeer [6] using kit while that of FSH, LH and testesterone were carried out using the method reported by Uotila et al. [7].

Table 1. Animal grouping

Groups	Number of animals	Treatment
1. (Normal Control Male)	6	Distilled Water
2. (Normal Control Female)	6	Distilled Water
3. (Male 200mg/kg)	6	Root extract of HF
4. (Female 200mg/kg)	6	Root extract of HF

Group 1 (normal control male group received distilled water as placebo),
Group 2 (normal control female group received distilled water as placebo),
Group 3 (test group male received oral dose of Holarrhena floribunda).
Group 4(test group female received oral dose of Holarrhena floribunda root extract).

2.3 Statistical Analysis

Results obtained from this study was analyzed by one-way analysis of variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups using SPSS windows. A significant change was considered acceptable at $P < 0.05$.

Table 2. Results of body weight changes of the animals

Groups	body weight changes (g)
1. Normal male control	53.00±03.00
2.Normal female control	43.00±15.57
3.Male(200 mg/kg)	30.00±11.14
4.Female(200 mg/kg)	69.00±01.10

Values are presented as mean ± SEM. n= 6; Result showing body weight variation of the animals

Table 3. Results of the effect of administration of *Holarrhena floribunda*root extract on lipid parameters in the respective groups

	CHOL. (mmol/l)	TRIG. (mmol/l)	HDL-C (mg/dl)	LDL-C (mmol/l)
Group 1 MC	4.02±0.41	1.30±0.05	30.01±0.82	2.26±0.37
Group 2 FC	3.87±0.22	1.15±0.03	29.98±0.77	3.27±0.47
Group 3 MT	5.75±0.09*[a]	2.11±0.15*[a]	39.88±1.24*[a]	4.17±0.35*[a]
Group 4 FT	5.80±0.10*[b]	2.10±0.12*[b]	38.20±1.83*[b]	4.06±0.21

*Values expressed as Mean ± SEM, *significant at p<.05. [a] significant at p<.05compared with group 1 (Male control). [b] significant at p<.05 compared with Group 2 (Female control). MC =male control group; FC =female control group; MT =male test group; FT =female test group.*

Table 4. Results of the effect of administration of *Holarrhena floribunda*root extract on hormonal indices in the respective groups

	Estradiol(E2) Pg/Ml	FSH(mIU/ml)	LH(mIU/ml)	TEST. ng/mL	PROG. ng/mL
Group 1 MC	-	0.51±0.06	16.44±0.31	8.14±0.50	-
Group 2 FC	7.83±0.35	0.38±0.07	15.50±0.15	-	0.18±0.03
Group 3 MT	-	0.88±0.06*[a]	19.88±1.46*[a]	10.68±0.64*[a]	-
Group 4 FT	8.75±0.17*[b]	0.77±0.04*[b]	21.09±0.79*[b]	-	0.33±0.05*[b]

*Values expressed as Mean ± SEM, *significant at p<.05. [a] significant at p<.05compared with group 1 (Male control). [b] significant at p<.05 compared with Group 2 (Female control). MC =male control group; FC =female control group; MT =male test group; FT =female test group.*

3. RESULTS AND DISCUSSION

The aim of the present study was evaluating the effects methanolic root extract of *Holarrhena floribunda* on the lipid profile and sex hormones which included ;follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estradiol and progesterone, fertility of rats. *Holarrhena floribunda*, a medicinal plant with ethnopharmacological properties used in the treatment of some cases of sterility/infertility in women.In the present study , The results of the lipid profile, there was increase in the levels of HDL-C in group 4. Estrogens are thought to increase HDL cholesterol by reducing hepatic triglycerides' lipase activity that catabolizes HDL cholesterol% [8]. Many studies of either gender have demonstrated that the risk of atherosclerosis is inversely related to blood levels of HDL cholesterol: the higher the level of HDL cholesterol, the lower will be the risk. It is indicated that for every 1 mg/dL rise in HDL cholesterol, the risk for developing cardiovascular disease decreases by 2–3 % [8].

