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EFFECTS OF AQUEOUS EXTRACT OF *Andira inermis* STEM BARK ON SOME REPRODUCTIVE PARAMETERS OF MALE WISTAR RATS

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ABSTRACT

Andira inermis stem bark has been used by herbal practitioners in the treatment of many ailments. Its Pharmacological activities include Anti-oxidant activity, haematological activities, hypoglycaemic activity and antiplasmodia activities. This study seeks to evaluate the effect of aqueous stem bark extract of Andira inermis on some gonadal hormones, sperm count and sperm

INTRODUCTION

Research on the therapeutic potential of plants has surged over the years, with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases. *Andira inermis* is a nitrogen fixing tree that is commonly grown as an ornamental. It has a handsome spreading crown, evergreen foliage; showy pink flowers and responds easily to management. *Andira inermis* is a multiple use tree that has not been extensively used in agroforestry or other reforestation programs because of relatively slow growth rates. *Andira inermis* is a legume that belongs to the Papilionoideae sub-family. The numerous common names that *Andira inermis* has are related to its widespread distribution, many uses and botanical characteristics. Names include Dog Almond in English, Gwaska in Hausa, Daluhi/Geloki/Kainiki in Fulfulde, and cabbage angelin, partridge wood or cabbage bark in the United States (Ngulde *et al.,* 2015). A. *inermis* grows in a

motility. Thirty-two male Wistar rats weighing 100-120g were randomized into Four (4) groups consisting eight (8) rats each. The animals were allowed to undergo acclimatization for 14days. Animals in group 1 served as the control, group 2, 3 and 4 were the treatment groups. Group 1 received free animal feed and water. Group 2, 3 and 4 received 100, 250 and 500mg/kg aqueous extract of Andira inermis stem bark administered orally daily for 90days. Twenty-four hours after the last administration, rats were sacrificed and cardiac puncture was used to collect the blood for centrifugation. The extract produced a significant ($P<0.05$) increase in serum testosterone, follicle stimulating hormone, and luteinizing hormone respectively at 100mg/kg body weight but had no significant effect on luteinizing hormone and testosterone hormone at 250mg/kg and 500mg/kg bodyweight compared to normal control. At 250mg/kg and 500mg/kg treatment, follicle stimulating hormone was significantly decreased compared to control. Sperm counts decreased significantly from 22.13±0.53million/ml to 13.00±1.16 million/ml and sperm motility from 11.25±0.41 million/ml to 8.00 ± 0.59 million/ml after treatment. Data was expressed as the mean ± SEM. Statistical comparisons between various treatment groups was determined using one-way analysis of variance (ANOVA).

KEYWORDS: Andira inermis, Gonads, Hormones, Testosterone, Sperm count

wide range of habitats from evergreen tropical rain forest to dry savanna vegetation, on well drained sandy soils as well as on poorly drained clay soils, in plains and on hill slopes. It is found in riparian zones, along rivers and in areas with a highwater table. It grows in alluvial forests in Central America but may be found in drier areas. It is found along roadsides, riverbanks, woodlands and pastures, from sea level to 900m above sea level (Orwa *et al*., 2009). It is a beautiful tree grown mostly in tropical America and West African countries including Nigeria.

During an investigation on the phytochemical spectrum of *A. inermis*, isoflavones from both stems and leaves were identified as formononetin, prunetin, biochanin A, calycosin, genistein, and pratensein, respectively (Ludmila *et al*., 2019).

Ethnomedicinal uses of *Andira inermis*

The stem bark of *A. inermis* is reported to have vermifuge, purgative (expelling intestinal worms) and narcotic properties. Fresh seeds are toxic and cannot be eaten. They are used as an anthelmintic to reduced vomiting. Herbal healers use the bark of the tree to cure constipation, reduce fever and as a strong laxative.

Andira inermis Pharmacological activities includes anti-oxidant activity in form of a blanket recommendation (soothing effect for the generally ill) by most herbalists as a result of the presence of natural antioxidants widely distributed in AI (Xu *et al.*, 2017). The haematological indices after the administration of A.I extract suggest a desirable effect for anaemia as it improves significantly red blood cell count (RBC), haemoglobin concentration (HB) as well as the pack cell volume (PCV) (Egua *et al.*, 2021). In the wild, this tree also offers a suitable environment for some plant epiphytes like orchids, bromeliads, mosses and ferns. An excessive dose of its extract can cause vomiting, fever, insomnia and even death. African populations are confronted with chronic diseases emergence whose treatment and follow-up constitute more economic problem. Hence it becomes paramount that the bioactive phytochemicals in *Andira inermis* are subjected to research, while also subjecting them to toxicity profiling (Quazim *et al.*, 2021).This study is designed to evaluate the subchronic toxicity of the aqueous *Andira inermis* stem bark extract on some male gonadal hormones.

Nutritional/Phytochemical analyses of *Andira inermis*

The aqueous methanolic extract of *Andira inermis* stem bark was found to contain saponins, terpenes, tannins, steroids, flavonoids, carbohydrates and alkaloids as shown in table 1. The phytochemicals present in the plant shows the richness of the plant in phytochemicals which may have bioactive properties (Rui 2013). And it has been reported that the common phytoconstituents found in medicinal plants with antidiabetic potential include polyphenols, flavonoids, terpenoids, tannins, alkaloids, saponins (Yatoo *et al.*, 2017). Some of which are present (terpenes, tannins, flavanoids, and alkaloids) in *A. inermis*.

