

Steroidogenic Effects and Phytochemical Profile of Aqueous Extract of *Tetracarpidium conophorum* (Mull. Arg.) & Hutch Kernels in Male Rats

Herman Akassa^{1,2*}, Bonaventure Max Lazare Peneme^{1,2}, Constantin Moukouma^{3,4}, Dieu-Merci Bevel Gallo Mongo^{3,4}, Rachie Osandze^{1,2,3}, Arnaud Wilfrid Etou Ossibi^{1,3}, Ange Antoine Abena^{1,5}

¹Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Republic of the Congo

²Laboratory of Animal Physiology, Faculty of Science and Technology, Marien Ngouabi University, Brazzaville, Republic of the Congo

³Departement of Pharmacopeia and Traditional Medicine, National Institute for Research in Health Sciences (IRSSA), Brazzaville, Republic of the Congo

⁴Chemistry Unit of the Plant and Life, Faculty of the Sciences and Techniques, Marien Ngouabi University, Brazzaville, Republic of the Congo

⁵Faculty of Applied Sciences, Denis Sassou Nguesso University, Kintélé, Republic of the Congo

Email: *hermanakassa@gmail.com

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Abstract

The main aim of this study was to evaluate the steroidogenic effects and phytochemical profile of aqueous extract of *Tetracarpidium conophorum* in male rats. Assays of biochemical reproductive parameters (total cholesterol, serum testosterone, FSH and LH) were carried out using Cypress and ELISA kits respectively. The chemical screening of the extract was conducted through tube reactions, the principle of which involves adding a reagent to a quantity of the extract, and subsequently observing the resulting reactions such as precipitation and color change. The results obtained showed that oral administration of the aqueous extract of *Tetracarpidium conophorum* (250 and 500 mg/kg) produced a significant increase ($p < 0.05$; $p < 0.01$) in testicular weight compared with the control lot. The aqueous extract of *Tetracarpidium conophorum* kernels at the doses studied produced a very significant decrease in total cholesterol levels ($p < 0.001$) and a significant increase ($p < 0.05$; $p < 0.01$) in serum testosterone and FSH levels. However, no variation was observed in LH levels. Phytochemical analysis of the extract revealed the presence of alkaloids, flavonoids, tannins, steroids, triterpenes, sugars, anthraquinones and saponosides. These results suggest that the aqueous extract of *Tetracarpidium conophorum* kernels could have hypocholesterolemic and steroidogenic potential. A study of the aqueous extract of *Tetracarpidium conophorum* on semen quality and oxidative stress parameters deserves to be conducted.

Keywords

Tetracarpidium conophorum, Steroidogenic, Chemical Screaming

1. Introduction

The endocrine system is made up of a set of glands (endocrines) that secrete mediator molecules called hormones. In particular, they regulate the functioning of the reproductive system, as well as the growth and development of tissues and organs [1]. However, this hormonal balance can be disturbed by the presence of chemical substances foreign to a living organism. These xenobiotic substances from natural or anthropogenic sources interfere with the functioning of the endocrine system (endocrine disruptors), which can lead to undesirable effects in organisms, their offspring or sub-populations [2] that can induce hypogonadism. This androgen deficiency is also accompanied by impaired sexual activity. Although erections are possible, there appears to be a direct relationship between hypogonadism and an alteration in the number and quality (rigidity) of nocturnal erections [3]. Ejaculation and libido disorders are also very common. In hypergonadotropic hypogonadism, testicular endocrine function is impaired. The main conditions causing hypergonadotropic hypogonadism are Klinefelter's syndrome (genetic anomaly causing one of the most frequent forms of hypogonadism in men, characterized by testicular dysgenesis), Myotonic Dystrophy (or Steiner's disease), a congenital disorder which, in 80% of cases, is accompanied by impaired testicular endocrine function, or anorchidism, characterized by the absence of testes in a boy with a normal karyotype, leading to severe androgen deficiency. There are two main alternatives to this problem, namely the use of synthetic drugs on the one hand, and medicinal plants on the other. Herbal medicine is an alternative practice referring to the use of plant parts or their extracts and essential oils for medicinal purposes. It has grown in recent times and has also attracted the attention of many types of research worldwide. As a result, natural plant products have been used to improve male fertility and manage certain physiological disorders. This is because they are rich in numerous natural antioxidants such as phenols, flavonoids, trapezoids and Xanthos. They are readily available, inexpensive and relatively safe. *Tetracarpidium conophorum* belongs to the Euphorbiaceae family and is prized above all for its aphrodisiac potential. It is used in Congolese pharmacopoeia to treat male infertility.

