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Effects of Aqueous Extract of *Tetracarpidium conophorum* (Mull. Arg.) & Hutch Kernels on Semen Quality and Oxidative Stress Parameters in Male Rats

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Abstract: The aim of the present study was to evaluate the effects of the aqueous extract of *Tetracarpidium conophorum* kernels on semen quality and oxidative stress parameters in male rats. 20 rats were randomly divided into 4 lots of 5 animals each, and lots 1 and 2 were given distilled water and the reference molecule for 28 days, while lots 3 and 4 were treated with the aqueous extract of *Tetracarpidium conophorum* kernels (250 and 500 mg/kg/po). The results show that administration of aqueous extract of *Tetracarpidium conophorum* kernels (250 and 500 mg/kg/po) to rats produced significant dose-dependent increases ($p < 0.05$; $p < 0.01$) in sperm count and motility compared with the control lot treated with distilled water. On the other hand, pH and sperm vitality showed no significant variation ($p > 0.05$). Administration of the aqueous extract of *Tetracarpidium conophorum* kernels at the doses studied resulted in no significant variation ($p > 0.05$) in MDA and CAT concentrations compared with distilled water. However, there was a significant increase ($p < 0.05$; $p < 0.001$) in GSH, and GPX concentrations at the respective doses of 500, 250 and 500 mg/kg of *Tetracarpidium conophorum* aqueous extract compared to rats given distilled water. Chemical analysis of the aqueous extract of *Tetracarpidium conophorum* kernels revealed the presence of polyphenols and total flavonoids with anti-free radical and antioxidant effects. These results suggest that the aqueous extract of *Tetracarpidium conophorum* may have spermatogenic and antioxidant potential.

Keywords: *Tetracarpidium conophorum*, Oxidative Stress, Semen

1. Introduction

Infertility is conventionally defined by the World Health Organization (WHO) as the inability to conceive naturally after one year of frequent unprotected mating [1]. Epidemiological data show that around 15% of couples experiencing difficulties in having children consult a doctor [2]. From the point of view of responsibility, in most cases, the woman is systematically incriminated [3]. In fact, around 40% of cases of male infertility are thought to be due to very high levels of free radicals of endogenous or exogenous origin in the seminal fluid [4]. Free radicals are physiological

products of cellular metabolism, which can become deleterious to spermatozoa through either increased production, or failure to degrade or eliminate them [5]. Recent studies have shown that male germ cells are highly sensitive to environmental factors (pesticides, heavy metals and synthetic drugs) [6-8], which have adverse effects on animal and human reproduction, resulting in the production of large quantities of free radicals that cause oxidative stress, leading to infertility or male sterility [9, 10]. To remedy this situation, many of the drugs currently in use are derived from plants producing metabolites that can treat male infertility, which has also become a public health problem [11]. The treatments proposed by modern medicine are often too costly, inaccessible, of questionable efficacy and sometimes present numerous side effects [12, 13]. *Tetracarpidium conophorum* belongs to the Euphorbiaceae family and is particularly prized for its aphrodisiac potential, being used in the Congolese pharmacopoeia for the treatment of male infertility.

Tetracarpidium conophorum has been the subject of a number of research projects, notably in the animal feed sector [14]. Studies characterizing the potential of *Tetracarpidium conophorum* seeds have also been carried out [15], as have studies on the toxicological effects of the aqueous extract of *Tetracarpidium conophorum* nuts in rats [16]. However, the effects of aqueous extract of *Tetracarpidium conophorum* kernels on semen quality and oxidative stress parameters have not yet been investigated. Therefore, the present study was initiated with the main objective of evaluating the effects of aqueous extract of *Tetracarpidium conophorum* kernels on semen quality and oxidative stress parameters in male rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Materials

Tetracarpidium conophorum kernels from Lekana in the Plateaux department (Republic of Congo) were supplied by vendors at the Lekana market in 2023. Identification of *Tetracarpidium conophorum* was carried out at the Institut National de Recherche en Sciences Exactes et Naturelles (I. R. S. E. N.).

Figure 1. *Tetracarpidium conophorum kernels.*

2.1.2. Animal Material

Three (3)-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 a standard diet, with free access to water and a 12-hour/12-hour (12/12) nocturnal-diurnal lighting cycle.