Phytochemical	Inference
Saponins	+ +
Terpenes	+
Tannins	+
Steroids	+
Flavonoids	+
Anthraquinones	–
Carbohydrates	+
Alkaloids	+

Key
+ Present in trace quantity, ++ Present in moderate concentration, +++ Present in high concentration - Abscence

Table 1: Phytochemical constituents of aqueous methanolic extract of *Andira inermis* stembark (Yatoo *et al.*, 2017)

Isoflavones

Isoflavones are the secondary

metabolite formed by symbiotic relationship with the rhizobia bacteria and the defense responses of leguminous plant. Isoflavones are synthesized as part of the phenylpropanoid pathway, the same biosynthetic pathway of flavonoid biosynthesis (Barnes, 2010). Phenylalanine converts 4-hydroxycinnamoylCoA by reaction with malonylCoA. Chalcone synthase catalyzes the reaction of this intermediate to convert to 4,2',4',6'-tetrahydrozychalcon (naringenin chalcone) and the combined enzyme reaction of chalcon synthase and chalcone reductase convert this intermediate to 4,2',4'-trihydrozychalcone (isoliquiritigenin). And then, chalcone isomerase catalyzes the ring closure of the heterocyclic ring to form 7, 4'-dihydrozyflavone (liquirintigenin) and 5, 7, 4'-trihydroxyflavone (naringenin). The B-ring is moved from the 2-position to 3-position by isoflavone synthase. Isoflavone dehydratase removed water to generate the 2, 3 double bonds in the heterocyclic ring. The products generated by this reaction are daidzein (7, 4'-dihydroxyisoflavone) and genistein(5,7,4'-trihydroxyisoflavone).

Absorption of Isoflavones

After isoflavones ingestion, isoflavones are hydrolyzed in intestinal wall by intestinal enzymes or intestinal micro-organism with glycosidase, and the conjugated isoflavones are changed to bioactive aglycones such as daidzein, genistein, and glycitein by hydrolysis.

Functions of Isoflavones

- **Hormone-like functions**

  -Estrogenic and anti-estrogenic function: isoflavones have an estrogenic effect and an anti-estrogenic effect because of similarity of structure with 17β-estradiol. Based on the similarity with 17-β-estradiol, isoflavones can bind to estrogen receptor (ER) such as ER-α and ER-β. ER-binding properties of isoflavones indicate that they have the potential to affect intracellular signaling mechanisms which is important for regulating cellular growth and protection.

  -Interaction with steroid hormone metabolisms and transport: isoflavones inhibit the activity of 5α-reductase, which catalyzes the conversion of testosterone to 5α-dihydrotestosterone, and CYP19 (aromatase), which mediates the conversion of testosterone to estradiol in low isoflavones concentration. In contrast, when isoflavones concentration is high, aromatase activity is rather increased to increase estradiol conversion (Ludmila *et al.,* 2019).

- **Antioxidant function**

  Genistein has antioxidant properties as scavenger of radicals and chelaters of metals. This function is occurred by affecting gene expression of enzymes that

react with antioxidants such as catalase and superoxide dismutase, and inhibiting with secondary oxidant production such as hydrogen peroxide or hypochlorous acid. Genistein is more active as antioxidant than daidzein due to having its third hydroxyl group in the C-5 position and equol is another better antioxidant action than its precursor compounds due to the absence of the 2,3-double bond in conjunction with a loss of the 4-oxo group which is enhancing antioxidant properties. Isoflavone metabolism pathway in intestine and liver affect the antioxidant properties of isoflavones and the metabolites. Although genistein significantly reacts with superoxide dismutase, catalase, and glutathione peroxidase, isoflavone metabolites such as equol, 8-OH-daidzein, O-desmethylangolensin (O-DMA), and 1,3,5-trihydroxybenzene have also played a role as potent scavengers. These isoflavone metabolites are highly chelating with Ferrous compounds relative to genistein and daidzein (Yu *et al.*, 2012).

- **Anti-inflammation function**

  Isoflavones genistein inhibit deregulated activation of NF-kB (nuclear factor kappa-B), which can suppress inflammation. Suppression in NF-kB activation may occur through inhibiting IkBα kinase, leading to inhibition of phosphorylation and degradation of IkBα and consequent NF-kB DNA binding by p65 nuclear translocation.

  Genistein directly affect to inhibit interleukin-8. Some isoflavone extracts from plant sources affect to suppress NF-kB and NO activation and may down-regulate several inflammation related genes such as COX2 (cytochrome oxidase-2), MMP-9, ICAM1 (inter-cellular adhesion molecule 1), iNOS to reduce inflammation (Prasad *et al.,* 2010).

Sex hormones

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. 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. 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. If the brain is unable to produce gonadotropic releasing hormone (GnRH), this disorder results in a lack of testosterone and thereby inhibits spermatogenesis. The lack of GnRH causes a group of disorders known as hypogonadotropic hypogonadism (hypogonatropisms). 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, normalize testosterone concentration, and stimulate the development of secondary sexual traits. 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 (Dasofunjo *et al*., 2020).

Luteinizing hormone (LH)

Reproduction is controlled by the hormones functional in the hypothalamic-pituitary-gonadal (HPG) axis. In the male they concern the maintenance of testicular testosterone (T) production and spermatogenesis by the two pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Luteinizing hormone is a glycoprotein hormone that is co-secreted along with follicle-stimulating hormone by the gonadotrophin cells in the adenohypophysis (anterior pituitary gland). Luteinizing hormone is a part of a neurological pathway comprised of the hypothalamus, the pituitary gland, and gonads. In this pathway, LH release is stimulated by gonadotropin-releasing hormone (GnRH) and inhibited by estrogen in females and testosterone in males. LH contributes to the maturation of primordial germ cells. In males, LH causes the Leydig cells of the testes to produce testosterone. Gonadotroph cells have large round cell bodies with prominent Golgi apparatus and endoplasmic reticulum. These cells are diffusely spread out and comprise around 10 to 15% of the functional anterior pituitary cell mass. LH is made up of an alpha and beta subunit. The alpha subunit of LH is made up of 92 amino acids, and the beta subunit is made up of 120 amino acids. Combined, these two subunits have a mass of 28kDa (Choi, 2014). The hypothalamus secretes GnRH in a pulsatile manner, which stimulates the secretion of LH. Gonadal steroids, estrogen, progesterone, and testosterone exert negative feedback, thus decreasing the secretion of LH (Sayed, 2022).

Function of Luteinizing hormone

In males, LH stimulates testosterone release by the Leydig cells of the testes. In females, LH stimulates steroid release from the ovaries, ovulation, and the release of progesterone after ovulation by the corpus luteum (Sayed, 2022).