Tetracarpidium conophorum has been the subject of a number of research studies, notably in the field of animal nutrition [4]; studies have been carried out to characterize the potential of *Tetracarpidium conophorum* seeds [5] and the toxicological effects of the aqueous extract of *Tetracarpidium conophorum* nuts in rats [6]. However, there have been no studies evaluating the androgenic potential of the bark of the aqueous extract of *Tetracarpidium conophorum* kernels. With this in mind, the main aim of this study was to evaluate the steroido-

genic effects and phytochemical profile of aqueous extract of *Tetracarpidium conophorum* in male rats.

2. Materials and Methods

2.1. Plant Material

Tetracarpidium conophorum kernels from Lekana in the Plateaux department (Republic of the Congo) were supplied by vendors at the Lekana market in 2023. *Tetracarpidium conophorum* was identified at the National Institute for Research in Exact and Natural Sciences (IRSEN).

2.2. Animal Material

Three-month-old male Wistar rats weighing between 200 and 250 g were used. These rodents were bred at the animal house of the National Institute for Research in Health Sciences (IRSSA) and fed in the standard way with free access to water and a nocturnal-diurnal (12/12) lighting rhythm.

2.3. Preparation of the Aqueous Extract of *Tetracarpidium conophorum* Kernels

The aqueous extract of *Tetracarpidium conophorum* kernels was prepared by maceration. *Tetracarpidium conophorum* kernels were stripped of their hulls or skins, and then air-dried in the laboratory at room temperature (28°C - 30°C) for 21 days. They were then crushed and ground in a mortar to obtain a homogeneous powder. One hundred grams of powder were mixed with 1000 mL of distilled water. The resulting mixture was then placed under a magnetic stirrer (model L-73) for 48 hours. The resulting macerate was filtered through Whatman n°3 filter paper and absorbent cotton. The filtrate obtained was concentrated in a water bath thermostated at 55°C for 3 days, yielding 3.5 g of brown-colored dry extract, which was stored at +4°C in an Appolo Brant brand refrigerator for pharmacological testing.

2.4. Preparation of the Reference Androgen Solution.

Testosterone enanthate (Androtardyl) as an intramuscular injectable solution was used. The dose required, according to the manufacturer's instructions, is 3.6 mg/kg in man or 0.54 mg for a 200 g rat. To facilitate administration, two successive dilutions yielded a 2.5 mg/ml solution. A single dose of 0.2 ml of this solution was administered intramuscularly to each animal [7].

We use the following formula to determine the volume: $V = \frac{D \times P}{C}$, avec: V: Volume (mL), D: Dose (mg/kg), C: Concentration of the reference molecule (g/mL).

2.5. Evaluation of the Steroidogenic Effects of the Aqueous Extract of *Tetracarpidium conophorum* Kernels

To evaluate the steroidogenic effects of this extract, twenty (n = 20) rats were

randomly divided into 4 lots of 5 animals each and received during 28 days various experimental doses of the aqueous extract of *Tetracarpidium conophorum* kernels (250 and 500 mg/kg po), testosterone enanthate (molecule reference 3, 6 mg/kg i.m) and distilled water (control, 1 mL/100g po).

NB: The reference molecule was administrated once intramuscularly for 28 days according to the use of the product.

2.5.1. Effect of Aqueous Extract of *Tetracarpidium conophorum* Kernels on Androgen-Dependent Sex Organ Weight

This study was carried out to assess the effects of the presumed trophic extract on the weight of androgen-dependent organs. The weight, size and secretory functions of the testes, epididymides, seminal vesicles, vas deferens and penis are regulated by androgens. After 28 days of per os administration at doses of 250 and 500 mg/kg of *Tetracarpidium conophorum*, the animals per lot were sacrificed by overdose with Cooper's ether (anesthesia) by inhalation of between 08:00 a.m. and 10:00 a.m. The testes, epididymides, vas deferens, seminal vesicles and penis were removed, cleared of fatty material and then weighed using a branded precision balance (Mettler-Tdedo) of capacity (160 g) and accuracy (10^{-3} g). [8] [9].

2.5.2. Effect of Aqueous Extract of *Tetracarpidium conophorum* Kernels on Biochemical Parameters

Serum collection

24 hours after the last treatment with *Tetracarpidium conophorum* extract (250 and 500 mg/kg), the animals were anesthetized with Cooper's ether by inhalation of between 08:00 a.m. and 10:00 a.m. Whole blood of each animal was collected through orbital route using hematocrit tubes and stored in dry tubes without anticoagulant for 4 hours. The supernatant collected after 4 hours was distributed to the marked tubes and stored at -20°C for determination of serum total cholesterol, testosterone, FSH and LH.

