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(RESEARCH ARTICLE)



## Evaluating the effect of ethanol leaf extract of *Gongronema latifolium* on some reproductive hormones of male Wistar rats

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### Abstract

This research was aimed at evaluating the effect of ethanol leaf extract of *Gongronema latifolium* on some reproductive hormones of male Wistar rats. Eighteen (18) male Wistar rats weighing 120-200 g were divided into three (3) groups of six (6) animals each. The animals were allowed to undergo acclimatisation period for seven days before the start of the research. Animals in group A served as the control, while group B and C were administered 100 and 200 mg/kg body weight of ethanol extract of *G. latifolium*, respectively. The administration was performed for 14 days. Twelve hours after the last administration, the rats were sacrificed and cardiac puncture procedure was used to collect the blood for some reproductive hormonal analysis. The extract produced a ( $P < 0.05$ ) significant increase in serum testosterone, follicle stimulating hormone, and luteinizing hormone respectively at 100mg/kg and 200mg/kg body weight compared to normal control and progesterone produced a significant ( $P < 0.05$ ) decrease in all experimental groups compared with normal control. The extract was found to contain alkaloids, saponins, flavonoids, steroids, triterpenoids, tannins, and glycosides at varying concentrations. The rich phytochemicals in the extract of *Gongronema latifolium* strongly contribute in enhancing sexual health and libido in males. It is possible to use the plant extract in the management of erectile related dysfunction.

**Keywords:** Erectile dysfunction; FSH; Libido; LH; Testosterone

### 1. Introduction

Sex hormones are specific regulatory molecules which modulate reproduction, growth and development, as well as the maintenance of internal environments and the production, use and storage of energy [1]. They are regulators of reproductive functions. Sex hormones, like periodontal tissue, have important effects on the nervous and cardiovascular systems and are significant determinants of the growth and integrity of the skeleton and oral cavity [2, 3]. The hypothalamic-pituitary - gonadal axis is known as the male reproductive hormone axis. It consists of three major components: the hypothalamic, pituitary and testicular glands [4, 5]. This axis works regularly to provide the right concentration of hormones for male sexual development and functions. Any abnormality in the system can lead to infertility or sexual dysfunction [6, 7]. If the brain is unable to produce gonadotropic releasing hormone (GnRH), this disorder results in a lack of testosterone and thereby inhibits spermatogenesis [8, 9]. The lack of GnRH causes a group of disorders known as hypogonadotropic hypogonadism (hypogonadotropism) [10, 11]. Gonadotropin-releasing hormone deficiency treatment choices include the use of sex hormones, gonadotropins and gonadotropin-releasing hormone injections. Testosterone injections are mainly used to improve testicular growth, normalise testosterone concentration, and stimulate the development of secondary sexual traits [12]. Similarly, pituitary's inability to produce sufficient amounts of luteinizing hormone and follicular stimulating hormone results in a failure to stimulate the synthesis of testosterone and spermatozoa or sexual dysfunction [13, 14]

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Erectile dysfunction is becoming a major calamity in Africa. It is slowly eating deep into the life of individuals' especially in married homes and is causing problems that have led to broken homes, separation and divorce not only in Africa but across the globe. The global estimated prevalence has been on the rise. It has been projected that the number of men with this condition will rise to 322 million by 2025 and 35%-47% in Nigeria [15].

While the use of allopathic pharmaceutical products has demonstrated a substantial improvement in the treatment of sexual disorders, a large number of side effects occur at the same time, including heart rhythm disturbances, suicidal impulses, psychiatric disorders and tremors [16]

As any agent capable of stimulating the sexual urge, inducing venereal desire and increasing pleasure and results, an aphrodisiac is defined [17]. Typically, aphrodisiacs are divided into two groups: preparations for psycho-physiological stimulation (olfactory, visual, tactile and aural) and internal agents, primarily derived from plants (food, alcohol and love potion) [17]. Side effects are correlated with various synthetic substances widely used to enhance sexual desire and efficiency, such as dopamine, amyl nitrite, and sildenafil citrate [18]. In developing countries, the search for new compounds from medicinal plants is being intensified, due to low side effects, easy availability, low cost, and high efficacy.

African medicinal plants such as *Gongronema latifolium* are known for both nutritional and medicinal value. The tropical rainforest plant *Gongronema latifolium* (Utazi) belongs to the Asclepiadaceae family and to the genus *Gongronema* [19]. It is an edible plant which, when cut, produces milky latex with a green leaf, yellow flower and stem. In particular, when eaten fresh, it has a distinctive sharp, bitter and slightly sweet taste.