Mechanism of action of Luteinizing hormone

Luteinizing hormone acts by binding to a G- protein coupled receptor, which in turn activates adenylyl cyclase. Adenylyl cyclase, an enzyme, then produces cyclic-AMP, thus

increasing its intracellular concentration, which then activates a kinase molecule called protein kinase A (PKA). PKA then phosphorylates specific intracellular proteins that subsequently achieve the end physiological actions of LH like steroid production and ovulation. Studies have shown that this testicular dysfunction and altered testosterone concentration results from higher levels of LH in the plasma and lower amounts of secretory LH pulses seen in men with end-stage renal disease when compared to healthy subjects or men who underwent a successful renal transplant. This fact is significant because the pulsatile secretion of LH is necessary for gonadotropin receptors of the testes to function properly. Furthermore, sustained high levels of LH in the blood and testes can cause a loss of gonadotropin receptors in the testes (Raju *et al.,* 2013). It has been found that low LH levels in the body can result in poor outcomes in assisted reproductive technology (ART). Thus, patients who have low endogenous LH, such as with hypogonadotropic hypogonadism, can have an increase in efficacy of ART with exogenous LH treatment.

Follicle Stimulating Hormone

Spermatogenesis is a concerted sequence of events during maturation of spermatogonia into spermatozoa. The process involves differential gene-expression and cell-cell interplay regulated by the key endocrine stimuli, i.e., follicle- stimulating hormone (FSH) and luteinizing hormone (LH)-stimulated testosterone. FSH affects independently and in concert with testosterone, the proliferation, maturation and function of the supporting Sertoli cells that produce regulatory signals and nutrients for the maintenance of developing germ cells. The testicular target cells of FSH are the Sertoli cells present in the seminiferous tubules. LH stimulates Leydig cell T production, and FSH stimulates in Sertoli cells, in synergy with T, the production of regulatory molecules and nutrients needed for the maintenance of spermatogenesis. Hence, both T and FSH regulate spermatogenesis indirectly through Sertoli cells. Sertoli cells form both structurally and biochemically a supporting environment for the maturing germ cells. Their number is determined by FSH action, in rodents during fetal and neonatal life, and in primates at neonatal and peri-pubertal age (Sharpe *et al.,* 2003). In both rodents and primates, FSHR expression starts during the second half of gestation, though the lack of ligand (FSH) and cAMP responsiveness imply that the receptor is initially functionally inactive. However, after the onset of fetal pituitary FSH production and activation of the receptor, the hormone plays a major role in Sertoli cell proliferation. During peri-puberty, the rising FSH concentration triggers the second phase of Sertoli cell proliferation, and the concentration of circulating FSH correlates strongly with Sertoli cell number and testis size in adulthood. In the

absence of FSH or FSHR (follicle-stimulating hormone Receptor), the Sertoli cell number is considerably decreased, by 30–45%, in comparison to normal testicular development. This is of high importance, as the Sertoli cells number determines the quantity of sperm produced; a Sertoli cell is able to support a certain maximum number of germ cells. Classical studies on animal models indicate that Sertoli cells proliferate until a finite number and differentiate toward puberty. Prepuberty, together with increasing FSH secretion, FSHR expression begins to fluctuate along with the stage of spermatogenesis. This is associated with maturation of the Sertoli cell population and completion of the first cycle of sperm maturation. In the postpubertal testis, FSH together with T evokes in Sertoli cells signals to propagate germ cell maturation, to provide antiapoptotic survival factors and to regulate adhesion complexes between germ cells and Sertoli cells. The lack of FSH or FSHR in mice does not lead to sterility, albeit it decreased testis size, reflecting reduced Sertoli cell number and capacity to support and nurture germ cells. While FSH influences solely the proliferation of Sertoli cells, T and FSH impact additively on the germ cells' entry into meiosis and stimulate synergistically its completion and entry into spermiogenesis (Oduwole *et al*., 2018).

FSH Regulates Genes Involved in Proliferation, Structure, and Function of Sertoli Cells. The regulatory system of the two gonadotropins, their feedback regulation, organization and interaction between germ and somatic cells, pose a challenge for dissecting the influence and target genes of a single factor, such as FSH. Sertoli cells present with a prominent fluctuating gene expression patterns along the seminiferous epithelial cycle. The general phenomenon is that FSH mostly elevates the expression of a large number of Sertoli cell genes (Soffientini *et al.*,2017).

FSH stimulates many genes including Krüppel-like factor 4(Klf4). KLF4 is a transcription factor that can be used to reprogram Sertoli cells to pluripotent stem cells, but it also plays a significant role in timing and accuracy of Sertoli cell differentiation. Logically for the supportive role of FSH in spermatogenesis, it also regulates and limits the massive wave of germ cell apoptosis during the first round of spermatogenesis. This process is apparently crucial to maintain the critical cell number between some germinal cell stages and Sertoli cells, and its lack brings about sterility. Pituitary-derived FSH provides indirect structural and metabolic support for development of spermatogonia into mature spermatids via its membrane-bound receptor in Sertoli cells. In addition to proliferation and differentiation of Sertoli cells, FSH regulates the structural genes involved in the organization of cell-cell junctions as well as genes required for the metabolism and transport of regulatory and nutritive substances from Sertoli to germ cells. Although FSH is not a mandatory requirement for the completion of spermatogenesis in rodents, its

deficiency, nevertheless, leads to significant reduction in sperm quantity (Oduwole *et al*., 2018).

Testosterone

Testosterone is a Sex steroid which is a crucial hormone for the proper development and function of the body; it regulates sexual differentiation, the secondary sex characteristics, and sexual behavior patterns. Following the conversion of cholesterol to pregnenolone, there are several possible pathways the body can use to produce androstenedione. However, the human body appears to preferentially use the pathway that involves the conversion of pregnenolone to dehydroepiandrosterone (DHEA), which occurs at approximately a fourfold greater rate than the pathway involving progesterone. Androstenedione is then converted to testosterone via 17-hydroxysteroid-3 (17HSD3) in the testis.

Finally, cytochrome P450 family 19 (often called aromatase) converts testosterone to estradiol in the Leydig cells of the testicles. While it is often stated that 90–95% of total androgen production is from the testes, it has been well documented that tissue such as the prostate can produce approximately 50% of its own androgens, without a significant release of the hormone into the circulation. This process can occur through so-called peripheral conversions, where a precursor, such as DHEA is converted to testosterone at a site other than the testis, owing to the presence of 3b-hydroxysteroid dehydrogenase and 17HSD3 or 17HSD5 (Hooper *et al*., 2017).