2.2. Methods

2.2.1. 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, then air-dried in the laboratory at room temperature (28-30°C) for 21 days. They were then crushed and ground in a mortar to obtain a homogeneous powder. One hundred grams (100g) of powder was mixed with 1000 mL of distilled water. The resulting mixture was then placed on 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 on 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 Apollo Brant refrigerator for pharmacological testing.

2.2.2. Preparation of the Reference Androgen Solution

Testosterone enanthate (Androtardyl) for intramuscular injection was used. The dose required, in accordance with the manufacturer's instructions, is 3.6 mg/kg in man, or 0.5mg for a 200 g rat. To facilitate administration, two successive dilutions were made to obtain a 2.5mg/ml solution. A single dose of 0.2 ml of this solution was administered intramuscularly to each animal [17]. 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]. To determine the volume administered, we used the following formula:

$$V = \frac{DxP}{C},$$

where: V: Volume (mL), D: Dose (mg/kg), C: Concentration of the reference molecule (g/mL)

2.2.3. Evaluation of the Effects of Tetracarpidium conophorum Kernel Extract on Semen Quality and Oxidative Stress Parameters in Rats

To assess the effects of *Tetracarpidium conophorum* kernel extract on semen quality and oxidative stress parameters in rats, 20 rats were randomly divided into 4 lots of 5 animals each and fed for 28 days:

a. Distilled water at a dose of 1 ml/100 g/po) (negative control lot);
b. Testosterone enanthate at 3.6 mg/kg/in (reference molecule lot);
c. Aqueous extract of *Tetracarpidium conophorum* kernels

at 250 and 500 mg/kg/po. respectively. Note: The reference molecule was administered once intramuscularly for a period of 28 days, in accordance with the drug's instructions.

(i). Effects of Aqueous Extract of Tetracarpidium conophorum Kernels on Some Semen Characteristics

The effects of the aqueous extract of *Tetracarpidium conophorum* kernels on semen quality were studied in order to assess its impact on sperm function (sperm motility, concentration and pH). To this end, after the animals had been sacrificed by ethyl ether overdose and the organs removed, the tail of the right epididymis of each rat was excised, weighed and dilacerated in a Petri dish containing 10 ml of 0.9% NaCl solution, then incubated in a water bath at 36°C [3, 10].

(ii). Effect of Tetracarpidium conophorum Kernel Extract on Sperm Motility

Motility is an important indicator of sperm vitality [3, 10]. Indeed, good quality sperm should contain at least 60-70% motile sperm with a motility grade or coefficient of 4 or 5 [18]. Sperm motility was assessed by direct examination of the solution. Twenty microliters 20µl of this solution were placed between slide and coverslip at ×40 magnification. Motile and immobile spermatozoa were counted on 12 randomly selected microscopic fields, and the percentage of motile spermatozoa was determined using the following formula [19].

$$\%\ of\ motile\ spermatozoa\ = \frac{\text{Number of motile spermatozoa}}{\text{Total number of spermatozoa}} \times 100$$

(iii). Effect of Aqueous Extract of Tetracarpidium conophorum Kernels on Sperm Concentration

Sperm counts were carried out using the Thoma cell. For this purpose, semen was diluted 100-fold with a formalin solution (35%) to fix the sperm and render them immobile during counting (for counting purposes). Ten 10µl microliters of this diluted semen were placed in the Thoma cell chamber (surface area = 0.2 mm² and depth = 0.1 mm), and covered with a cover slide (and covered with a cover slip). Counting was performed under a microscope (Leica DM 750) at ×40 magnification. Sperm were counted in five large squares. This operation was repeated 2 times, and the average thus calculated was used to determine the number of sperm per epididymal tail, according to the following formula: Number of sperm per tail = number of sperm per mm^3 × 1000 × 10, where 10 is the volume of the solution in ml and 1000 is the conversion factor from mm^3 to ml [3, 10].

(iv). Effect of Extract on Sperm pH and Vitality

The pH was measured not from pure spermatozoa, but directly from spermatozoa suspended in 2 ml of physiological water, and the pH reading was taken using urine strips. Vitality was assessed using a specific methodology: preparation of the slide used to count live and dead spermatozoa; staining of spermatozoa, then observation under an optical microscope (objective 40×) to read 200 spermatozoa. On smears, live spermatozoa are stained red with 2% eosin, while dead spermatozoa are not stained. [8, 10].