**Figure 1** The plant of *Gongronema latifolium*

It is then taken as an aphrodisiac and as a purgative for colic and stomach pain and to treat symptoms of worm infection [22]. Due to its composition of different active chemicals, *Gongronema latifolium* is believed to possess good medicinal qualities. Some of the medicinal values of *Gongronema latifolium* have also been scientifically validated. Therefore, since sexual hormones play a significant role in reproduction, this present study determines the effect of *Gongronema latifolium* on some reproductive hormones of male Wistar rats.

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## 2. Material and methods

### 2.1. Plant material

Fresh leaves of *Gongronema latifolium* were collected from Obudu, Obudu Local Government Area, Cross River State, Nigeria. The leaves were taken to the Department of Botany, University of Calabar for identification and authentication. The Voucher number Herb/Bot/UCC/611 has been deposited for future reference at the Department's Herbarium.

### 2.2. Preparation of plant material

Fresh leaves of *Gongronema latifolium* were air-dried at room temperature for twenty (20) days, macerated and pulverised into powdery form using the blender and then sieved.

### 2.3. Ethanol extraction

Three hundred (300) g of powdered *Gongronema latifolium* leaves were dissolved in 1200 mL of ethanol water for 72 h in a refrigerator. After that, it was filtered with muslin cloth and filtered using Whatman filter No1. The filtrate was evaporated to dryness and the percentage yield was calculated and reconstituted into dosage and administered into rats.

### 2.4. Phytochemical screening

The qualitative phytochemical screening of *Gongronema latifolium* leaf was determined by the methods of [23, 24]

### 2.5. Experimental animals

Eighteen (18) Wistar albino rats (120-200) g were obtained from the Animal Holding Unit of the Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus, Yala Local Government Area, Cross River State, Nigeria. The animals were allowed to undergo acclimatisation period for seven days before the start of the research.

The rats were housed in a plastic cage. The animals were kept in well-ventilated room at temperature and relative humidity of  $29 \pm 2^\circ\text{C}$  and 70% respectively, with 12 h natural light-dark cycle and were allowed free access to standard feed and water *ad libitum*. Constant cleaning and removal of faeces and spilt feeds from cages was done daily to maintain good hygiene.

### 2.6. Experimental design

The experiment was conducted using Eighteen (18) male Wistar rats, ranging from 120-200 g. Group A served as the control, group B was administered with 100mg/kg body weight of ethanol extract of *G. latifolium*, group C 200 mg/kg body weight of the extract, The vehicle and extract were orally administered after 12 days of administration all rats were fasted for 24 h, the rats were sacrificed and cardiac puncture procedure was used to collect the blood.

### 2.7. Hormonal assay

Serum testosterone was estimated using Rapid lab kit (United Kingdom) for the enzyme linked immunosorbent assay (ELISA) while serum FSH, LH and progesterone were estimated using Dialab Kit (Austria) for ELISA for quantitative determination of FSH in the serum.

## 3. Results

The result depicts that the leaves of *G. latifolium* contain alkaloids, saponins, flavonoids, steroids, triterpenoid, tannins, glycosides at varying concentrations and the absence of both phlobatanins and anthraquinones (Table 1).

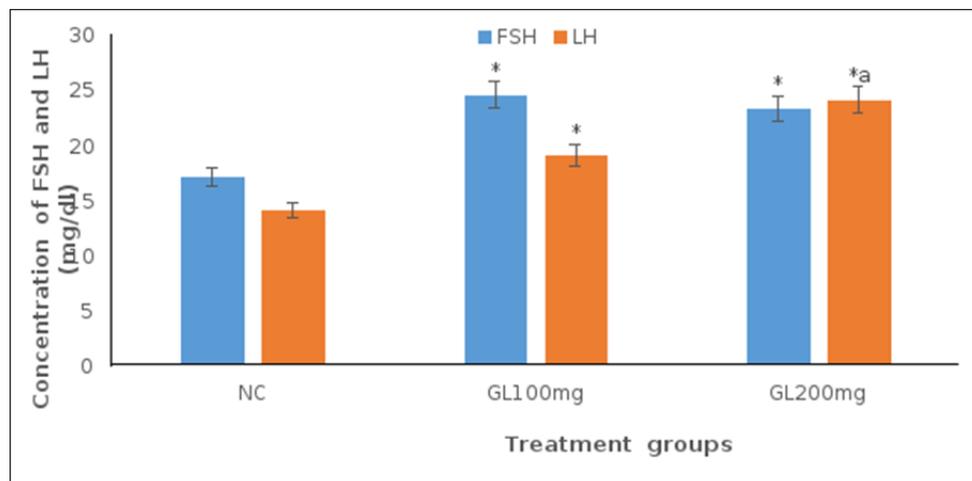
**Table 1** The phytochemical screening of *G. latifolium* leaf