Testosterone is a male hormone with significant impact on spermatogenesis (Amanatkar *et al*., 2014) Leydig cells of the testicles secrete testosterone, and the adrenals and is the most important androgen secreted into the blood (Mansoureh *et al*., 2016; Gauthan *et al*., 2002).

The system for the release and control of the testosterone hormone is known as the hypothalamic-pituitary-gonadal axis (HPGA). A recent advancement in the understanding of the HPGA was the discovery of kisspeptins, produced by the KISS1 gene, and their role in the regulation and secretion of gonadotropin-releasing hormone (GnRH). Infact, KISS1 is the initial signal for GnRH secretion, and is now universally recognized as the major central regulator of the HPGA. Kisspeptin neurons are located in the brain in two areas, the arcuate nucleus and the anteroventral periventricular nucleus. The kisspeptin receptors (KISS1R) are located on GnRH neurons in the preoptic area of the anterior hypothalamus, which stimulates GnRH release into the hypothalamic-hypophyseal portal vein, connecting the hypothalamus to the anterior pituitary. At the anterior pituitary, the GnRH receptor (GnRHR) receives the signal to secrete luteinizing hormone into the

circulation, which is done in a pulsatile manner. Luteinizing hormone is then the signal for testosterone secretion, which as described above can occur in several tissues, predominantly the testis. Whe appropriate levels are reached, androgens as well as estrogens (which are synthesized from testosterone) provide negative feedback to the KISS1R to stop further secretion (Hooper *et al*., 2017).

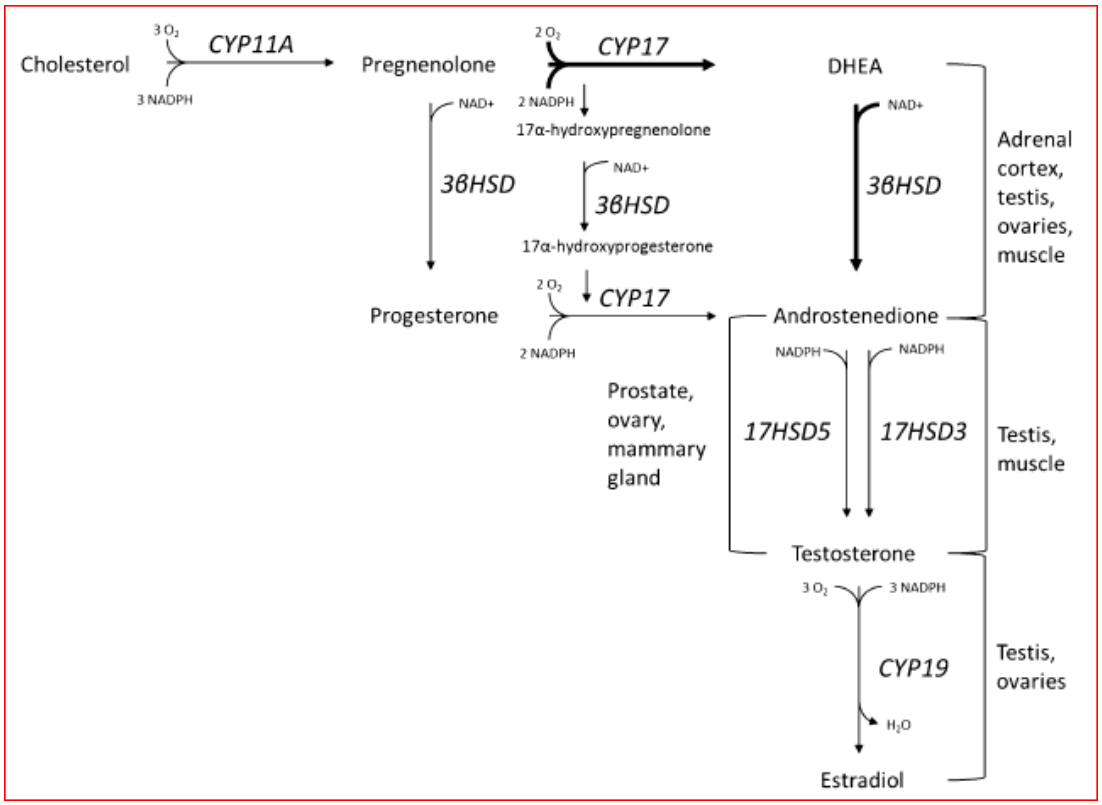

Fig. 1 Testosterone biosynthesis (Hooper *et al*., 2017)

High concentrations of T inhibit the release of GnRH, FSH, and LH, a negative feedback control. Conversely, when T concentrations are low, higher levels of GnRH, FSH, and LH are released.

Thus, reciprocal action of T with the hypothalamic and gonadotropic hormones is necessary for regulation of normal reproduction in the male. The Leydig cells synthesize and secrete T less than 30 minutes after the onset of an LH episode. This T secretion is short and pulsatile, lasting for a period of 20 to 60 minutes. It is believed that pulsatile discharge of LH is important for two reasons. First, high concentration of T within the seminiferous tubule is essential for spermatogenesis. Second, Leydig cells become unresponsive to sustained high levels of LH believed to be caused by reduction in the

number of LH receptor. In fact, continual high concentrations of LH result in reduced secretion of T. It is believed that chronically high systemic concentrations of T suppress FSH secretion. Sertoli cells function is FSH dependent. Thus, their function is compromised when FSH is reduced.

The periodic reduction in T allows the negative feedback on FSH to be removed (Hooper *et al.*, 2017).

Substances known to affect Gonadal Hormones

In mammals, the effects of estrogen are mainly mediated by two different estrogen receptors, ERα and ERβ. These proteins are members of the nuclear receptor family, characterized by distinct structural and functional domains, and participate in the regulation of different biological processes, including cell growth, survival and differentiation. The two-estrogen receptor (ER) subtypes are generated from two distinct genes and have partially distinct expression patterns. Their activities are modulated differently by a range of natural and synthetic ligands. Some of these ligands show agonistic or antagonistic effects depending on ER subtype and are described as selective ER modulators (SERMs). Accordingly, a few phytochemicals, called phytoestrogens, which are synthesized from plants and vegetables, show low estrogenic activity or anti-estrogenic activity with potentially anti-proliferative effects that offer nutraceutical or pharmacological advantages (Sylvain *et al.,* 2017).