(v). Determination of Oxidative Stress Parameters

a. Preparation of epididymal homogenates

Harvested organs were weighed and ground in porcelain mortars. The 50 mM Tris-HCl buffer solution for epididymides was added to the mortars to obtain 20% homogenates. These homogenates were centrifuged at 4000 rpm for 40 minutes. The supernatant collected was aliquoted and stored in a freezer (-32°C) for the determination of some oxidative stress parameters.

b. Catalase assay (CAT)

Principle

The method used to determine the enzymatic activity of CAT is that of [20]. CAT catalyzes the destruction of hydrogen peroxide into water and oxygen. CAT activity was measured at 240 nm using a spectrophotometer, by observing the variation in optical density following H_2O_2 dismutation at an incubation temperature of 25°C.

c. Glutathione peroxidase (GPx) determination

Principle

To estimate the enzymatic activity of glutathione peroxidase, we used the method which is based on the reduction of H_2O_2 in the presence of reduced glutathione (GSH), which is converted into a (GSSH) (transforming the latter into oxidized glutathione (GSSH)) in the presence of GPx according to the following reaction:

$$H_2O_2 + 2GSH \xrightarrow{GPx} GSSH + 2H_2O$$

d. Malondialdehyde (MDA) determination

Principle

Lipid peroxidation in the kidney is assessed by measuring malondialdehyde (MDA) according to the colorimetric method [21]. MDA is one of the end products of polyunsaturated fatty acid (PUFA) degradation under the influence of free radicals generated during stress. In a hot, acidic environment (pH 2 to 3, 100°C), a molecule of MDA reacts with two molecules of thiobarbituric acid (TBA) to form a pink-colored complex (read at 532 nm).

e. Reduced glutathione (GSH) determination

Principle

The determination of GSH is based on a colorimetric method [21]. The deglutathione determination is based on the oxidation of GSH by 5, 5'- Dithiobis 2-nitrobenzoic acid (DTNB), releasing thionitrobenzoic acid (TNB) which absorbs at 412 nm.

(vi). Determination of Total Polyphenols and Flavonoids

a. Determination of total polyphenols

Total polyphenols were determined using a spectrophotometer. We determined the optical densities of our extracts and compared the results with those obtained using a gallic acid standard of known concentration. The determinantion was carried out as follows: 0.1 ml plant extract was introduced into a test tube, to which was added 0.9 ml distilled water, 0.9 ml Folin-Ciocalteu reagent (1N) and immediately 0.2 ml Na_2CO_3 solution (20%). The resulting mixture was incubated at

room temperature for 40 minutes, protected from light. Absorbance was then measured with a spectrophotometer at 725 nm against a methanol solution used as a blank. It should be noted that a straight-line calibration curve was made prior to analysis with gallic acid under the same conditions as the samples to be analyzed. The results obtained were expressed in mg gallic acid equivalent per 100 grams of dry matter (mgEGa/100 gMs). [22].

b. Determination of total flavonoids

Total flavonoids were also determined using a spectrophotometer, as follows: 250 µl of extract and 1 ml of distilled water were successively introduced into a test tube. At the initial time (0 min), 75 µl of $NaNO_2$ solution (5%) was added, followed by 75 µl of $AlCl_3$ (10%) 5 min later. After 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water were successively added to the mixture. The absorbance of the resulting mixture was directly measured with a UV-visible spectrophotometer at 510 nm, and the results expressed as mg Rutin equivalent per 100 grams of dry matter (mgERu/100g Ms). A calibration curve was established using standard Rutin solutions prepared at different concentrations [23].

2.2.4. 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 test to compare test groups. Results are expressed as mean ± standard error, with $p < 0.05$ as the threshold of significance.

3. Results

3.1. Effect of Tetracarpidium conophorum Aqueous Extract on Some Semen Characteristics

Table 1 summarizes the effect of aqueous extract of *Tetracarpidium conophorum* on a number of semen characteristics. The results show that administration of aqueous extract of *Tetracarpidium conophorum* (250 and 500mg/kg/po) significantly ($p < 0.05$; $p < 0.01$) increased sperm count and mass motility compared with control rats given distilled water, and did not significantly ($p > 0.05$) alter sperm pH and vitality.