Phytochemicals	<i>G. latifolium</i>
Alkaloids	++
Saponins	+++
Flavonoids	++
Steroids	+
Tannins	+
Triterpenoids	+
Glycosides	++
Phlobatanins	-
Antraquinones	-

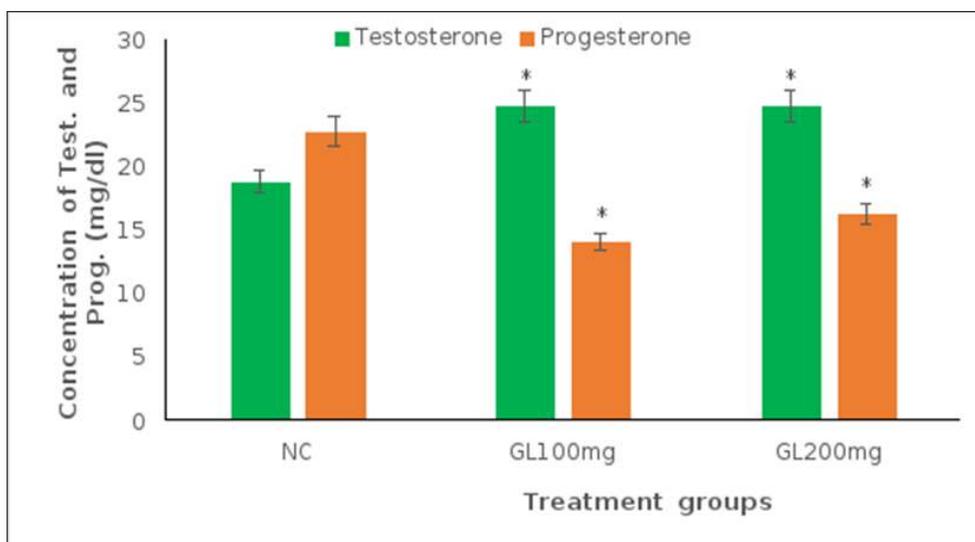
Where: + = present; ++ = moderately present; +++ = highly present; - = absent

The result also indicates the effect of ethanol extract of *G. latifolium* on some reproductive hormones following extract administration, it was observed that the extract produced a significant increase ( $P < 0.05$ ) in the concentration of serum follicle stimulating hormone (FSL) and luteinizing hormone (LH) at 100mg/kg and 200mg/kg body weight compared to normal control (Figure 2).

Likewise, the extract produced a significant increase ( $P < 0.05$ ) in serum testosterone at both 100mg/kg and 200mg/kg body weight compared with the normal control. More so, the extract produced a significant decrease ( $P < 0.05$ ) in serum progesterone in the experimental groups compared with the control (Figure 3).



**Figure 2** Effect of *Gongronema latifolium* ethanol extract on Follicle stimulating hormone (FSL) and Luteinizing hormone (LH) of experimental animals. Values are expressed as means + SEM; (n = 5 rats per group). \* = significantly different from NC ( $P < 0.05$ ), a = significantly different from GL100mg ( $P < 0.05$ ). Legend: NC, normal control that received normal saline; GL100mg = Group that received 100mg/kg b.w ethanol extract of *G. latifolium*, GL200 = Group, which received 200mg/kg b.w ethanol extract of *G. latifolium*.



**Figure 3** Effect of *Gongronema latifolium* ethanol extract on testosterone and progesterone of experimental animals. Values are expressed as means  $\pm$  SEM; (n = 5 rats per group). \* = significantly different from NC ( $P < 0.05$ ). Legend: NC = normal control that received normal saline, GL100mg = Group that received 100mg/Kg b.w ethanol extract of *G. latifolium*, GL200 = Group that received 200mg/Kg b. w ethanol extract of *G. latifolium*.

#### 4. Discussion

Medicinal plants and other ethnobotanical can stimulate reproductive hormones due to the activity of the phytonutrients in the herbal therapies or by either binding to hormone receptors, which result in conformational changes that will enhance the physiological function of the hormones or bind to enzymes that are involved in the synthesis of such reproductive hormones.