Hormone assays (Testosterone, LH and FSH)

Serum testosterone, FSH and LH were determined using the ELISA Accu-Diag™ Kit (Diagnostic Automation Inc). (Enzym Linked Immunoassay) techniques, using commercial kits (testosterone kit CYPRESS, LH and FSH kit CYPRESS), following the manufacturer's instructions.

2.6. Research into Chemical Families

Chemical screening of *Tetracarpidium conophorum* aqueous extract was carried out using tube reactions [10] [11]. The principle is based on the addition of a reagent to a quantity of extract and subsequent monitoring of the resulting reactions: precipitation, change in coloration.

2.6.1. Alkaloids

5 ml aqueous extract is placed in a test tube. Add 1 ml 1N hydrochloric acid and a few drops of reagent. A red precipitate is formed (with Dragendoff's reagent); a

yellowish precipitate (with Mayer's reagent).

2.6.2. Tannins

From 5 ml of a 5% infusion (15 mn), add an aqueous solution of iron(III) chloride (1 ml). In the presence of tannins, a greenish or blue-blackish coloration develops.

2.6.3. Flavonoids

Place 5 ml of 5% infusion, 5 ml hydrochloric acid solution (HCl), 1 ml isoamyl alcohol and a few magnesium chips in a test tube. The appearance of an orange-pink indicates the presence of flavones:

- Purplish pink indicates the presence of flavanones.
- Red indicates the presence of flavanols.

2.6.4. Sterols and Triterpenes (Liebermann-Buchard Reaction)

Dissolve the dry extract (10 ml) obtained in 1 ml acetic anhydride and add 1 ml chloroform. Divide the solution between two test tubes, one of which serves as a control. Using a pipette, place 1 ml sulfuric acid in the bottom of one. A brownish-red or violet ring forms where the two lipids come into contact, and the supernatant layer turns green or violet, revealing the presence of sterols and triterpenes.

2.6.5. Reducing Compounds

In a beaker, we dry-evaporated 10% aqueous decoctate (5 ml) in a water bath. To the decoctate residue, add Fehling's liqueur (1 ml). The result is a red precipitate characteristic of reducing compounds.

2.7. Statistical Analysis of Collected Data

Statistical analysis of the data collected was carried out using Analysis of Variance (ANOVA), Student's t-test and Mann-Whitney to compare the "test" groups. Results were expressed as mean \pm standard error with $p < 0.05$ as the significance threshold.

3. Results

3.1. Effect of Aqueous Extract of *Tetracarpidium conophorum* Kernels on Androgen-Dependent Sex Organs Weight

The weight of androgen-dependent organs (epididymides, vas deferens, seminal vesicles and penis) in animals treated with aqueous extract of *Tetracarpidium conophorum* (250 and 500 mg/kg/ po) showed no variation from the control lot. However, testicular weight increased significantly ($p < 0.05$; $p < 0.01$) compared to that of control rats treated with distilled water. This increase is comparable to that of the reference molecule (**Table 1**).

3.2. Effect of Aqueous Extract of *Tetracarpidium conophorum* Kernels on Total Cholesterol Levels

The effects of aqueous extract of *Tetracarpidium conophorum* (250 and 500

mg/kg po) on serum total cholesterol levels are plotted in **Figure 1**. These results show that administration of aqueous extract of *Tetracarpidium conophorum* (250 and 500 mg/kg po) resulted a decrease significant ($p < 0.001$) dose-dependent in serum total cholesterol levels compared with animals from the control lot.

3.3. Effect of Aqueous Extract of *Tetracarpidium conophorum* Kernels on Testosterone Levels

Figure 2 below shows the effects of *Tetracarpidium conophorum* aqueous extract on testosterone levels. **Figure 2** shows that gavage of rats with aqueous extract of *Tetracarpidium conophorum* (250 and 500 mg/kg po) caused a significant increase ($p < 0.05$; $p < 0.01$) in serum testosterone levels compared with control rats treated with distilled water. This increase is comparable to testosterone enanthate.

Table 1. Effect of aqueous extract of *Tetracarpidium conophorum* kernels on androgen-dependent sex organs weight.