Many natural and synthetic chemicals in the environment and in food have been reported with hormonal activity, particularly showing estrogenic potency. These compounds are called endocrine disrupting chemicals (EDCs). A lot of EDCs are generated from human activities. For example, polycyclic aromatic hydrocarbons (PAH), such as polychlorinated dibenzo-p-dioxins and dibenzofurans, or polychlorinated biphenyls (PCBs), which are the most persistent and widespread in the environment. Bisphenol A, nonylphenol and ethinyl estradiol were also reported to be among the major environmental estrogens. A series of experimental and epidemiological studies over the past decades have suggested that these environmental contaminants can interfere with normal hormonal processes and induce deterioration of the reproduction function in males and females. Furthermore, numerous natural molecules present in vegetables and plants possess estrogen- and antiestrogen-mimetic activities. These natural molecules are mainly phytoestrogen and isoflavones, the most widely consumed. The most abundant isoflavones are genistein and daidzein.

Against environmental stresses and aggressions, plants produce secondary metabolites belonging to the large family of polyphenols, which have many biological activities, such

as antioxidant, antifungal and antibiotic properties. All of these compounds contain one or several aromatic rings with at least one hydroxyl group. Hydroxyl groups can be free, but most of the time they are engaged in another function with an ester, ether or a glycoside. Among these compounds, phytoestrogens have a structural similarity with 17β-estradiol and could bind both ERs.

The anti-estrogenic activity of phytoestrogens is due to their structural similarity with 17-β-estradiol (E2), as well as interacting with ERs, phytoestrogens can affect the secretion of gonadotropin-releasing hormone (GnRH). Phytoestrogens could disrupt the endocrine system by interfering with the hypothalamic–pituitary–gonadal axis, which controls estrogen secretion. The hypothalamus releases GnRH and stimulates the pituitary to produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH), gonadotropins that promote the secretion of estrogen, progesterone, and testosterone by the ovaries or testes. Low estrogen levels are a signal for the hypothalamus to release GnRH, whereas high levels provide a negative feedback. Therefore, the presence of exogenous compounds structurally similar to E2 may interfere with this system (Domínguez-López *et al*., 2020).

Phytoestrogens have also been reported to affect sex hormones through ER-independent mechanisms of action, such as by altering hormone-binding globulin (SHBG) levels. Circulating estrogens and androgens are mostly bound to albumin and SHBG, with only a small fraction remaining free. As estrogens and androgens are only biologically active in their free form, SHBG affects steroidal activity. In vitro studies have shown that isoflavonoids stimulate the synthesis of SHBG by liver cancer cells (Jefferson 2010).

The effects on reproduction are not dose-response but are influenced by the type of isoflavone and period. There are variations in the serum concentration of hormones and action on their negative feedback on the hypothalamic-pituitary-testicular axis in males. Reproductive functions are also affected by spermatogenesis, such as decreased sperm count, lower reproductive performance, reduced litter size, low sperm production, and reduced seminal vesicle size (Sleiman *et al*., 2021).

MATERIALS AND METHOD

Collection of Plant Sample and Identification

The plant sample was collected in Lugda, Fufore LGA of Adamawa State, Nigeria. The sample was botanically identified and authenticated by a taxonomist in Plant science unit of Modibbo Adama University, Yola Adamawa State. Plant sample collected was air-dried at room temperature and weighed several times until a constant weight was obtained. The sample was pulverized in a mortar and pestle into fine powder.

Preparation of Plant Crude Extract

The grounded sample (1000g) was soaked in distilled water, sieved with muslin piece and allowed to dry using a water bath. The crude extract was placed in a labeled sealed container and stored at normal laboratory conditions until required for reconstitution and administration.

Reconstitution of crude extract Sample for Administration

Aqueous extract was prepared by dissolving 3000mg (3g) crude sample in 60ml distilled water to constitute 50mg/ml of sample. Appropriate dosage to be given to rat in respective groups based on their weight was calculated, measured and administered accordingly.

Experimental animals

Thirty-two (32) apparently healthy young Wister male albino rats weighing between 100 – 120g were used for this study. The rats were kept at animal's house under normal environmental conditions and maintained with free access to pelletized growers/finisher feed. The rats were handled according to regulations guiding experimental animals handling ethics. The animals were allowed to acclimatize for two weeks (14 days).

TABLE 2: Experimental animals

GROUP	DESCRIPTION	TREATMENT
I	Normal/control	0.00 mg/kg *Andira inermis* daily for 90days
II	Treatment I	100 mg/kg *Andira inermis* daily for 90days
III	Treatment II	250 mg/kg *Andira inermis* daily for 90days
IV	Treatment III	500 mg/kg *Andira inermis* daily for 90days

Administration of feed and sample

Simple random sampling technique was used in grouping the rats into four (4) groups of 8 animals each in a Complete Randomize Design (CRD) and housed in conventional wire mesh cages. Aqueous sample of *Andira inermis* (AI) was administered orally daily by intubation using intravenous cannula tube at doses of 0, 100, 250 and 500 mg/kg body weight to the respective rats in their respective groups by single forced oral feeding once per day for a period of 90 days

Collection and preparation of Blood Samples

At about 24 hours after the last treatment, the animals were subjected to 12 hours fasting, anaesthetized by dropping each individual animal in a plastic jar saturated with

chloroform vapour. Blood sample was collected through cardiac puncture into labeled centrifuge tubes without anticoagulant and was allowed to clot then centrifuged at 4000g for ten minutes to obtain serum which was used for hormonal assay.

Hormonal assay

Serum gonadal hormones concentration was determined using Rapid Quantitative Test kit (Finecare) which is a fluorescence immunoassay used along with Finecare™ FIA Meter.

Determination of Testosterone

Method: Serum testosterone concentration was determined by the method of Bertoft (1984).