From the results in Table 4, estradiol increased in group4 compared to the control group2, any disturbance in the equilibrium level of these hormones may lead to loss of implantation and may cause infertility [9,10]. It has also been reported that conceptus synthesis and release of estrogens is essential for establishment of pregnancy and, endometrial exposure to estrogen prior to the normal conceptus secretion results in total pregnancy loss [11,12,13]. From this present work, it can be seen that *in vivo* exposure of the uterus to *Holarhena floribunda* extracts for 40 days may have contributed to the set up of some modifications in the uterine endometrium that transforms it from a non receptive to a receptive phase allowing for the implantation and development of the blastocyst. Indeed, many studies have suggested that phytosterols may have effects on the reproductive system and in particular that they possess estrogenic activity [14,15]. With this view, which implies a potentiating effect of *H. floribunda* on endogenous estrogen's activity, agrees with some reported data in the literature, [1], and further supports the suspected hypothesis of pro-implantation and pro-development properties proposed above. Further studies on the rat isolated estrogenized and non estrogenized uterus will help to complete and better ascertain these potentiating effects of *Holarrhena floribunda*.

From this work, administration of methanolic extract of (H.F) promotes fertility by reducing post implantation loss and by increasing litter size in female Wistar rat strain. The result of FSH obtained increased in the various 1 and 3 respectively. Follicle-stimulating hormone is the central hormone used in mammalian reproduction, essential forgonadal development and maturation at puberty as well as gamete production during the fertile phase of life [16]. It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells. The values of testesterone obtained shows significant increase in the test group3 compared to the control group1.Testosterone, being an important androgen plays several roles in various aspects of sexual maturation, behavior, spermatogenesis, differentiation, and maintenance of accessory sex organs [17]. The synthesis and release of androgens is dependent on the pituitary gonadotrophins, which are FSH and LH. Both FSH and LH are essential for testicular function and spermatogenesis [17]. The LH also increased between the test group and control groups. The LH is the main tropic regulator of Leydig cell function without which androgen production is not possible, [18]. Follicule stimulating hormone stimulates maturation of the Graafian follicle while leutinizing hormone causes it to synthesize testosterone which is then converted to estrogen by aromatase [19]. Progesterone also increased in group4 compared to the control group 2.Progesterone which is produced in the ovaries,placenta, and adrenal glands, helps to regulate themonthly menstrual cycle, prepares the body for conception and pregnancy [20], as well as stimulate sexual desire [17]. The hormone also encourages the growth of milk-producing glands in the breast during pregnancy. High progesterone levels arebelieved to be partly responsible for symptoms of premenstrual syndrome (PMS), such as breast tenderness, feelings of bloat and mood swings. The feedback inhibition of GnRH secretion by estrogens and progesterone provides the basis for the most widely-used form of contraception. Such feedback inhibition of GnRH prevents the mid cycle surge of LH and ovulation [17]. Estradiol stimulates the growth of the uterine. The results of this investigation suggest that root extract of *H. floribunda* contained potent agents with abilities to increase serum FSH, LH, testosterone and progesterone concentration. The increase in FSH, LH, testosterone and progesterone levels would have been due to some potent agents in

the extract that stimulate the synthesis and subsequent release of these hormones in the anterior pituitary gland/ovary or probably promote cholesterol catabolism. Follicle stimulating and luteinizing hormones play essential roles in the control of mammalian reproductive function. In female mammals, FSH stimulates ovarian follicle growth and maturation, as well as E_2 synthesis by granulosa cells, whereas LH stimulates androgen production by theca cells and ovulation of the dominant follicle(s) [21]. Women with loss-of function due to mutations in the FSHB or FSH receptor (FSHR) genes present clinically with primary or secondary amenorrhea and associated arrest in follicle development at the pre-antral stage [22,21]. In this condition, moderate doses of *Holarrhena floribunda* extract will be of immense benefit. In males, LH stimulates androgen production by interstitial Leydig cells, although FSH targets Sertoli cells in the testes to regulate spermatogenesis.

4. CONCLUSION

From the results obtained in this research, it showed that the extract improved the level of sex hormones in both the male and female rats.

ETHICAL APPROVAL

Authors received ethical approval according to international /university standard.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/28087