Table 1. *Effect of Tetracarpidium conophorum hydroethanol extract on some semen characteristics.*

Sperm characteristics	Traitements			
	Negative control 1mL/100g	Testosterone enanthate 3.6mg/kg	A. T. C extract. 100mg/kg	A. T. C extract 250mg/kg
pH	6.5±0. 30	6.6 ±0.45ns	6. 3 ±0.20ns	6. 2±0.26 ns
Number of sperm / epididymal tail ($\times 10^6$)	13.33±0.88	14±1.15ns	21.33±2.02*	23±2.88*
Sperm mass mobility (%)	26.66±6.66	33.33±4.08ns	81.66±6.12**	85±2.04**
Sperm vitality (%)	73.33±3.33	80±5.77ns	78.33±12.01ns	81.66±4.40ns

Values are means ± MSE, with n = 5. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$ indicating a significant difference from controls (distilled water, ns: $P > 0.05$ indicating non-significant difference from control (distilled water) and A. T. C: Aqueous *Tetracarpidium conophorum*

3.2. Effect of Tetracarpidium conophorum Aqueous Extract on Oxidative Stress Parameters

The effect of *Tetracarpidium conophorum* aqueous extract on some oxidative stress parameters is shown in Table 2. It shows that oral administration of the aqueous extract of *Tetracarpidium conophorum* kernels did not lead to any significant variation ($p > 0.05$) in Malondialdehyde (MDA) and Catalase (CAT) concentrations, whatever the doses used, compared with animals from the control lot. On the other hand, there was a significant increase ($p < 0.05$; $p < 0.001$) in the concentrations of reduced Glutathione (GSH) and Glutathione peroxidase (GPX) at the respective doses of 500, 250 and 500 mg/kg of aqueous extract of *Tetracarpidium conophorum* compared with rats fed distilled water.

Table 2. *Effect of Tetracarpidium conophorum aqueous extract on oxidative stress parameters.*

Oxidative stress parameters	Distilled water (1 ml/100 g)	testostérone Enanthate (3.6mg/mL)	Extrai*t AT. C* (250 mg/kg)	Extrait AT. C (500 mg/kg)
MDA (µmol)	0.57 ± 0.01	0.61± 0.01ns	0.60 ± 0.04ns	0.59 ± 0.01ns
CAT (µmol H_2O_2/min)	0.52 ± 0.38	0.17± 0.05ns	0.16 ± 0.17ns	0.17 ± 0.08ns
GSH (Nm)	2.39 ± 0.09	2.63 ± 0.08ns	2.73 ±0.14ns	2.89 ± 0.15*
GPX (µmol de GSH)	0.42 ± 0.00	1.60 ± 0.01***	2.36 ± 0.01***	0.82± 0.00***

Values are means ± MSE, with n = 5. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$ indicating significant difference from controls (distilled water), ns: $P > 0.05$ not significantly different from controls (distilled water) and A. T. C: Aqueous *Tetracarpidium conophorum,* Malondialdehyde: (MDA), Catalase (CAT), Reduced Glutathione (GSH) and Glutathione peroxidase (GPx),

3.3. Chemical Analysis of Tetracarpidium conophorum Aqueous Extract

The results of quantitative UV-visible spectrophotometer analyses of the *Tetracarpidium conophorum* aqueous extract studied are shown in Figure 2. This figure shows that total polyphenol contents are quantitatively higher than total flavonoid contents, with values of 340 mg EAG/gMS and 18

mg EO/g MS respectively.

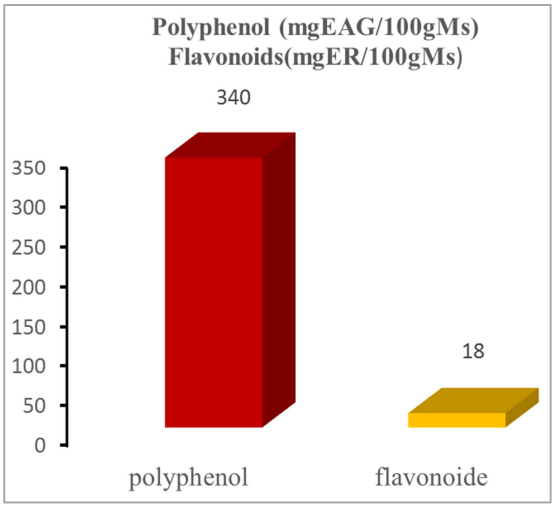

Figure 2. *Determination of total polyphenols and flavonoids.*

4. Discussion

The study of some sperm characteristics and oxidative stress parameters is considered a useful indicator for assessing the effects of plant extracts on testicular function. The main aim of the present study was to evaluate the effects of the aqueous extract of *Tetracarpidium conophorum* kernels on semen quality and oxidative stress parameters in male rats. The results obtained show that aqueous extract of *Tetracarpidium conophorum* in rats induces significant dose-dependent increases in sperm count and motility compared with the control lot treated with distilled water. Our results are in agreement with those of [24, 25]. Similar results were also observed in rats treated with the aqueous extract of *Pausinystalia yohimbe* and the hydroethanol extract of Strychnos camptoneura respectively [3, 26].