Principle

The Finecare™ Testosterone Rapid Quantitative Test is a fluorescence immunoassay using a competitive method for quantitative analysis of testosterone in serum/plasma. When the serum is added to sample well of the test cassette, the fluorescence-labeled anti-Testosterone monoclonal antibodies on the sample pad bind to Testosterone in blood specimen and form immune complexes. As the fluorescence-labeled anti- Testosterone monoclonal antibodies not bound to Testosterone migrate on the nitrocellulose membrane by chromatography, it can be captured by Testosterone-BSA conjugated antigens that have been immobilized on test line. Thus, the more Testosterone in the sample, the less unbound fluorescence-labeled anti- Testosterone monoclonal antibodies is accumulated on testline. Signal intensity of fluorescence inversely reflects the amount of testosterone and Finecare™ FIA Meters show testosterone concentrations (ng/ml) in sample.

Procedure

The test cassette, detection buffer and sample were allowed to equilibrate to room temperature prior to testing. The ID chip was inserted into the chip port of meters making sure that the cassette lot number matches with ID chip lot number. 75µL of sample Pipetted into the detection buffer tube and mixed thoroughly with the addition of 75µL of sample into the sample well of the test cassette. The timer was set for 15minutes and countdown began right after adding sample mixture into the sample well at room temperature. The test cassette was inserted onto the test cassette holder of meter, and "Test" Pressed to start testing. Meter scanned the sample-loaded test cassette immediately, and results displayed on the screen of meter and printed out by clicking "Print".

Determination of Follicle Stimulating Hormone

Method: Serum Follicle Stimulating Hormone was determined by the method of Cox (2012).

Principle

The Finecare™ FSH Rapid Quantitative Test is based on fluorescence immunoassay technology. The Finecare™ FSH Rapid Quantitative Test uses a sandwich immunodetection method. When serum is added into the sample well of the test cartridge, the fluorescence-labeled detector anti-FSH antibodies on the sample pad bind to FSH antigen in blood specimen and form immune complexes. As the complexes migrate on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibodies and FSH are captured to detector anti-FSH antibodies that have been immobilized on test strip. Thus, the more FSH antigen in blood specimen, the more complexes accumulated on test strip. Signal intensity of fluorescence of detector antibodies reflects the amount of captured FSH (mIU/mL) in sample.

Procedure

Before testing, the "use" mode was activated and ID chip inserted into Finecare™ FIA System with addition of 75µL of sample into the detection buffer tube followed by thorough shaking, mixing the sample and a subsequent addition of 75µL of sample into the sample well of the test cartridge. The timer was set for 15minutes and countdown began right after adding sample mixture into the sample well at room temperature. The test cartridge was then inserted into the test cartridge holder of meter, and "Test" Pressed to start testing. Meter starts scanning the sample-loaded test cartridge immediately, and results displayed on the screen of meter and result was printed out by clicking "Print".

Determination of serum Luteinizing Hormone

Method: Serum Luteinizing Hormone was determined by the method of Cox (2012).

The Finecare™ LH Rapid Quantitative Test is based on fluorescence immunoassay technology. The Finecare™ FSH Rapid Quantitative Test uses a sandwich immunodetection method. When serum is added into the sample well of the test cartridge, the fluorescence-labeled detector anti-LH antibodies bind to LH antigens in blood specimen and form immune complexes. As the complexes migrate on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibodies and LH are captured to detector anti-LH antibodies that have been immobilized on test

strip. Thus, the more LH antigen in blood specimen, the more complexes accumulated on test strip. Signal intensity of fluorescence of detector antibodies reflects the amount of LH captured (mlU/mL) in sample.

Procedure

The "use" mode was activated before testing and ID chip inserted into Finecare™ FIA System with addition of 75µL of sample into the detection buffer tube followed by thorough shaking to mix the sample and a subsequent addition of 75µL of sample into the sample well of the test cartridge. The timer was set for 15minutes and countdown began right after adding sample mixture into the sample well at room temperature. The test cartridge was then inserted into the test cartridge holder of meter, and "Test" Pressed to start testing. Meter starts scanning the sample-loaded test cartridge immediately, and results displayed on the screen of meter and was printed out by clicking "Print".

SPERM ANALYSIS

Sperm count

Method: The sperm count analysis was carried out using the wet preparation method as described by Alex (2014).

Principle

Most accurate method of determining sperm concentration is volumetric dilution and hemocytometry. Gently mixing the semen sample using a positive displacement pipette before the volume is withdrawn is essential for an accurate determination of sperm concentration.

A fixed volume of a liquefied semen aliquot is used and fixed sperm counted in a Neubauer hemocytometer chamber. Dilution of 1:19 is usually employed. Dilutions may be made in small, clean, glass or plastic vials.

Procedure

The whole epididymis of each rat was dissected, teased at few sites with a scalpel blade; the scalpel blade was allowed to extend into, but not through, the lumen of the duct, and blood vessels avoided and diluted in 5mL of 10% normal saline at room temperature. The sperm was allowed to diffuse into the medium for 0.5 to 15 min and the sperm count determined using Neubeur counting chamber and compound light microscope. This was achieved using Computer-assisted sperm analysis method (CASA) which is a computer system that requires a high-resolution camera connected to a phase-contrast microscope.

The analysis of a single view-field takes 1 second. Sperm concentration and morphology was examined with the provided CASA system. Semen analysis was conducted with strict adherence to the program settings provided by the manufacturer. The settings of the program will be installed and controlled by a Microptic representative, according to the Microptic CASA manual (Talarczyk-Desole *et al*., 2017)

Sperm Morphology

Method: The sperm morphology was determined using the staining technique described by Dibal *et al*, 2020

Procedure:

Several smears of the sperm sample were prepared on glass slides, allowed to dry at room temperature, and fixed with ethanol. The smears were stained with crystal violet stain ($C_{25}H_{30}ClN_3$), and washed after 5minutes and allowed to dry at room temperature. All the slides were observed under light microscope at 400x magnification. (Dibal *et al*., 2020). The characteristics that were examined are the shape and size of sperm cells.

Statistical analysis

Data was expressed as the mean ± SEM. Statistical comparisons between various treatment groups was determined using one-way analysis of variance (ANOVA). $P \leq 0.05$ was considered as significant.