Androgenic Parameters Dependent (g)	Treatment			
	Distilled Water (1 mL/100g)	Testosterone Enanthate (3.6 mg/kg)	<i>Tetracarpidium conophorum</i> (250 mg/kg)	<i>Tetracarpidium conophorum</i> (500 mg/kg)
Testes	3.33 ± 0.03 ^{ns}	3.56 ± 0.03 ^{**}	3. ± 0.06 [*]	3.60 ± 0.00 ^{**}
Epididymides	0.53 ± 0.08 ^{ns}	0.46 ± 0.03 ^{ns}	0.43 ± 0.03 ^{ns}	0.36 ± 0.03 ^{ns}
Vas Deferens	0.4 ± 3.92 ^{ns}	0.4 ± 3.92 ^{ns}	0.2 ± 1.96 ^{ns}	0.2 ± 1.96 ^{ns}
Seminal Vesicles	1.46 ± 0.06 ^{ns}	1.43 ± 0.08 ^{ns}	1.46 ± 0.06 ^{ns}	1.46 ± 0.08 ^{ns}
Penis	0.36 ± 0.06 ^{ns}	0.5 ± 0.05 ^{ns}	0.36 ± 0.03 ^{ns}	0.33 ± 0.03 ^{ns}

Note: Values are means ± SEM, with n = 5; *: $p < 0.05$; **: $p < 0.01$, significant difference from controls (distilled water), ns: $p > 0.05$, non-significant difference from controls (distilled water).

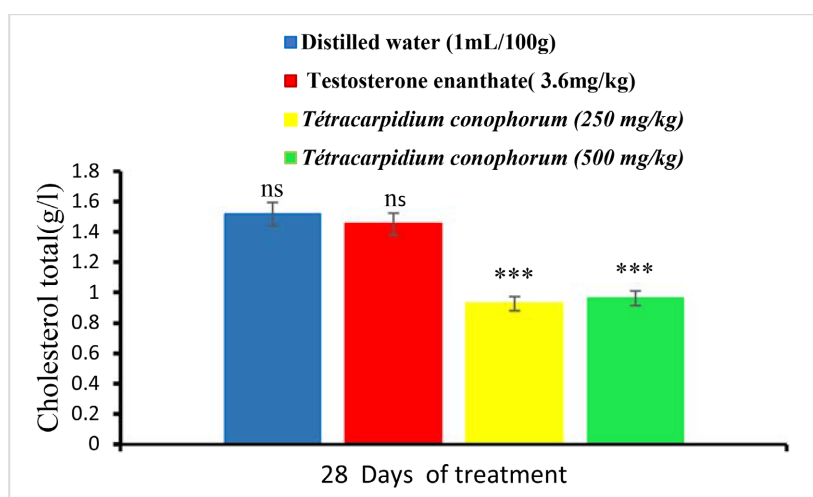


Figure 1. Effect of aqueous extract of *Tetracarpidium conophorum* kernels on total cholesterol levels. ns: $p > 0.05$, non-significant difference from control (distilled water); ***: $p < 0.001$, significant difference from control (distilled water).

3.4. Effect of Aqueous Extract of *Tetracarpidium conophorum* Kernels on LH Levels

The effects of *Tetracarpidium conophorum* aqueous extract on LH levels are shown in **Figure 3**. The results show that administration of *Tetracarpidium conophorum* aqueous extract to rats at the doses studied produced no significant variation compared with rats treated with distilled water.

3.5. Effect of Aqueous Extract of *Tetracarpidium conophorum* Kernels on FSH Levels

The effects of aqueous extract of *Tetracarpidium conophorum* on FSH levels are summarized in **Figure 4**, which shows that oral administration of aqueous extract of *Tetracarpidium conophorum* (250 and 500 mg/kg po) to rats produced a significant ($p < 0.05$) dose-dependent increase in FSH levels compared with the