Similarly, a significant dose-dependent increase in sperm motility and concentration was observed by [27, 28] in rats treated respectively with aqueous extract of Zanthoxylum macrophylla and ethanolic extracts of Chlorophytum borivilianum from Tribulus Terrestris with Anacyclus. Oral administration of the aqueous extract of *Tetracarpidium conophorum* in rats produced no significant changes in pH or sperm vitality compared with the control lot treated with distilled water. Our results are close to those obtained by [8] in drug-treated rats. This pH is normal and reflects a normal state of the seminal vesicles and prostate, as an acidic pH is indicative of an altered prostate [10] in drug-treated rats. The improvement in semen characteristics observed could also be linked to the antioxidant properties of the extract.

Sperm membranes are particularly rich in polyunsaturated fatty acids, making them especially sensitive to reactive oxygen species (ROS) derived from oxygen metabolism. As well as acting on lipids, ROS can also damage proteins and DNA. These molecules can lead to lipid peroxidation of the sperm plasma membrane, problems in the course of capacitation or the acrosomal reaction, and loss of motility, potentially resulting in infertility [10]. Antioxidant compounds present in plants could therefore protect sperm DNA from free radicals and improve sperm characteristics. The aqueous extract of *Tetracarpidium conophorum* is said to contain a number of phenolic compounds (flavonoids, tannins, etc.) revealed by chemical screening [29], with chemical analysis revealing the presence of total polyphenols and flavonoids, which confer antioxidant properties and act as free-radical scavengers [30], limiting the negative impact of free radicals on spermatozoa. The high protein content of the extract is also thought to improve semen characteristics [14, 15].

In fact, it has been reported that proteins are macromolecules that are among the most essential metabolic nutrients and contribute to sperm nutrition and maturation [10]. The results of this study show that oral administration of the aqueous extract of *Tetracarpidium conophorum* kernels produced no significant variation in Malondialdehyde (MDA) and Catalase (CAT) concentrations, whatever the dose used, compared with control animals. These results confirm the possible antioxidant effects of the aqueous extract of *Tetracarpidium conophorum* kernels. Our results are similar to those obtained by [31, 32] in guinea pigs exposed to Cypermethrin and treated respectively with ethanol extract of Mangifera indica leaves and ethanol extract of Bersama engleriana leaves on oxidative stress and reproductive parameters in male Guinea pig (Cavia porcellus) exposed to cypermethrin. Indeed, phenolic compounds present in the aqueous extract of *Tetracarpidium conophorum* contribute directly to antioxidant actions as they are considered the most important antioxidant components. [33]. Such molecules and their actions can prevent lipid peroxidation of lipid membranes, thereby reducing the concentration of MDA and the activities of antioxidant enzymes (SOD and CAT). The non-variation in MDA and CAT concentration could explain the concomitant increase in GSH and GPX levels, which are involved in their degradation [31].

5. Conclusion

The main aim of this study was to evaluate the effects of *Tetracarpidium conophorum* aqueous extract on some semen characteristics and oxidative stress parameters. The results show that aqueous *Tetracarpidium conophorum* extract improves semen characteristics and reduces the harmful effects of oxidative stress. These results suggest that aqueous *Tetracarpidium conophorum* extract is likely to have spermatogenic and antioxidant potential. This justifies its use in traditional Congolese pharmacopoeia for the treatment of male infertility.

References

[1] World Health Organization., 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition). Geneva: World Health Organization.

[2] Trussell J., 2013. Optimal diagnosis and medical treatment of male infertility. Semin Reprod Trussell J. Med. 31: 6-235.