RESULT AND DISCUSSION

Effect of administration of *Andira inermis* aqueous stembark extract on male sex hormones

TABLE 3: Hormonal Assay of Male Rats administered *Andira inermis* extract

DOSAGE mg/kg	Leutenizing hormone mIU/mL	Follicle stimulating hormone mIU/mL	Testesterone ng/mL
CONTROL	1.39 ±0.06	12.87 ±0.24	0.40 ±0.05
100	4.75 ±0.20*	110.13 ±1.63*	6.87 ±0.30*
250	1.13 ±0.04	22.92 ±0.7*	0.33 ±0.40
500	1.16 ±0.09	4.23 ±0.42*	0.28 ±0.01

Mean ± Standard error; n=8

*Statistically significant ($p<0.05$) Compared to Control

Table 3 shows the result of hormonal assay of male rats after the administration of *Andira inermis* aqueous extract for 13weeks (3months). The result indicates a statistically significant ($P < 0.05$) increase in Luteinizing Hormone of experimental rats administered 100mg/kg extract (group 2) when compared with the control group.

The treatment groups 3 & 4 shows no significant change in the Luteinizing Hormone assay when compared with the control. The result in table 3 also indicates a significant increase in testosterone of experimental rats administered 100mg/kg extract (group 2) when compared with control group. The treatment groups 3&4 (250 & 500mg/kg) showed no significant change in the hormone Testosterone as at when compared to control. The result also shows a statistically significant increase ($p<0.05$) in follicle stimulating hormone assay in treatment group 2 (100mg/kg), and then indicate a statistically significant downward change (decrease) in follicle stimulating hormone assay in treatment groups 250mg/kg and 500mg/kg as compared to the corresponding control group.

Effect of administration of *Andira inermis* aqueous stembark extract on sperm parameters.

TABLE 4: Sperm Parameters in Male Rats administered *Andira inermis* extract

Dose mg/kg	Sperm count million/ml	Sperm mortility million/ml	Sperm morphology million/ml
CONTROL	22.13 ± 0.53	11.25 ± 0.41	15.25 ±0.62
100	16.13 ±1.06*	8.75 ±0.50*	11.00 ±0.46*
250	17.88 ±0.64*	9.00 ±0.38*	10.88 ±0.44*
500	13.00 ±1.16*	8.00 ±0.59*	7.00 ±0.80*

Mean ±Standard error; n=8

*Statistically significant ($p<0.05$) Compared to Control

Table 4 displays morphometric alterations of sperm cells. The table shows that Sperm counts decreased significantly from 22.13±0.53 million/ml to 13.00 ±1.16 million/ml in *Andira inermis* treated rats of 90 days as compared to the corresponding control group.

Andira inermis affected sperm motility by significantly decreasing it in male rats from 11.25 ± 0.41 million/ml to 8.00 ±0.59million/ml after treatment for 90days. This decrease was observed in all treatment groups as compared with the control group.

The above table (4) also shows statistically affected sperm morphology from 15.25 ±0.62million/ml to 7.00 ±0.80million/ml of the rat's sperm cells causing a decreased calculated morphology in all the treatment groups as compared to the control group.

DISCUSSION

Luteinizing hormone (LH) is a hormone produced by the pituitary gland in the brain. In males, LH stimulates the production of testosterone. In this study, the effect of *Andira inermis* stembark extract was examined as shown in Table 3. The result of Luteinizing hormonal assay of male rats after the administration of extract for 13weeks(3months) indicates a statistically significant ($P < 0.05$) increase in Luteinizing Hormone of experimental rats administered 100mg/kg extract (group 2) when compared with the control group and other treatment groups.

The treatment groups 3 and 4 (administered 250 and 500mg/kg extract) shows no significant change in the Luteinizing Hormone assay when compared with the control. These phenomena can be explained by the effect of genistein (which is abundantly present in the extract) on the hypothalamic-pituitary-gonadal (HPG) axis, upon which normal estrous cyclicity is dependent (Jefferson *et al.,* 2005).

The result in table 3 indicates a significant increase in testosterone of experimental rats administered 100mg/kg extract (group 2) for 13 weeks when compared with control group and other treatment groups. The treatment groups 3 and 4 (250 & 500mg/kg) showed no significant change in the hormone Testosterone as at when compared to control. 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 as observed in this study at 100mg/kg has been associated with a moderate but significant increase in sexual desire and penile function (Vingren *et al.,* 2010). Clinical data on testosterone show that a slightly increased testosterone level in adult males, results in an enhanced sexual desire and arousability (Valdes-Socin *et al*., 2017). 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 (Jameson *et al*., 2015).

In this research work, the observed increase in serum testosterone suggests that the *Andira inermis* stembark extract possesses a sex enhancing potential due to the presence of phytoconstituent such as flavonoids and alkaloid or saponin with relation to the dosage (100mg/kg). The result in this study also shows a statistically significant increase ($p<0.05$) in follicle stimulating hormone assay in treatment group 2 (100mg/kg), and then indicate a statistically significant downward change (decrease) in follicle stimulating hormone assay in treatment groups 3 and 4 (250,500mg/kg) as compared to the corresponding control group.

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 (Jiang *et al*., 2017).

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 body weight indicates that the extract improved secondary sexual characteristics, sexual health with libido at that moderate dose of 100mg/kg but decreases sexual function at 250mg/kg and 500mg/kg (Dasofunjo *et al*., 2020). This may be as a result of Phytoestrogens in more concentration and duration.

Phytoestrogens affect male reproductive hormones (FSH) by binding to ER α (estrogen receptor alpha) and ER β (estrogen receptor beta) and mimicking estradiol's conformational structure. They also act on the male reproductive system by behaving as an antioxidant or tyrosine kinase inhibitor and by interfering with the androgen receptor pathway and affects spermatogenesis in males (Giwercman, 2011).