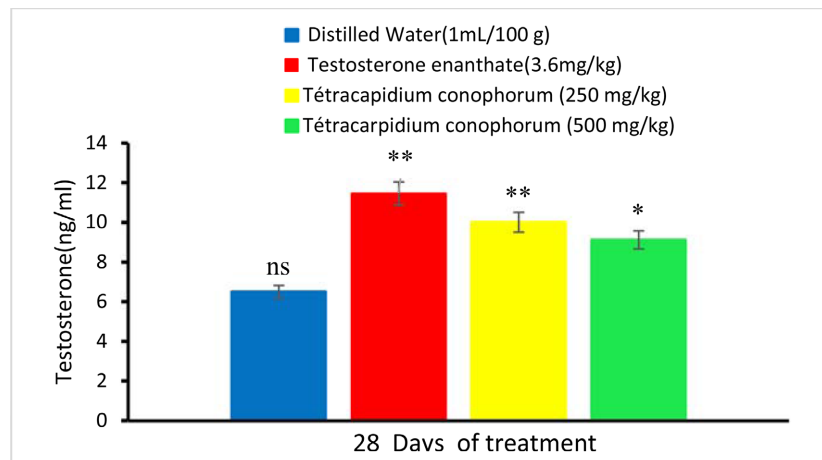


Figure 2. Effect of aqueous extract of *Tetracarpidium conophorum* kernels on testosterone levels. ns: $p > 0.05$, non-significant difference from control (distilled water); *: $p < 0.05$, significant difference from control (distilled water); **: $p < 0.01$, significant difference from control (distilled water).

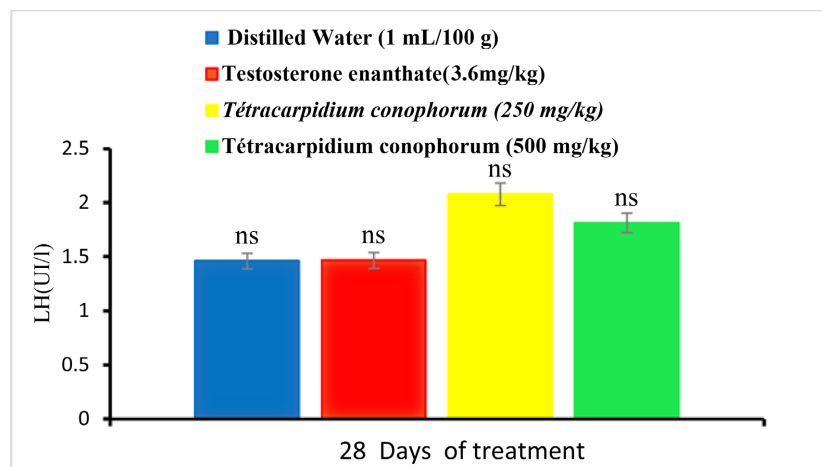


Figure 3. Effect of aqueous extract of *Tetracarpidium conophorum* kernels on LH levels. ns: $p > 0.05$, non-significant difference from control (distilled water).

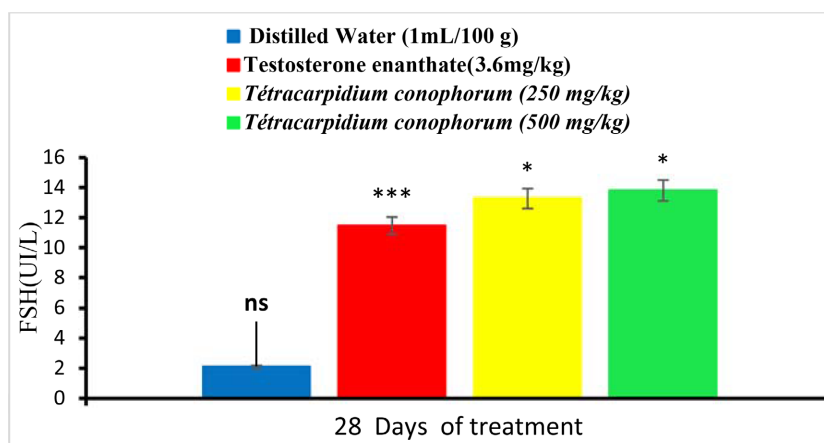


Figure 4. Effect of aqueous extract of *Tetracarpidium conophorum* kernels on FSH levels. ns: $p > 0.05$, non-significant difference from control (distilled water); *: $p < 0.05$, significant difference from control (distilled water); ***: $p < 0.001$, significant difference from control (distilled water).

Table 2. Chemical screening results.

Families Searched	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Triterpenes	+
Sugars	+
Anthraquinone	-
Saponosides	-

Note: +: presence; -: absence.

control lot treated with distilled water. This increase was highly significant ($p < 0.001$) in rats treated with the reference molecule.

3.6. Searching for Chemical Families

The phytochemical screening of the aqueous extract of *Tetracarpidium conophorum* kernels is presented in **Table 2**. The obtained results show that analysis of the extract showed the presence of the following chemical families: alkaloids, flavonoids, tannins, steroids, triterpenes, sugars, anthraquinones and saponosides.