[3] Akassa H., Makemba Nkounkou G. S., Nkoua Badzi C., Etou Ossibi A. W., Tamboura H. H., Abena A. A., 2019. Effects of aqueous extract of Pausinystalia yohimbe trunk bark on semen quality and reproductive performance in male wistar rats. Int. J. of Multidisciplinary and Current research. 7: 732-740.

[4] Agarwal A., Said T., 2005. Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. BJU Int; 95: 7-503.

[5] Methorst C., Huygue E., 2014. Members of the Andrology and Sexual Medicine Committee of the Association Française d'Urologie. Oxidative stress and male infertility: pathophysiology and therapeutic interest of antioxidants. Progress in Urology, Vol. 24: 4-10.

[6] Ngoula F., Guemdjo Tekam M., Kenfack A., Tadondjou Tchingo CD., Nouboudem S., Ngoumtsop H., Tsafack B., Teguia A., Kamtchouing P., Galeotti M., Tchoumboue J., 2017. Effects of heat stress on some reproductive parameters of male cavie (Cavia porcellus) and mitigation strategies using guava (Psidium guajava) leaves essential oil. J Therm Biol. 64: 67-72.

[7] Kenfack A., Akono N. E., Ngoula F., Nounamo J. G. A., Kamtchouing P., Tchoumboue J., 2018. Post Exposure Effects of Propoxur (Agricultural Pesticide) on Male Fertility in Wistar Rat. Iranian Journal of Toxicology; 12 (3): 21-27.

[8] Landry M. M., Mbongo, J. A., Bimangou J. D. D., Mokondjimobé, E., Iloki L. H., and Abena, A. A., 2019. Latrogenic male infertility with psychotropic drugs in rats: case of haloperidol and clomipramine. International journal of scientific research, (8): 1-3.

[9] Huyghe H., Bonal M., Daudin M., Droupy S., 2013. Sexual dysfunctions and infertility. Progress in Urology Elsevier Masson.; 23: 745- 751.

[10] Akassa H., Peneme B. M. L., Morabandza C. J., Etou ossibi A. W, and Abena A. A, 2022. Evaluation of the androgenic potentialities of the hydro-ethanolic extract of the trunk barks of Strychnos camptoneura (longaniaceae) in the wistar rat. world journal of pharmacy and pharmaceutical sciences; 11: 145-156.

[11] Azonbakin S., Dangbemey P., Osseni R., Yaude, S. A., Kora F., Adovoekpe D., Djego F., Laleye A., Gbaguidi F., 2021. Enquête ethnobotanique sur les plantes utilisées dans le traitement de l'infertilité masculine au Benin. International Journal of Biological and Chemical Sciences. 15. 1667-1677.

[12] Zohoun T. H., Flenon J., 1997. Can traditional medicine and the African pharmacopoeia provide an alternative to the current prohibitive costs of modern medicine? In Pharmacopoeia and Traditional African Medicine. U. B. Press.: Lomé; 3-15.

[13] Byg A., Baslev H., 2001. Diversity and use of palms in Zahamena, eastern Madagascar. Biodivers Conserv., 10: 951–70.

[14] Ntsoumou VM., Adzona PP., Bati J. B., Kengue T., Mabanza -Mbanza B. B., Saboukoulou AJ., Ndinga AF., Guembo J. R., and Banga -Mboko H., 2021. Evaluation of the substitution of Glycine meal max (L.) Merr. (Soja) by the Tetracarpidium conophorum (Mull. Arg.) & Dalz on the performance of laying hens. Int. J. Biol. Chem. Sci.: 15 (6): 2526-2542.

[15] Ossoko J PL., Dzondo G. M., Mvoula T. D., et al., 2021 Assessment of the nutrtional potential of the kernels of the seeds of Tetracarpidium conophorum collected in Lékana in the Départementof Plateaux in the Republic of Congo; J. Bio. Innov. ; 10 (4): 1102- 1110.

[16] Akomolafe SF., Oboh G., Oyeleye S. I., and Tosin A., Olasehinde T. A., 2017. Toxicological Effects of Aqueous Extract From (African Walnut (Tetracarpidium conophorum) Leaves in Rats Journal of Evidence-Based Complementary & Alternative Medicine.; 22 (4): 919-925.

[17] Akassa H., Peneme B. M. L., Etou Ossibi A. W., Morabandza C. J., Kanga M. R., Goma Dinouvanza A., and Abena A. A.,2023. Androgenic and Antioxidant Effects of Ethanolic Extract of Strychnos camptoneura (longaniaceae) Trunk Bark in Male Wistar Rats. American Journal of Pharmacological Sciences, 11(1): 8-14.