It has been reported that several phytochemicals have been implicated in enhancing sexual function in animal models. For example, saponins are responsible for aphrodisiac activity in *Fadogia agrestis* (Schweinf. Ex Hiern) and *Tribulus terrestris* (Linn.) so are alkaloids in *Pausinystalia yohimbe* (K. Schum) and *Microdesmis keayana* (J. Leonard) [25]. Similarly, pro sexual stimulatory property of *Mondia whitei* Hook (Skeels) has also been attributed to its steroid and triterpene contents [26]. These bioactive agents display aphrodisiac activity either by increasing androgen biosynthesis and secretion or by acting directly on the central nervous system in order to modulate the function of animal neurotransmitters and gonadal tissues. Specifically, saponins enhance androgen production [27] whereas alkaloids increase the dilation of blood vessels in the sexual organs, or increase nitric oxide that plays a key role in central erection and central sexual stimulation [28] Interestingly, some of these phytochemicals such as saponins and steroids were also detected in the leaf extract in this study that suggests that they are responsible in enhancing sexual behaviour by either binding to hormone receptors, resulting in a conformational change that will enhance the physiological function of the hormones or bind to enzymes that are involved in the synthesis of such reproductive hormones to enhance its production. Moreover, it has been documented that by boosting testosterone production and preventing metabolic degradation, flavonoids facilitate male sexual behaviour [29]. Terpenoids are associated with the stimulation of penile erection and the enhancement of the sexual performance [30]. Kim *et al* [31] demonstrated that saponin facilitated the relaxation of the corpus cavernosum muscles by stimulating the L-arginine/nitric oxide pathway. These bioactive components affect the central nervous system by activating neurotransmitters or the periphery by stimulating the release of nitric oxide [32]. Therefore, it appears that phytochemical content present in *Grongolema latifolium* have androgen stimulatory action responsible for the increase in sex hormones, which enhance its sex-enhancing activity and spermatogenesis.

Testosterone is a male hormone with significant impact on spermatogenesis [33]. Leydig cells of the testicles secrete testosterone, the adrenals and ovaries, and is the most important androgen secreted into the blood [34, 35]. Testosterone deficiency is presented with delayed puberty or regression of previously established male characteristics that depend on testosterone, such as hair distribution, potency, and libido. An elevated level of testosterone has been associated with a moderate but significant increase in sexual desire and penile function [36]. Clinical data on testosterone show that a slightly increased testosterone level in adult males, results in an enhanced sexual desire and arousability [37]. The level of testosterone has been reported to be related to LH and FSH such that increase in the levels of the gonadotropins results in a corresponding increase in testosterone [38]. In this present research work, the observed increase in serum testosterone suggests that the extract possesses a sex enhancing potential due to the presence of phytoconstituent such as flavonoids and alkaloid or saponin.

FSH stimulates testosterone spermatozoa development and promotes seminiferous tubule formation. FSH is responsible for the development, growth, pubertal maturation and reproductive processes of the human body [39]. Excess secretion of FSH is responsible for early puberty, whereas deficiency causes infertility and underdevelopment of gonads. From this study, the observed increase in serum FSH at 100mg/kg and 200mg/kg body weight indicates that the extract improved secondary sexual characteristics, sexual health with libido.

LH stimulates the production of testosterone from Leydig cells in males. Decreased secretion of LH can result in the breakdown of gonadal function (hypogonadism). In males, this disorder is usually manifested as failure in the development of normal numbers of sperm. In females, amenorrhoea is commonly observed. FSH and LH are collectively responsible for the development and maintenance of secondary sexual character in males [40, 41,42]. From this work, increase in serum LH in all experimental groups suggests that the extract aid gametogenesis, via the hypothalamic axis.

Progesterone is an endogenous sex hormone and progestogen involved in humans and other species' menstrual cycle, pregnancy, and embryogenesis [43]. Greater than normal levels indicate pregnancy. Adrenal or ovarian cancer, molar pregnancy, or overproduction of hormones by the adrenal glands may also be demonstrated by elevated levels. Lower than normal levels indicate amenorrhoea. Abnormally low levels of progesterone can also cause problems with ovulation. From this work, the decrease in serum progesterone following the administration of the extract acknowledges the fact that the plant extract increases libido in males and not females as progesterone activity is mainly associated with females.

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## 5. Conclusion

Conclusively, the extract of *Gongronema latifolium* enhances sexual health and libido in males. It is possible to be used in the management of erectile related dysfunction.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors have declared no conflict of interest.

### *Statement of ethical approval*

Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Nigeria (approval number FBMS/CRUTECH/20/018).

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