4. Discussion

The main aim of this study was to evaluate the steroidogenic effects and phytochemical profile of aqueous extract of *Tetracarpidium conophorum* in male rats. The results obtained showed that administration of the aqueous extract of *Tetracarpidium conophorum* kernels to rats at the doses studied produced a sig-

nificant increase in testicular weight compared with the control lot given distilled water. However, no variation was observed in the weights of the epididymides, vas deferens, seminal vesicles and penis. These results are close to those obtained by [8] in rats treated with aqueous extract of *Pausinystalia yohimbe* at doses of 100 and 250 mg/kg respectively. Our results contradict those obtained by [12], which observed a non-significant increase in testicular weight in rats treated with ethanolic extract of *Strychnos camptoneura* trunk bark. This difference was attributed to the type of extract, the different plant organs, the extraction techniques used and the presence or absence of the various secondary metabolites in the plant. Gavage of rats with the aqueous extract of *Tetracarpidium conophorum* kernels (250 and 500 mg/kg po) produced a significant increase in serum testosterone levels compared with control rats treated with distilled water. These results are similar to those of [13] in rats treated with *Tetracarpidium conophorum* (300 and 400 mg/kg), and are also in agreement with [8] [14], which showed that the treatment with aqueous extract of *Zanthoxylum macrophylla* in rats respectively and *Strychnos camptoneura* in rats caused an increase in serum testosterone levels. The aqueous extract kernels of *Tetracarpidium conophorum* could have a steroidogenic action on the hypothalamic-pituitary-testicular axis, thus stimulating the synthesis of testosterone by Leydig cells. Androgens, whose main hormone is testosterone, have anabolic action, causing an increase in protein synthesis and therefore muscle mass. The aqueous extract of *Tetracarpidium conophorum* could have a steroidogenic action on the hypothalamic-pituitary-testicular axis, thus stimulating the synthesis of testosterone by Leydig cells. Cholesterol is the precursor lipid in testosterone synthesis [8]. The very significant decrease in cholesterol levels suggests that the aqueous extract of *Tetracarpidium conophorum* kernels probably has hypolipidic and cardioprotective potential. The very significant decrease in cholesterol levels would justify the significant increase in testosterone levels. This plant contains sterol and it has been established that plant sterols exert their hypocholesterolemic effect by inhibiting intestinal absorption of dietary as well as endogenous cholesterol. However, the molecular mechanisms for such inhibition are still not fully understood. It is generally accepted that the presence of increased quantities of plant sterols compete with cholesterol for micellar solubility, thereby lowering the amount of cholesterol available for absorption by intestinal mucosal cells. Plant sterols have been shown in clinical trials to block absorption sites in human thus helping to reduce cholesterol level in humans [15]. These results show that administration of aqueous extract of *Tetracarpidium conophorum* (250 and 500 mg/kg po) results in a highly significant dose-dependent decrease in serum total cholesterol levels compared to animals in the control lot [15]. These results corroborate those of [15], who also obtained a significant decrease in cholesterol levels in adults (adults) who consumed *Tetracarpidium conophorum* nuts. The results show that administration of the aqueous extract of *Tetracarpidium conophorum* at the doses studied in rats produced no significant variation in LH le-

vels compared with rats treated with distilled water. However, oral administration of aqueous extract of *Tetracarpidium conophorum* kernels (250 and 500 mg/kg po) in rats produced a significant dose-dependent increase in LH levels compared with the control group treated with distilled water. The increase in FSH levels indicates that the aqueous extract of *Tetracarpidium conophorum* kernels may have potential spermatogenic effects. The improvement in hormonal parameters and serum cholesterol levels observed in this study is therefore attributed to the presence of secondary metabolites in the extract. Indeed, phytochemical analysis of the extract revealed the presence of the following chemical families: alkaloids, flavonoids, tannins, steroids, triterpenes, sugars, anthraquinones and saponosides. These phytochemical compounds have several pharmacological potentialities [16] [17] [18] [19]. Indeed, the steroidal nature of saponins could facilitate an intermediary role in the androgen production pathway [20]. Other studies have shown that flavonoids have antioxidant properties and are reported to elevate androgen levels in animals; they may also contribute to the observed aphrodisiac effect [21] [22].

5. Conclusion

These results suggest that the aqueous extract of *Tetracarpidium conophorum* kernels has hypocholesterolemic and steroidogenic potential. A study of the extract aqueous *Tetracarpidium conophorum* on semen quality and oxidative stress parameters deserves to be conducted.

Statement of Ethical Approval

The present research work does not contain any studies performed on animal/human subjects by any of the authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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