[18] Dérivaux V., Ectors F., 1986. Animal domestic reproduction. 3e édition. Cobay Louvain. La Neuve; Liège: 1-439.

[19] Ngoula F. Effects of organophosphorus and carbamate pesticides on some reproduction parameters in the adult male rat. PhD thesis, University of Yaoundé I; Cameroun, 163p. 2008.

[20] Sarika C., 2022. Evaluation of ameliorative effect of quercetin and candesartan in isoprenaline induced myocardial infarctionWorld Journal of Pharmaceutical Research; 11 (2): 1409-1412.

[21] Uhegbu F. O., Imo C., and Ugbogu A. E., 2015. Effect of aqueous extract of Piper guineense seeds on some liver enzymes, antioxidant enzymes and some hematological parameters in albino rats. International Journal of Plant Science and Ecology; 1: 167-171.

[22] Brand Williams W., Cuvelier M. E., and Berset C., 1995. Use of free radical method to evaluate antioxidant activity. Technology 28: 25-30.

[23] Waterhouse A. L., 2001. Determination of total phenolics. Current Protocols in Food Analytical Chemistry; I1.1.1-I1.1.8.

[24] Ngandjui A., Ngaha N. M., Kenmogne H., Koloko B. L., Massoma L. D., 2017. Evaluation of the fertility activity of the aqueous leaves extract of Zanthoxylum macrophylla (Rutaceae) on male rats. J Phytopharmacol; 6 (5): 277-281.

[25] Syed A H., Ahsan H., Furqan N., Yang W., Hafiz A., Rasul S., and Yuanda S., 2018. Evaluation of the Spermatogenic Activity of Polyherbal Formulation in Oligospermic Males. BioMed Research International: 1-10.

[26] Akassa H, Samlan O. A. N., Peneme B. M L., Goma D. A. L., Kanga M. R., and Abena A. A., 2023. Spermatogenic and antiradical activities of the hydro-ethanolic extract of Strychnos camptoneura (longaniaceae) trunk barks in Wistar rats orld Journal of Advanced Research and Reviews, 17 (01), 386–394.

[27] Dariush H. M., Bagher M., Pardis H., Paria A., Ensieh S., Parsova T., Mahmoud H., Mahmoud M., 2019. Improvement of fertility parameters with Tribulus Terrestris and Anacyclus Pyrethrum treatment in male rats. Int Braz J Urol; 45 (5): 1043-1054.

[28] Maïmouna C. K., Blaise W. K, I., 2021. Effect of Different Parts of Kigelia africana Fruit Aqueous Extracts on Sperm Parameters and Testis Advances in Reproductive Sciences; 9: 171-188.

[29] Chikezie., Uchechukwu N., 2017. Phytochemical and Proximate Compositions of Tetracarpidium conophorum [African Walnut] Seeds, International Journal of Research Studies in Biosciences; 5 (10): 25-31.

[30] Knasmüller S., Nersesyan A., Misík M., Gerner C., Mikulits W., Ehrlich V., Hoelzl C., Szakmary A., Wagner K. H., 2008. Use of conventional and -omics based methods for health claims of dietary antioxidants: a critical overview. Br. J. Nutr; 99: 3-52.

[31] Nounamo Guiekep A. J., Kenfack A., Ngoula F., Vemo, N. B., Nguemmeugne, K. S., Tedonkeng P. E.,. 2019. Attenuating effects of Mangifera indica leaves ethanolic extract against acetamiprid induced reproductive toxicity in male guinea pigs Veterinary Research Forum. 10 (3) 187-192.

[32] Vemo B. N., Kenfack A., Ngoula F., Akono N. E., Kodjio N., Guiekep N. A. J., Tsambou megnimeza A. M., and Teguia A., 2017. Effects of ethanol extract of Bersama engleriana leaves on oxidative stress and reproductive parameters in male Guinea pig (Cavia porcellus) exposed to cypermethrin Int. J. Biol. Chem. Sci. 11 (5): 2243-2253.

[33] Badmus J. A., Adedosu T. O., Fatoki J. O., et al., 2011. Lipid peroxidation inhibition and antiradical activities of some leaf fractions of Mangifera indica. Acta Pol Pharm; 68 (1): 23-29.