Cooper in 2019 reported that phytoestrogens such as isoflavones exhibit a similar chemical structure to 17 β-estradiol as reported in this study. The two major soy isoflavones, namely, daidzein and genistein, bind to estrogen receptor beta (ER β), suggesting beneficial effects on fertility, although they are weak estrogens compared to endogenous E. The soy isoflavones act by two pathways; the hormonal and nonhormonal pathways, that involve the arrest or alteration of cellular growth through tyrosine kinase or epigenetics (Cooper *et al*., 2019; Chorosho *et al*., 2023). As endocrine cells, they secrete the 39-kDa glycoprotein inhibin, which feeds back on the anterior pituitary gland to suppress FSH synthesis and release. Elevated serum FSH is a reliable indicator of germinal epithelial damage (Tesarik *et al*., 2000).

Follicle Stimulating Hormorne is necessary for maintaining normal sperm count and function. Studies have shown that FSH deprivation not only lowers sperm count but also affects the quality of the remaining sperm (Orlowski *et al*.,2023). Elevated levels of FSH are associated with unresponsive gonads or hyperfunctioning pituitary adenomas. Low levels of FSH as observed in the treatment groups of 250 and 500mg/kg of *Andira inermis* stembark aqueous extract may be associated with either hypothalamic or anterior pituitary dysfunction.

Effect of administration of *Andira inermis* aqueous stembark extract on sperm count as displayed in Table 4 shows alterations of sperm cells. The table shows that Sperm counts decreased significantly from 22.13 ± 0.53 million/ml to 13.00 ±1.16 million/ml in *Andira inermis* treated rats of 90 days as compared to the corresponding control group. Exposure to exogenous estrogen either during development or adulthood, was said to induce

structural and functional changes in the male reproductive tract. Exposure of neonatal rats to estrogenic chemicals reduces sperm concentrations, (Goyal *et al.,* 2003), Sertoli cell number (Atanossova *et al*., 2005), and gene expression (Adachi *et al*., 2004).

Similar structural and functional abnormalities can be induced by anti-estrogen treatment of adult rats. It has also been demonstrated that dietary phytoestrogen exposure through a soy-containing rodent feed, influences spermatogenesis independently of the hypothalamo–pituitary–gonadal axis (Robertson *et al*., 2002).

Feeding a diet of high phytoestrogen content to adult rats, not previously exposed to elevated dietary phytoestrogen, was said to disrupts normal spermatogenesis by increasing apoptosis of developing germ cells. Spermatogenesis describes a process of mitotic clonal proliferation, genetic reduction by meiosis, and cell differentiation and remodeling. It has also been well established that the development of germ cells is dependent on testosterone and FSH, the absence of both hormones increasing germ cell apoptosis as can be observed in the results of this study.

Furthermore, there was evidence of stage-dependent changes in apoptosis between the high and low treatment groups. Increased germ cell apoptosis was observed with increase in concentrations of the extract across the treatment groups. This induction of apoptosis suggests, therefore, that the extract is anti-estrogenic since exposure of male rats to high phytoestrogen content increases germ cell apoptosis reducing testicular sperm numbers. This disruption of spermatogenesis may be independent of the hypothalamo–pituitary–testicular axis and is likely to be due to disruption of paracrine and/or autocrine actions of estrogen in the testis. (Oliveira et al., 2001)

This study's investigation into the effects of *Andira inermis* stembark extract on sperm motility reveal that *Andira inermis* stembark affected sperm motility by significantly decreasing it in male rats from 11.25 ± 0.41 million/ml to 8.00 ±0.59million/ml after treatment for 90days. This decrease was observed in all treatment groups as compared with the control group. The results indicate significant decline in sperm motility and morphology of the rats exposed to the extract for 90days as observed in table (4) which shows a statistically affected sperm morphology from 15.25 ±0.62million/ml to 7.00±0.80million/ml of the rat's sperm cells causing a decreased calculated morphology in all the treatment groups as compared to the control group. Normal sperm morphology was significantly affected by increased percentage of sperm with detached head, sticky cells and increased abnormal sperm tail morphology. These findings are similar to those reported from both animal studies (Amara *et al.*, 2008) and research involving humans (Wang *et al*., 2016). Various mechanisms may explain the reduced sperm quality induced by the extract since alteration in sperm parameters could be attributed to direct effect on

testicular tissue which leads to reproductive dysfunction such as reduced sperm count, motility and morphology (de Souza *et al.*, 2010). The extract may specifically disrupt Sertoli-germ cell tight junctions and thus leads to the failure of spermatogenesis. Profound testicular damage displays destruction of the seminiferous tubules and progressive sloughing of immature germ cells which result in abnormalities in early sperm development (Zhang *et al*., 2010). Furthermore, exposure to phytoestrogens may affects steroid hormone actions involved in the regulation of reproductive processes. The maintenance of normal steroidogenic enzymes activity is required for proper testicular steroidogenesis and spermatogenesis (Adamkovicova *et al.*, 2016).

CONCLUSION

The highlighted potential effects of *Andira inermis* stembark extract on male reproductive hormones, specifically follicle-stimulating hormone (FSH), indicated that the extract showed a significant increase in serum FSH levels at a moderate dose of 100mg/kg, which may contribute to improved sexual health and libido. However, higher doses of the extract (250mg/kg and 500mg/kg) resulted in a significant decrease in FSH levels, which could negatively impact sperm count and function. This decrease may be attributed to an increased concentration and duration of phytoestrogens in the extract, which can bind to estrogen receptors and interfere with androgen receptor pathways. Overall, these findings suggest the potential for *Andira inermis* stembark extract as a natural supplement for improving male reproductive health, but caution must be taken regarding dosage and duration of use.

Andira inermis stembark extract used in this study had a significant effect on sperm count, motility and morphology in male rats. Sperm motility decreased, and abnormal sperm morphology with detached head, sticky cells, and abnormal sperm tail morphology was observed in the treated rats, compared to the control group. The mechanisms behind the reduced sperm quality may include direct effects on testicular tissue, disruption of Sertoli-germ cell tight junctions, and phytoestrogen exposure affecting steroid hormone actions involved in the regulation of reproductive processes. These findings highlight potential reproductive dysfunctions associated with *Andira inermis* stembark extract exposure and suggest the need for further research into the effects of plant-based compounds on male reproductive health. The study showed that aqueous stembark extract of *Andira inermis* distorted the histomorphology of the testis of adult male Wistar rats. As such, males within reproductive age should be cautious in its longtime use.

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